



**EFFECT OF LIPOPHILIZATION ON THE ANTIOXIDANT ACTIVITY OF
CARVACROL, QUERCETIN AND VANILLIN WITH CONJUGATED LINOLEIC
ACID**

**EFEECTO DE LA LIPOFILIZACIÓN SOBRE LA ACTIVIDAD ANTIOXIDANTE DE
CARVACROL, QUERCETINA Y VAINILLINA CON ACIDO LINOLEICO
CONJUGADO**

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Received: October 18, 2018; Accepted: January 7, 2019

Abstract

The structure and physicochemical and antioxidant properties of carvacrol, quercetin and vanillin were modified by a lipophilic reaction with conjugated linoleic acid (CLA). The chemical structure of the precursor and lipophilized antioxidants was determined by ¹H NMR. The derivatives of carvacrol-CLA and quercetin-CLA showed the highest yields of lipophilization. The lipophilized systems had greater lipophilicity (log *P*, log *S* and hydrophilic-lipophilic balance) and greater antioxidant activity than the polyphenol precursors, being the carvacrol-CLA and vanillin-CLA complexes that presented the highest antioxidant activity. The results suggest that the lipophilization reaction improved the lipophilicity and antioxidant activity of the compounds involved and that these lipophilized derivatives could have applications in the food, cosmetic and medical industries.

Keywords: Antioxidant activity, esterification, CLA, hydrophobicity, lipophilicity.

Resumen

La estructura y las propiedades fisicoquímicas y antioxidantes de carvacrol, quercetina y vainillina se modificaron mediante una reacción de lipofilización con ácido linoleico conjugado (CLA). La estructura química de los antioxidantes precursores y lipofilizados se determinó por ¹H RMN. Los derivados de carvacrol-CLA y quercetina-CLA mostraron los mayores rendimientos de lipofilización. Los sistemas lipofilizados tuvieron mayor lipofilicidad (log *P*, log *S* and equilibrio hidrofílico-lipofílico) y mayor actividad antioxidante que los precursores polifenólicos, siendo los complejos carvacrol-CLA y vainillina-CLA los que presentaron la mayor actividad antioxidante. Los resultados sugieren que la reacción de lipofilización mejoró la lipofilicidad y la actividad antioxidante de los compuestos involucrados y que estos derivados lipofilizados podrían tener aplicaciones en las industrias alimentaria, cosmética y médica.

Palabras clave: Actividad antioxidante, esterificación, CLA, hidrofobicidad, lipofilicidad.

1 Introduction

Natural phenolic compounds, such as carvacrol, quercetin and vanillin, are polyphenolic compounds with potential biological properties associated with beneficial health effects, like antioxidant,

chelating, free radical scavenging, anti-inflammatory, antiallergic, antimicrobial, antiviral, anticarcinogenic and antiobesity (Frankel *et al.*, 1993). These and other essential oils have been used in the preparation of emulsions, encapsulation and have been incorporated into edible films or other products due to their antimicrobial and antioxidant properties (Bautista-Baños *et al.*, 2018; Ocampo-Salinas *et al.*, 2016).

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<https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n2/Ortega>
issn-e: 2395-8472

As antioxidants, phenolic compounds may also protect cells against oxidative stress (Zhong and Shahidi, 2011). The stability and antioxidant activity depend on several factors, such as the structure of the phenolic compound, the pH, temperature, and their interactions with other synergists or antagonists present.

Although polyphenols exhibit high antioxidant activity *in vitro*, a low bioavailability in a cellular environment has been demonstrated due to their hydrophilic nature, which limits the passage through the cell membrane (Bernini *et al.*, 2017). The ability to cross biological membranes and reach the site of action in the cell, as well as its release and absorption, are dependent on its solubility and hydrophilic-lipophilic balance (HLB) (Cruz *et al.*, 2017). In addition, its hydrophilic nature negatively affects its efficacy in lipophilic systems, such as fats, oils, cosmetic formulas and lipophilic biological systems.

Therefore, the lipophilization reaction can improve lipophilicity, solubility and miscibility in lipid systems. Lipophilization is the modification of a substrate via esterification with a lipophilic moiety, resulting in a new molecule with greater affinity to non-polar compounds and with a modified HLB (Figueroa-Espinoza and Villeneuve, 2005). This reaction can be carried out by different methods, including *N*-hydroxysuccinamide esters (Haque *et al.*, 1982), succinic or acetic anhydrides (Messinger *et al.*, 1987) or fatty acid chlorides (Roussel-Philippe *et al.*, 2000). However, there are unproven reactions, such as Fischer esterification, which is a type of simple esterification involving the formation of a hydroxyl group ester and a carboxyl in the presence of an acid catalyst (Offenhauer, 1964).

The functional and antioxidant properties of lipophilized compounds are associated with the length and the type of the fatty acid chain (Matemu *et al.*, 2011; Silva *et al.*, 2000; Laguerre *et al.*, 2010). Thus, lipophilization with long chain fatty acids resulted in better functional properties (Mendoza-Sanchez *et al.*, 2018). It has been reported that the esterification of omega-3 polyunsaturated fatty acids (PUFAs) to natural phenolics protects the PUFAs from oxidation, contributing to the bioactivity of derived esters (Mbatia *et al.*, 2011). Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of *c*9-, *c*12-octadecadienoic acid that occurs naturally in products from ruminant animals (dairy, meat). CLA has been reported to act as a metabolic regulator, hypocholesterolemic, antiobesity, antiatherogenic and anticarcinogenic agent, antioxidant, as well as exert

favorable effects in the prevention and treatment of certain nourishing allergies (Pariza, 2004). There are currently several commercial products available that contain CLA and polyphenols, with the promise of increasing the metabolic demand of the individual ingredients, as coadjuvants in the defense against oxidative stress and as dietary supplements for fat loss.

It is interesting to know the effect and mechanisms of lipophilization on the interaction and structural modification of hydrophilic antioxidants with CLA as a lipophilic antioxidant that allows incorporation in food, cosmetic or pharmaceutical systems. Thus, this work aimed to increase the lipophilicity of hydrophilic antioxidants and create a synergistic effect by combining two natural antioxidants. The lipophilized derivatives, obtained by esterification of different antioxidants (carvacrol, quercetin and vanillin) with CLA using Fisher's reaction, were evaluated for their antioxidant and lipophilic properties.

2 Materials and methods

2.1 Materials

Conjugated Linoleic Acid (CLA) (75% pure) as a free fatty acid was a kind gift Pharma Nutrients, Inc., (Lake Bluff, IL). The antioxidants carvacrol, quercetin, vanillin; as well as the reagents of trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripiridyl-s-triazine (TPTZ), 2,2'-azinobis-(3-ethylbenzthiazolin)-6-sulfonic acid (ABTS), ethyl acetate (AcOEt), sulfuric acid (H₂SO₄), hexane, methanol (MeOH) were obtained from Sigma-Aldrich.

2.2 Lipophilization of polyphenol compounds

Lipophilization was performed by a Fisher esterification reaction using concentrated sulfuric acid (H₂SO₄) as a catalyst and tetrahydrofuran (THF) as a solvent (Offenhauer, 1964), using a molar ratio of 1:1 (w:w) conjugated linoleic acid, and respective polyphenol (vanillin, carvacrol or quercetin). The purification was carried out by column chromatography using silica gel (100 to 200 mesh) as a stationary phase and 9:1 AcOEt/MeOH mobile phase (v:v), AcOEt/MeOH 8:2 (v:v), AcOEt/Hexane 7:3 (v:v), AcOEt/Hexane 6:4 (v:v) and AcOEt/Hexane 8:2 (v:v), obtaining the corresponding fractions in each of

the reactions. The yield of each one of the reactions was determined as the ratio of the real grams obtained in each of the reactions of the lipophilized systems; comparing them with the theoretical grams that should be obtained in each one of the corresponding reactions using the equation 1.

$$\text{yield (\%)} = \frac{\text{g lipophilized compound}}{\text{g theoretical lipophilized compound}} \times 100 \quad (1)$$

2.3 Optimization and evaluation of lipophilic reactions by ^1H NMR

For the optimization, identification and verification of the lipophilized systems, an analysis by Nuclear Magnetic Resonance (NMR) of ^1H was performed, this technique allowed to verify if the lipophilization reaction in each of the precursor compounds had been carried out. Nuclear Magnetic Resonance (NMR) studies were performed on a BRUKER spectrometer; model Ascend 500 MHz, using as solvents, deuterated methanol (CD_3OD) and deuterated chloroform (CDCl_3). To corroborate the formation of the lipophilized compound, the signals were assigned according to the chemical displacement of each of the hydrogens of the lipophilized systems and compared with the spectra of the antioxidant precursors in a brand spectrometer BRUKER, model Ascend 500 MHz.

2.4 Evaluation of antioxidant activity

2.4.1 Radical 2,2-difenil-1-picrilhidrazilo (DPPH)

The ability to capture radicals of precursors and lipophilized systems was determined through the DPPH radical method according to the method described by Sorensen *et al.* (2014). Carvacrol, vanillin and quercetin and lipophilized systems (carvacrol-CLA, vanillin-CLA and quercetin-CLA) were evaluated at different concentrations (5, 10, 15, 20 and 25 mM/L) in ethanol, absolute ethanol was used as the target and a 1:1 (v/v) solution of 300 μM DPPH and ethanol was used as a standard control, which represented 0% antioxidant activity. 100 μL of the samples were taken in triplicate and placed in a 96-well plate, then 100 μL of 300 μM DPPH in ethanol was added. The reaction mixture was stirred and incubated for 30 minutes to protect the light, finally the absorbance at 520 nm was measured against the control blank. The readings were

made in a microplate reader (Multiskan Spectrum Thermo Electron Corporation, USA). The results were reported as percentage of DPPH radical inhibition using the following equation (Eq. 2):

$$\text{Inhibition[\%]} = \left[1 - \left(\frac{A_1}{A_0} \right) \right] \times 100 \quad (2)$$

where: A_1 is the absorbance of the sample and A_0 is the absorbance of the standard.

2.4.2 Analysis of reducing power (FRAP)

The ferric/antioxidant reducing power (FRAP) analysis was performed in a 96-well microplate and the absorbance at 595 nm was measured to obtain the blank reading (initial reading). Subsequently, 5 μL of sample were placed in each well incubating for 8 minutes and the absorbance was measured at 595 nm (final reading). To obtain the FRAP value of each sample, the final reading will be left over from the initial reading. The measurements were made on a Multiskan Spectrum microplate reader (Thermo Electron Corporation). FRAP values were expressed as micromoles (μmoles) equivalents of TROLOX (a water soluble analogue of vitamin E)/L.

2.4.3 Inhibition by ABTS

The percentage of inhibition by ABTS was performed according to the methodology developed by Ré *et al.* (1999) in which radical $\text{ABTS}^{\bullet+}$ was obtained after the reaction of ABTS (7 mM) with potassium persulfate (2.45 mM, final concentration) incubated at room temperature (± 25 °C) and in the dark for 16 h. Once the radical $\text{ABTS}^{\bullet+}$ was formed it was diluted with ethanol until obtaining an absorbance value comprised between 0.70 (± 0.1) to 754 nm. The samples of polyphenols and lipophilized compounds were diluted with ethanol until an inhibition of 20 to 80% occurs, compared to the absorbance of the blank, after adding 20 μL of the sample. To 980 μL of $\text{ABTS}^{\bullet+}$ radical dilution, the absorbance 754 nm was determined at 30 °C, 20 μL of the sample was added and the 754 nm was measured after 1 min. The synthetic antioxidant of reference, Trolox, was evaluated in a range of concentrations of 0-25 mM in the same conditions. The results were expressed in TEAC (equivalent to μmol Trolox/L).

2.5 Lipophilicity characterization

The lipophilicity was assessed according to the following parameters: partition coefficients ($\log P$) and solubility ($\log S$), using the software OSIRIS Data Warrior v.4.4.3 of Actelion Pharmaceuticals Ltd. The Hydrophilic-Lipophilic Balance (HLB) of the lipophilized systems was calculated according to the method proposed by Davis, (1957) based on the assignment of a group number to the chemical groups that make up the surfactant using the following equation (Eq. 3):

$$HLB = 7 + \sum \text{Hydrophilic groups} + \sum \text{Lipophilic groups} \quad (3)$$

2.6 Statistic analysis

The analyzed data corresponding to the antioxidant activity were subjected to a two-way analysis of variance (ANOVA) using a Tukey comparison test.

3 Results and discussion

3.1 Chemical lipophilization of polyphenols and CLA

Structural assignment of all lipophilized systems was carried out using ^1H NMR, and two-dimensional experiments. Since CLA is constituted by a mixture of isomers, the product was a mix of lipophilized products, which was not subsequently purified. In the assignment, the representative bands of the said mixture of isomers are mentioned.

3.1.1 Lipophilization of carvacrol

The ^1H NMR spectra for carvacrol (Fig. 1a) and the lipophilized product of carvacrol-CLA (Fig. 1b) were assigned as follows. In the carvacrol spectrum, a doublet at $\delta = 1.2$ ppm was ascribed to the methyl groups ($-\text{CH}_3$) of the iso-propyl; the methine group ($-\text{CH}$, 9) occurred as multiplets at $\delta = 2.7$ ppm; the signal at $\delta = 2.15$ ppm corresponded to the third methyl group ($-\text{CH}_3$, 7); the single signal close to $\delta = 5$ ppm was assigned to the hydroxyl group ($-\text{OH}$); finally, the signals between $\delta = 6.5$ -7.0 ppm were assigned to the aromatic ring hydrogens ($-\text{CH}$, 1, 2, 4). The representative signals of the lipophilized

product included the terminal methyl group of the CLA chain ($-\text{CH}_3$, 30) at $\delta = 0.98$ ppm; multiplets in the region $\delta = 1.1$ -1.8 ppm, containing the methyls of the iso-propyl group ($-\text{CH}_3$, 10, 11), as well as the methylenes of the CLA chain ($-\text{CH}_2$, 16-19, 26-29); signals for the methylene groups ($-\text{CH}_2$, 25, 20 and 14), as well methyl 7 and methine 9, between $\delta = 1.9$ -2.4; the vinyl hydrogens of the CLA chain ($-\text{CH}$, 21-24) at $\delta = 5.2$ -6.1 ppm, and finally, the aromatic ring hydrogens ($-\text{CH}$, 1, 2 and 4) at $\delta = 6.2$ -6.9 ppm.

3.1.2 Lipophilization of quercetin

The ^1H NMR spectra for quercetin (Fig. 2a) and the lipophilized product of quercetin-CLA (Fig. 2b) were assigned as follows. In the quercetin spectrum, a signal was observed at $\delta = 4.95$ ppm, containing the hydroxyl groups of the system ($-\text{OH}$, 7, 8, 20-22); simple signals at $\delta = 6.2$ and 6.45 ppm were ascribed to methine 2 and methine 6, respectively; doublets at $\delta = 6.9$ and 7.65 ppm corresponded to methine 16 and methine 15, respectively, and finally, a simple signal at $\delta = 7.75$ ppm was assigned to methine 19.

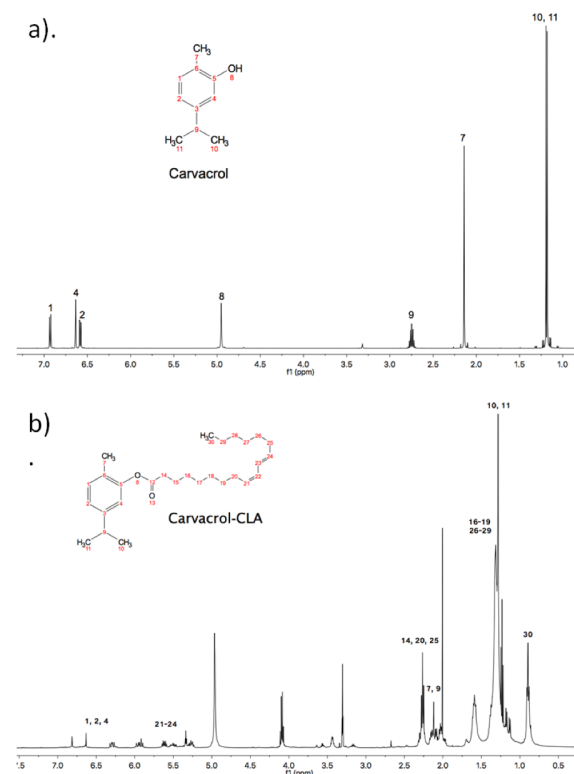


Fig. 1. ^1H NMR spectra of a) Carvacrol and b) Carvacrol-CLA lipophilized.

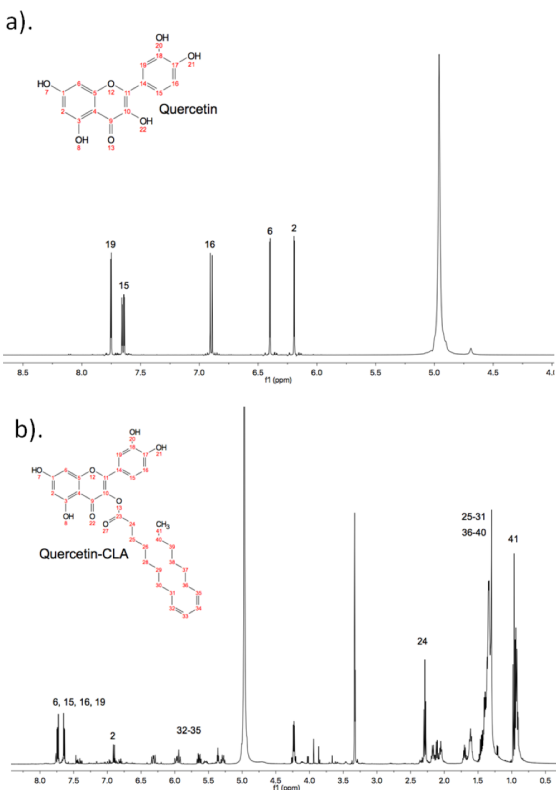


Fig. 2. ^1H NMR spectra of a) Quercetin and b) Quercetin-CLA lipophilized.

The spectrum of the lipophilized system displayed the methyl of the CLA chain ($-\text{CH}_3$, 41) at $\delta = 0.90$ ppm; the methylenes of the CLA chain ($-\text{CH}_2$, 25-31 and 36-40) in the region of $\delta = 1.1$ - 2.2 ppm; the signal for methylene 24 at $\delta = 2.3$ ppm; signals for the vinyl protons of the CLA chain ($-\text{CH}$, 32-35) at $\delta = 5.2$ - 6.4 ; methine 2 of the aromatic system at $\delta = 6.9$ ppm, and signals for protons 6, 15, 16 and 19 in the region of $\delta = 7.2$ - 7.8 ppm.

3.1.3 Vanillin lipophilization

The ^1H NMR spectra for quercetin (Fig. 3a) and the lipophilized product of quercetin-CLA (Fig. 3b) were assigned as follows. The vanillin spectrum showed a signal at $\delta = 3.95$ ppm, which was appointed to the methylene group ($-\text{CH}_3$, 12); at $\delta = 6.7$ - 7.5 ppm, the corresponding signals were assigned to the aromatic hydrogens ($-\text{CH}$, 1, 2 and 5); the broad signal at $\delta = 4.9$ ppm was ascribed to the hydroxyl group ($-\text{OH}$, 7), and finally, the signal at $\delta = 9.8$ ppm corresponded to the aldehyde group ($-\text{CHO}$, 9).

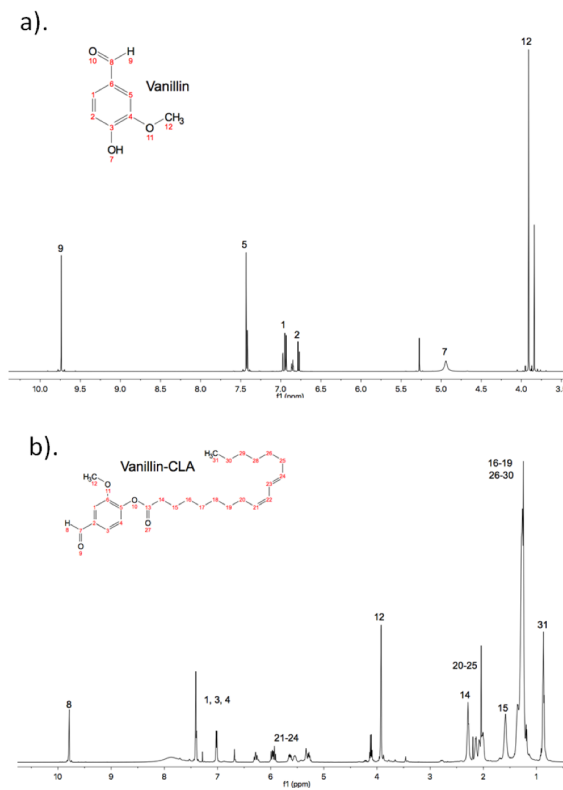


Fig. 3. ^1H NMR spectra of a) Vanillin and b) Vanillin-CLA lipophilized.

The spectrum of the lipophilized system included a signal at $\delta = 0.9$ ppm, which was assigned to the methyl of the CLA chain ($-\text{CH}_3$, 31); multiplets in the $\delta = 1.0$ - 1.5 range, corresponding to the methylenes of the CLA chain ($-\text{CH}_2$, 17-20 and 27-30); a signal at $\delta = 1.6$ ppm, corresponding to methylene 16; a signal at $\delta = 2.0$ ppm that was assigned to the methylenes 21 and 26; a signal at $\delta = 2.3$ ppm attributed to methylene 11; a signal at $\delta = 3.9$ ppm, which was assigned to the methoxyl group ($-\text{OCH}_3$, 8); a series of signals between $\delta = 5.1$ - 6.3 , corresponding to the vinyl hydrogens of the CLA chain ($-\text{CH}$, 21-24); signals between $\delta = 6.9$ - 7.5 , corresponding to the aromatic ring ($-\text{CH}$, 1, 3, 4), and finally, a simple signal at $\delta = 9.8$ ppm, which was assigned to the hydrogen of the aldehyde group ($-\text{CHO}$).

Table 1 shows that different yields presented in each of the lipophilization reactions, with the lipophilized carvacrol-CLA system exhibiting the greatest lipophilization yield (80%). This lipophilization performance is possibly due to the low incorporation by the long non-linear CLA chain (Young and Shahihi, 2017).

Table 1. Yields (%) for the lipophilization reaction of the polyphenols with CLA.

Lipophilized samples	Yield (%)
Carvacrol-CLA	80 ± 1.15
Quercetin-CLA	75 ± 1.07
Vanillin-CLA	70 ± 0.98

The data represent the average of n=3 replicates or measurements ± the standard deviation.

3.2 Antioxidant activity

Several methods are available to determine the antioxidant activity. Each of these tests evaluates the antioxidant activity of the test material from a different perspective. Consequently, it has been demonstrated that the best approach to estimating the antioxidant activity is using a combination of two or more complementary tests (Ozer *et al.*, 2018). In this work, antioxidant activity was evaluated *in vitro*, through reducing power (FRAP), and the capacity to inhibit DPPH⁺ and ABTS^{•+} free radicals. Both lipophilic and hydrophilic groups are present in the structure of the lipophilized compounds, so the challenge was to select appropriate analytical tools to assess the antioxidant properties (Sorensen *et al.*, 2014).

Carvacrol, quercetin and vanillin are recognized compounds that exhibit antioxidant activity and whose consumption is considered desirable due to its beneficial effects on health. Table 2 shows that antioxidant activity estimated by the inhibition of DPPH radicals by the precursor and lipophilized phenolic compounds depended on the phenolic compound used. The most potent lipophilized systems were carvacrol-CLA (87.96%) and vanillin-

CLA (85.21%) in comparison with their respective carvacrol, vanillin and CLA precursors. It has been reported that the antioxidant properties of lipophilized compounds are influenced by the molecular structure of the ring of the phenolic compounds involved (Rice-Evans *et al.*, 1997; Medina *et al.*, 2009), as well as the extension of the acid chain fatty acid used in the esterification reaction (Zhong and Shahidi, 2011; Liu *et al.*, 2000). This behavior may be because antioxidants possess a degree of hydroxylation and hydroxyl-specific groups presents in polyphenols and CLA confer some degree of radical stability. Thus, dihydroxyl substitutions have greater antioxidant activity than monohydroxy substitution, due to greater resonance stabilization (Zhou and Elias, 2013).

In contrast, the quercetin-CLA system showed a slightly lower antioxidant activity than the initial precursors. This observation could be because the groups involved in free radical scavenging activity of quercetin are the hydroxyl groups (OH), which are more available to react with the radicals, causing greater antioxidant activity compared to their lipophilized systems (Hazra *et al.*, 2008). Consistent with the DPPH values, the lipophilized carvacrol-CLA system exhibited the greatest reducing power (16941 μmol Trolox/L) and percentage inhibition of the ABTS^{•+} radicals (96.76%), indicating a greater antioxidant capacity compared to the carvacrol alone and with the other lipophilized compounds. The different antioxidant techniques were evaluated at various concentrations (5-25 mM/L). La antioxidant activity of the antioxidant precursors and the lipophilized compounds evaluated by the radical DPPH, FRAP and ABTS had a concentration dependent trend.

Table 2. Antioxidant properties of the phenolic compounds and derivates lipophilized with conjugated linoleic textacid (CLA).

Antioxidant	DPPH (% inhibition)	IC50* (mM/L)	FRAP (μmol Trolox/L)	ABTS (% inhibition)
Carvacrol	79.69 ± 0.70 ^{a,b}	8.27 ± 0.25 ^d	13739.83 ± 202.26 ^d	93.95 ± 0.90 ^c
Carvacrol-CLA	87.96 ± 1.40 ^c	7.54 ± 0.40 ^c	16941 ± 186.62 ^e	96.76 ± 0.60 ^d
Quercetin	78.96 ± 1.80 ^a	4.79 ± 0.55 ^a	14066.50 ± 380.01 ^d	98.24 ± 0.40 ^d
Quercetin-CLA	80.45 ± 1.10 ^{a,b}	4.15 ± 0.42 ^a	13308.17 ± 509.28 ^{c,d}	97.38 ± 0.29 ^d
Vanillin	77.53 ± 0.50 ^a	7.66 ± 0.15 ^c	10263.17 ± 213.33 ^b	90.94 ± 0.40 ^b
Vanillin-CLA	85.21 ± 0.10 ^c	6.17 ± 0.08 ^b	12914.83 ± 81.26 ^c	94.27 ± 0.20 ^c
CLA	77.58 ± 0.02 ^a	13.33 ± 0.10 ^e	3566.50 ± 203.36 ^a	68.87 ± 0.70 ^a

The data represent the average of n=3 replicates or measurements ± the standard deviation at a concentration of 25 mM. Equal letters in the same column mean no significant differences ($p < 0.05$). *IC50 determined on the basis of the DPPH (% Inhibition) radical.

The IC50 values were lower for the lipophilized derivatives quercetin-CLA (4.15 mM/L), vanillin-CLA (6.17 mM/L) and carvacrol-CLA (7.54 mM/L) compared to the corresponding precursor compounds quercetin (4.79 mM/L), vanillin (7.66 mM/L) and carvacrol (8.27 mM/L), suggesting a greater antioxidant activity. In contrast, CLA alone showed the highest value of IC50 (13.33 mM/L), indicating that their antioxidant activity is improved when it is bound to a polyphenolic compound. This finding can be attributed to the functional groups of these compounds, such as hydroxyls (OH) and double bonds, both of the aromatic rings, as well as the hydrocarbon chain provided by the CLA. These groups can act together, allowing the incorporation and neutralization of free radicals, thereby increasing the antioxidant activity in each case (Antolovich *et al.*, 2002).

3.3 Lipophilicity of polyphenols and lipophilized derivatives

The calculated $\log P$ and $\log S$ ($\text{clog } P$ and $\text{clog } S$) values were used to assess the lipophilicity of the polyphenol precursors and lipophilized products (Table 3). For the lipophilized compounds, the lipophilicity values estimated by $\text{clog } P$ varied between 8 and 9, whereas the precursor compounds presented values between 1 and 2. The $\text{clog } P$ values decreased in the order of carvacrol-CLA (9.94) > quercetin-CLA (8.74) > vanillin-CLA (8.27). All the lipophilized compounds exhibited $\text{clog } P$ values > 5 and $\text{clog } S$ values < -5, which indicates that these compounds are highly lipophilic and predicted to bind to plasma proteins (Kates, 2011). The enhanced lipophilicity of lipophilized systems may lead to their improved incorporation into the lipid bilayers of cell membrane and hence better bioavailability in the body, as well as greater potential in liposome-based drug delivery systems. The $\text{clog } P$ values found for lipophilized systems agree with the low water solubility values estimated through $\text{clog } S$ and indicate greater lipophilic than hydrophilic properties, which is due to the incorporation of the CLA molecule. According to the HLB values, all lipophilized derivatives had higher HLB values than the phenolic precursor compounds. The lipophilized derivatives carvacrol-CLA (0.001) and vanillin-CLA (0.250) had the lowest HLB values, when using the method of Davis (1957), reflecting a higher percentage of hydrophobicity, as reported by Griffin (1949).

Table 3. Calculated parameters of lipophilicity of antioxidants and lipophilized derivatives.

Antioxidants	$\text{clog } P$	$\text{clog } S$
Carvacrol	2.84	-2.53
Carvacrol-CLA	9.94	-6.99
Quercetin	1.49	-2.49
Quercetin-CLA	8.74	-6.76
Vanillin	1.77	-1.66
Vanillin-CLA	8.27	-6.12
Conjugated Linoleic Acid (CLA)	6.46	-4.32

$\text{clog } P$ = Partition coefficient and $\text{clog } S$ = Solubility coefficient.

Conclusions

The lipophilization of the carvacrol, quercetin and vanillin phenolic compounds with CLA was confirmed by ^1H NMR. The lipophilization changed the structure and properties of carvacrol, quercetin and vanillin, increasing their hydrophobicity and reducing their water solubility, thereby enhancing their functional properties. The lipophilized carvacrol-CLA and quercetin-CLA systems exhibited the best performance in the lipophilization reaction, with the lipophilized carvacrol-CLA system presenting the best yield and antioxidant activity in all three methods (ABTS, DPPH, FRAP). The antioxidant activity of all lipophilized derivatives was higher than the precursor polyphenols and CLA. Overall, all lipophilized systems showed enhanced lipophilicity properties ($\text{clog } P$, $\text{clog } C$ and HLB).

Acknowledgements

The first author thanks the support of the National Council of Science and Technology (CONACYT) for the grant awarded to study the Master of Food Sciences (Grant number 337414) and the project of Research and Development of Food Laboratory (L-IDEA, number 124229).

Nomenclature

A_1	absorbance of the sample
A_0	absorbance of the standard
TEAC	equivalent to $\mu\text{mol Trolox/L}$
NMR	Nuclear magnetic resonance
DPPH ⁺	2,2'-diphenyl-1-picrylhydrazyl radical
FRAP	Ferric reducing antioxidant power

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