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Phenolic Acids Enzymatic Lipophilization

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Lipophilization is the esterification of a lipophilic moiety (fatty acid or fatty alcohol) on different substrates (phenolic acid, sugar, protein, ...), resulting in new molecules with modified hydrophilic/lipophilic balance. This reaction can be obtained chemically or enzymatically using different enzymes. Phenolic acids possess interesting biological properties (antioxidant, chelator, free radical scavenger, UV filter, antimicrobial, ...), but because of their relatively low solubility in aprotic media, their application in oil-based products is limited. Therefore, the esterification of their carboxylic acid function with a fatty alcohol enhances their hydrophobicity and results in a multifunctional amphiphilic molecule. Enzymatic lipophilization of phenolic acids is nowadays studied for potential industrial applications. Different systems have been proposed to perform the reaction yield [free or immobilized enzymes (lipase, feruloyl esterase, tannase, etc.), free or added organic solvent, addition of surfactant, microemulsion system, etc.]. Some of the functional properties of these esters have been demonstrated. This review presents a panorama of the advances in this field.

Keywords: Phenolic acid; lipase; lipophilization; esterification; antioxidant; emulsion; multifunctional; additive

INTRODUCTION

A large number of phenols and phenol derivatives occur in nature, particularly in the plant kingdom. Phenolic acids and derivatives, together with flavonoids, stilbenes, and lignans, constitute the polyphenols, which are secondary plant metabolites and form part of the human diet. Polyphenols participate in the defense system of plants and possess antioxidant and several biological properties.

The phenolic acid family is composed by the cinnamic (C_6-C_3) and the benzoic (C_6-C_1) acid derivatives (**Figures 1** and **2**), characterized by containing a benzene ring substituted with one or more hydroxy or methoxy groups and a carboxylic group. The cinnamic derivatives also contain a propenoic chain (*1*). Phenolic acids are natural hydrophilic antioxidants, which occur ubiquitously in fruits, vegetables, spices, and aromatic herbs. They are of particular interest because of their potential biological properties, such as antioxidant, chelating, free radical scavenging, anti-inflammatory, antiallergic, antimicrobial, antiviral, anticarcinogenic, and UV filter properties (*2−8*).

PHENOLIC ACIDS AS ANTIOXIDANTS

According to Halliwell (*9*), an antioxidant is any substance that, when present at low concentrations compared to those of an oxidizable substrate (proteins, lipids, carbohydrates, DNA), significantly delays or prevents oxidation of that substrate. Antioxidants are frequently employed to prevent lipid oxidation in foods, cosmetics, and pharmaceutical products, as they slow the oxidation of unsaturated fats and oils and the concomitant formation of bad smells and tastes, preserving food quality. According to Frankel and Meyer (*10*), the requirements to select the most effective antioxidant for a food are to understand the true protective properties of the antioxidant, to identify the substrates being oxidized, the location of the antioxidant in the system, and the effect of other components on the antioxidant activity, and to determine the relevance of a model system to a real food.

Many sources of antioxidants are available in nature. Extracts rich in natural antioxidants as phenolic compounds can be obtained from raw vegetable materials by simple preparation, and they are not considered as chemicals by consumers; however, they are not pure, and composition has to be optimized. Often, a larger addition is necessary to have a good activity, which could be expensive, and they might impart off-flavors or color to the stabilized products. According to Shahidi (*11*), extracts from rosemary and tea have been commercialized in Japan, Europe, and North America, despite their higher cost in comparison with synthetic additives. More research on the safety of natural antioxidants to be used as additives in food have to be done, as not enough data on their biological activity are

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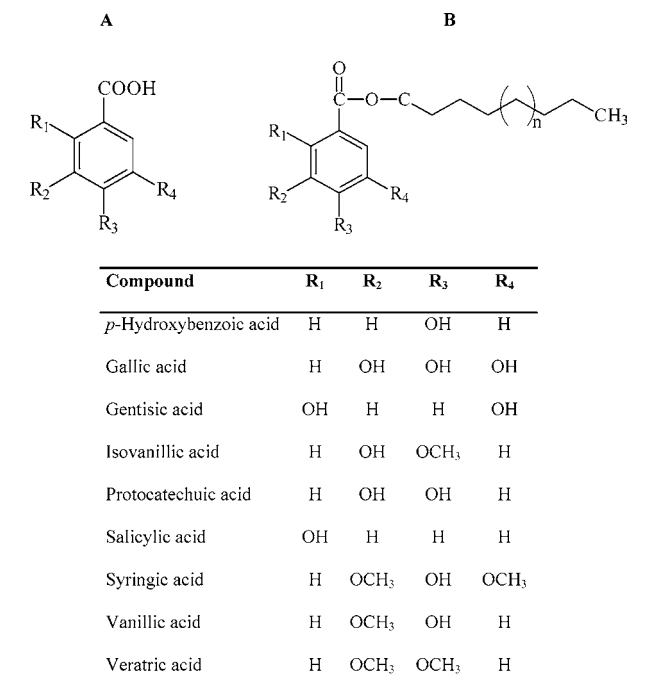† UMR IATE, CIRAD.
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A

B

Compound	R_1	R_2	R_3	R_4
p-Hydroxybenzoic acid	H	H	OH	H
Gallic acid	H	OH	OH	OH
Gentisic acid	OH	H	H	OH
Isovanillic acid	H	OH	OCH₃	H
Protocatechuic acid	H	OH	OH	H
Salicylic acid	OH	H	H	H
Syringic acid	H	OCH₃	OH	OCH₃
Vanillic acid	H	OCH₃	OH	H
Veratric acid	H	OCH₃	OCH₃	H

Figure 1. Chemical structures of the benzoic acid derivatives (**A**) and of the fatty alcohol esters of benzoic acid derivatives (**B**).

C

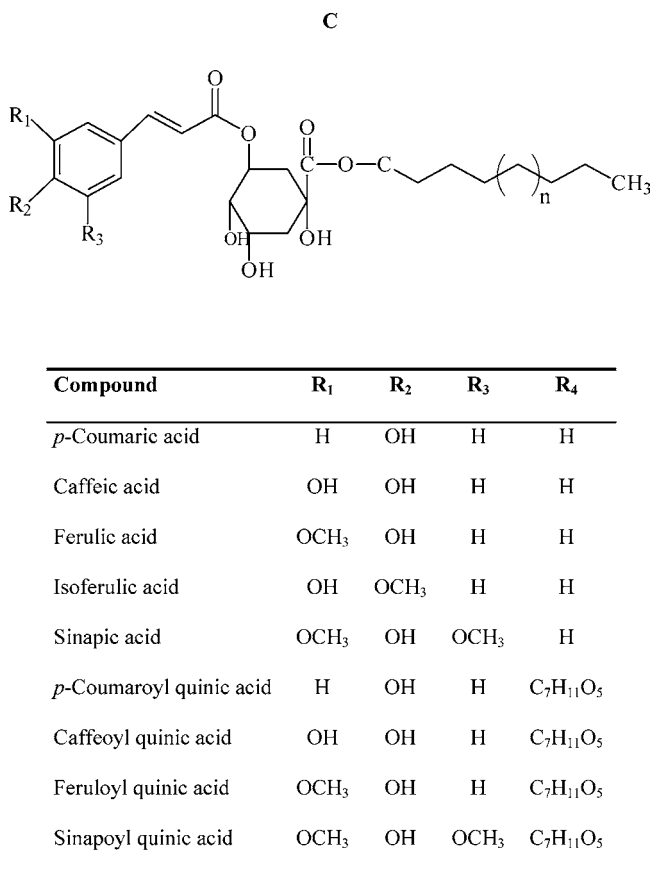| Compound	R_1	R_2	R_3	R_4
p-Coumaric acid	H	OH	H	H
Caffeic acid	OH	OH	H	H
Ferulic acid	OCH₃	OH	H	H
Isoferulic acid	OH	OCH₃	H	H
Sinapic acid	OCH₃	OH	OCH₃	H
p-Coumaroyl quinic acid	H	OH	H	$C_7H_{11}O_5$
Caffeoyl quinic acid	OH	OH	H	$C_7H_{11}O_5$
Feruloyl quinic acid	OCH₃	OH	H	$C_7H_{11}O_5$
Sinapoyl quinic acid	OCH₃	OH	OCH₃	$C_7H_{11}O_5$

Figure 2. Chemical structures of the cinnamic acid derivatives (**A**) and of the fatty alcohol esters of cinnamic acid derivatives with $R_4 = H$ (**B**) and $R_4 = C_7H_{11}O_5$ (**C**).

available (*12*). Synthetic antioxidants present some advantages as they are pure, efficient, readily available, and relatively cheap, present no adverse effect on taste, and are toxicologically safe, with a constant activity and guaranteed quality. They present some disadvantages; only a few approved substances are available on the market as these compounds may be toxic at high doses, and consumers consider them as chemicals and dangerous for human health.

Plant phenols are of considerable interest from the viewpoint of dietary antioxidant supplementation and food preservation (*13*). Hydroxyl groups associated with phenolic compounds are the most common, and often the most effective free radical scavengers in foods (*14*), as they may readily donate an electron or the hydrogen to intercept and convert free radicals to a more stable compound. In foods, the efficiency of antioxidants, and particularly of phenolics, is dependent on their structure, volatility, heat stability, and pH sensitivity (*2, 14, 15*). Lipids are commonly found in foods as dispersions, and the physical properties of lipid and water or air interfaces can influence oxidative reactions. The antioxidant effectiveness in multiphase foods is affected by important factors determined by interfacial phenomena governing the localization and orientation of antioxidants, by partitioning between the aqueous phase and the lipophilic phase, and by interacting with the emulsifier at the interface (*10*). According to Decker (*14*), the solubility of the antioxidant in relation to the site of oxidation is an important factor to be considered. Recently, several authors have worked on the chemical and enzymatic modification of phenolic acids to obtain multifunctional amphiphilic antioxidants.

INTEREST OF LIPOPHILIZATION

Because of the relatively low solubility of phenolic acids in aprotic media, applications for these natural antioxidants in oil-based food processing and cosmetics is limited. The hydrophobicity of phenolic acids can be enhanced by chemical or enzymatic lipophilization, by esterifying the carboxylic acid function of the phenolic acid with a fatty alcohol, to obtain an amphiphilic molecule, which should keep its original functional properties (antioxidant, antimicrobial, UV filter, etc.) (*16, 17*). Due to this amphiphilic nature, the functionalized phenolic acid could accumulate at oil−water or oil−air interfaces where oxidation is considered to occur, increasing the oil protection. The interest in this reaction is to modify or to perform the functional properties of the original phenolic compound.

Chemical and enzymatic esterification of phenolic compounds with acids or alcohols has been reported by several authors. The chemical synthesis (*17−20*) is difficult due to heat sensitivity and to the susceptibility of oxidation in alkaline media of phenolic acids. Furthermore, chemical reactions are unselective, resulting in unwanted side reactions; they involve many intermediary stages and purification steps to remove byproducts and catalyst residues, generating extra wastes to dispose of (*21*). On the other hand, the enzymatic synthesis offers the advantages of milder reaction conditions, minimization of side reactions and formation of byproducts, a selective specificity, a wider variety of pure synthetic substrates, fewer intermediary and purification steps, and a more environmentally friendly process (lower energy consumption and waste materials production) (*21, 22*). For this, it is possible to use lipases in low water media to esterify a fatty alcohol (acting as a nucleophile) to a phenolic compound (*7, 23−29*). Feruloyl esterases (*30, 31*), tannase (*32*), esterase, and cutinase (*27*) have also been used.

Even if enzymes are more expensive than chemical reagents, it should be possible to develop an economical and efficient enzymatic process to manufacture lipophilized phenolic acids, by using a packed bed reactor, which should allow the enzyme to be reused as long as it is active. A device to recycle the reaction mixture containing the fatty alcohol and the phenolic acid in optimized composition should be adapted to the reactor,

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to obtain the maximum esterification. The amount of enzyme in the reactor and the flow of reactants have also to be optimized, and water should be removed by using a vacuum or by adding molecular sieves in the reactor.

PHENOLIC ESTERS IN NATURE

Different kind of phenolic esters can be found in nature: methyl caffeate and *n*-alkyl-*p*-coumarates in the aerial parts and root extracts of *Artemisia assoana*, respectively (*33*); steryl esters of ferulic and *p*-coumaric acids in soybean and cereals (*34*– *38*); hexadecyl and octadecyl ferulates from the wound periderm of potato (*Solanum tuberosum*) (*39*); hexadecyl, octadecyl, and eicosyl esters of *p*-coumaric acid in the vine and root latex of sweetpotato (*40*); *n*-alkyl-*trans*-ferulates and *trans*-*p*-coumarates in the leaves of *Larix kaempferi* having alkyl groups from C_{22} to C_{26} (*41*); mixtures of fatty esters (C_{14}, C_{16}, C_{20}, C_{22}) of *p*-hydroxyphenylethanol and *p*-coumaryl alcohol in the pods of *Piliostigma thonningii* (*42*); docosyl caffeate in the halophytic plant *Halocnemum strobilaceum* (*43*); 1-*O*-(2,4-dihydroxy)- phenylacetyl glycerol in the seeds of *Nigella damascena* (*44*); phenolic fatty acid esters [*E* and *Z* isomers of *p*-coumaryl alcohol with primarily long-chain (C_{16}–C_{26}) saturated fatty acids] in the epicuticular wax of Gala apples, which could act as feeding repellents or as antifungal compounds (*45*); 2-methyl-2-butenyl *p*-coumarate and 3-methyl-3-butenyl ferulate in propolis (*46*); eicosanyl-*trans*-*p*-coumarate in roots of *Tanacetum longifolium* (*47*), etc.

PHENOLIC ESTERS AS ADDITIVES

Different esters of gallic acid [propyl (E310), octyl (E311), and dodecyl (E312) gallates] are widely used as food antioxidant additives (*48*–*50*). Octyl gallate possesses a broad antimicrobial spectrum, and it is an efficient antifungal (*51*, *52*). Several patent applications have been published concerning the chemical synthesis of phenolic acid lipophilic esters. Nakanishi et al. (*53*) reported a method to synthesize esters of caffeic acid with a phenethyl, butyl, hexyl, or ethyl alcohol, which would have anti- inflammatory and anticarcinogenic properties. Vercauteren et al. (*54*) provided a process for the chemical esterification of the phenol functions of flavanols with saturated or unsaturated fatty acids, with a view to cosmetic applications or pharmaceuti- cal preparations with lipophilic, antiradical, and antioxidizing properties. Taniguchi et al. (*19*) chemically produced alkyl ferulates, reacting ferulic acid with an alkanol (especially 2-ethyl-1-hexanol) in the presence of an acidic catalyst (*p*- toluenesulfonic acid) in an organic solvent (toluene). Cheetham and Banister (*55*) developed a method to produce caffeic acid and derivatives thereof as sunscreen agents. They obtained the hexyl caffeate from the trans-esterification of chlorogenic acid with hexane-1-ol in the presence of *Candida antarctica* lipase and using hexane as solvent. Hexyl caffeate exhibited a greater absorbance of UV A and UV B light than caffeic acid. They also chemically prepared ethyl caffeate and the hexyl ester of dimethoxycinnamic acid, which showed good absorption in the UV A and UV B regions. Nkiliza (*56*) deposed a patent on a process for esterifying a polyphenolic oligomeric extract of grape seeds by fatty acid chloride, to be used topically or orally in a cosmetic or a pharmaceutical product.

ENZYMATIC LIPOPHILIZATION OF PHENOLIC ACIDS WITH FATTY ALCOHOLS

Several authors have been working on the esterification of phenolic acids with fatty alcohols (**Table 1**).

Guyot et al. (*23*, *24*) synthesized some phenolic esters by direct esterification of fatty alcohols using a thermostable immobilized lipase B (EC 3.1.1.3) from *C. antarctica* (**Table 1**). This enzyme is adsorbed on a macroporous acrylic resin and presents some advantages: it can be used in batch and column reaction operations and in fixed-bed reactors; its optimum activity temperature is 40–60 °C; and it can be easily recovered from the reaction mix and recycled. Different phenolic acids (cinnamic, caffeic, dihydrocaffeic, ferulic, 3,4-dimethoxy- cinnamic, chlorogenic acid) and several fatty alcohols [butanol (C4), octanol (C8), dodecanol (C12), *cis*-9-octadecen-1-ol (oleyl alcohol) (C18:1)] were tested. These esterifications were carried out without added solvent, the role of which was ensured by the excess alcohol. Yields of the ester formation were calculated from the amount of substrate having reacted compared to the initial quantity of substrate.

When *n*-butanol was used, after 15 days of reaction at 60 °C, no esterification of caffeic acid occurred, and only traces of esterified ferulic acid were obtained. Results obtained after 7 days of reaction are summarized in **Figure 3** (*23*, *24*). For cinnamic acid, the highest yields were reached with butanol and octanol. For hydrocaffeic acid, yields were high with all of the alcohols. Ferulic acid esterification was only possible with alcohols containing more than eight carbons, but in a low yield. After 15 days of reaction, they obtained maximum yields of 13, 10, and 14%, when using octanol, dodecanol, and 9-octa- decen-1-ol, respectively. For the 3,4-methoxycinnamic acid, butanol was the best alcohol, after 7 days of reaction. More chlorogenic acid was esterified with octanol and hexadecanol than with dodecanol and *cis*-9-octadecen-1-ol after 7 days of reaction (**Figure 3**) (*23*), and after 30 days of reaction, maximum yields of 60% with octanol and 40% with dodecanol, hexade- canol, and 9-octadecen-1-ol were obtained (*24*) (**Table 1**). When using 2-methyl-2-butanol as the solvent (*24*), esterification yields were higher (55–75% depending on the fatty alcohol) than those obtained in a solvent-free medium after 30 days of reaction with chlorogenic acid. In summary, esterification was possible in the cinnamic series when the aromatic cycle was not para- hydroxylated (cinnamic and 3,4-dimethoxycinnamic acids), and when the side chain was saturated (dihydrocaffeic acid), para- hydroxylation had no effect. They concluded that the simulta- neous presence of a double bond on the side chain conjugated with the cycle containing a *p*-hydroxyl group inhibits the *C. antarctica* lipase. These results were later confirmed by Buisman et al.(*25*) and Stamatis et al. (*26*, *27*).

Stamatis et al. (*26*) investigated the *C. antarctica* and the *Rhizomucor miehei* lipase esterification of cinnamic acid deriva- tives (cinnamic, caffeic, coumaric, ferulic, and *p*-hydroxybenzoic acids) and ascorbic acid with 1-octanol in nonaqueous media using different solvents (acetone, 2-methyl-2-propanol, and 2-methyl-2-butanol). They observed that for both lipases, higher yields were obtained in the solvent-free system. Cinnamic acid esterification yields were better for *R. miehei* lipase when using long-chain alcohols and for *C. antarctica* when using medium or high chain lengths.

Buisman et al. (*25*) studied seven lipases to esterify func- tionalized phenols (cinnamic and benzoic acid derivatives) with fatty alcohols (C_4, C_6, or C_{12}) to synthesize lipophilic antioxi- dants, in solvents of different polarities (**Table 1**). They observed low ester yields (1–3%) when using lipases from *Humicola lanuginosa*, *Candida cylindracae* (*rugosa*), *Rhizopus*, *Geotri- chum candidum*, *Candida rugosa* AY30, and *Pseudomonas* species. With lipase B from *C. antarctica,* yields from 2% (diethyl ether, 34 °C) to 85% (*n*-pentane, 50 °C) were obtained

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Table 1. Reaction Conditions and Yields of Enzymatic Lipophilization of Phenolic Acids with Alcohols

reference	conditions	phenolic acid	fatty alcohol	yield (%)
Guyot et al. (*23*)	phenolic acid (100 mg) fatty alcohol (7.5 mL) lipase (2.5% w/w based on reaction mix) 60 °C 15 days	caffeic cinnamic dihydrocaffeic 3,4-dimethoxycinnamic ferulic	*n*-butanol	0 97 78 60 traces

Buisman et al. (*25*)	cinnamic acid (0.067 mmol)
	fatty alcohol (1 equiv)
	solvent (2 mL): diethyl ether,
	   tert-butyl methyl ether, 1-butanol,
	   cyclohexane, *n*-pentane
	lipase (10 mg)
	34 or 50 °C
	5 days
		cinnamic	1-butanol in:
			   diethyl ether
			   tert-butyl methyl ether
			   1-butanol
			   cyclohexane
			   n-pentane
			1-hexanol:
			   n-pentane
			1-dodecanol:
			   n-pentane
				
				2
				2
				6
				68
				85
				
				70
				
				68

Buisman et al. (*25*)	benzoic acid derivatives (0.067 mmol) fatty alcohol (0.067 mmol) solvent (2 mL): diethyl ether lipase (10 mg) 34 °C >7 days	*p*-hydroxybenzoic gentisic gallic vanillic syringic	1-hexanol	<2

Stamatis et al. (*26*)	phenolic acid (50–500 mg)
	aliphatic alcohols or acetone (5 mL)
	lipase (150 mg)
	50 °C
	12 days
		cinnamic
		caffeic
		o-coumaric
		m-coumaric
		p-coumaric
		ferulic
		p-hydroxybenzoic
			1-octanol with
			  C. antarctica or
			  R. miehei
				82 or 59
				traces
				21 or 18
				38 or 32
				25 or 22
				11 or 30
				≤6

Guyot et al. (*24*)	phenolic acid (106.3 mg) fatty alcohol (7.5 mL) lipase (1.5% w/w based on reaction mix) 60 °C 30 days	chlorogenic[a]	octanol dodecanol hexadecanol 9-octadecen-1-ol	60 40 40 40
Guyot et al. (*24*)	phenolic acid (106.3 mg) fatty alcohol (2.8 mmol) solvent (10 mL): 2-methyl-2-propanol lipase (1.2% w/w based on reaction mix) 60 °C 30 days	chlorogenic[a]	octanol dodecanol hexadecanol 9-octadecen-1-ol	75 70 60 55
Compton et al. (*28*)	phenolic acid (0.1 M) fatty alcohol (0.1 M) solvent (5 mL): 2-methyl-2-propanol lipase (100 mg) 60 °C, under nitrogen 13 days	ferulic	1-octanol	14
Giuliani et al. (*30*)	phenolic acid (220 μM) fatty alcohol (9 mL) CTAB (100 mM)/hexane/pentanol (0.86 M) water-in-oil microemulsion feruloyl esterase (135 nM) 40 °C, pH 6.0 8 h	ferulic	*n*-pentanol	60
Stamatis et al. (*27*)	phenolic acid (0.3–3 mmol) fatty alcohol (5 mL) lipase, esterase, or cutinase (30 mg/mL) 45 °C 12 days	cinnamic *p*-coumaric ferulic *p*-hydroxyphenylpropionic	1-octanol	8–82 10–25 3–30 29–97
Topakas et al. (*31*)	*F. oxysporum* feruloyl esterase *n*-hexane/1-propanol/water (47.2:50.8:2.0 v/v/v) microemulsions 30 °C 224 h (9.33 days)	cinnamic *p*-coumaric ferulic *p*-hydroxyphenyl acetic *p*-hydroxyphenylpropionic	1-propanol	traces 13 16 75 70
Yu et al. (*32*)	phenolic acid (0.1 mmol) fatty alcohol (1 mL) organic solvent (2 mL) tannase (1 g) 40 °C 12 h	gallic	1-propanol	0–44

[a] 5-*O*-Caffeoylquinic acid.

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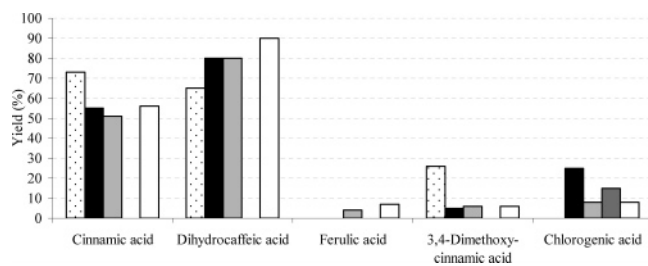*J. Agric. Food Chem.,* Vol. 53, No. 8, 2005 **2783**

Figure 3. Esterification of phenolic acids by different fatty alcohols at 60 °C after 7 days of reaction: butanol (dotted bar); octanol (black bar); dodecanol (light gray bar); hexadecanol (dark gray bar); 9-octadecen-1-ol (white bar) [adapted from Guyot et al. (*23, 24*)].

by esterifying cinnamic acid with 1-butanol and using different solvents after 5 days of reaction. The use of a low-polar solvent such as *n*-pentane improved the esterification yield. They explain that the more polar solvents deactivate the enzyme by sorbing water. The best yield (97%) was obtained by Guyot et al. (*23*), by carrying out the reaction in pure 1-butanol during 15 days. Solvent-free process is preferable, because of the cost of eliminating organic solvent traces in the final product. Yields of 70 and 68% were obtained when cinnamic acid was esterified with 1-hexanol or 1-dodecanol in *n*-pentane. Yields below 2% were obtained after >1 week of reaction when various hydroxy- and methoxy-substituted benzoic acid derivatives were esterified with 1-hexanol. These authors confirmed the inhibiting effect of the electron-donating substituents conjugated to the carboxylic group in these phenolic acids. They suggested that the electron-donating effects intrinsically deactivate the electrophilic carbon center of the carboxylic group for nucleophilic attack of the alcohol and that steric hindrance could reduce the overall esterification reaction rate.

Stamatis et al. (*26, 27*), Compton et al. (*28*), Giuliani et al. (*30*), and Topakas et al. (*31*) worked the lipophilization of ferulic acid.

Stamatis et al. (*26*) esterified ferulic acid with 1-octanol using *C. antarctica* and *R. miehei* lipases, obtaining 11 and 30% yields, respectively, after 12 days of reaction at 50 °C. When different enzymes are used, the yields of ferulic acid esterification to 1-octanol after 12 days at 45 °C were 11% (*C. antarctica* lipase), 30% (*R. miehei* lipase), 8% (*C. rugosa* lipase), 3% (*Fusarium oxysporum* esterase), and 5% (*Fusarium solani* cutinase, EC 3.1.1.74) (**Table 1**). These enzymes were also able to catalyze the esterification of cinnamic, *p*-coumaric, and *p*-hydroxyphenylpropionic acids with relatively high rates.

Compton et al. (*28*) used a *C. antarctica* lipase to synthesize *n*-octyl ferulate using 2-methyl-2-propanol as the solvent at 60 °C, during 13 days, and obtained a 14% yield (**Table 1**). They screened other commercially available esterases (*Mucor miehei*, porcine and bovine pancreas), without success.

Giuliani et al. (*30*) used the feruloyl esterase (EC 3.1.1.73) from *Aspergillus niger* to synthesize pentylferulate, working in a cetyltrimethylammonium bromide (CTAB)/hexane/pentanol water-in-oil microemulsion system and obtained a high yield (60%) (**Table 1**). According to these authors, in the microemulsion, conformational constraints imposed by the micellar system on the protein structure keep the enzyme in the more active conformation. This microemulsion system holds the alcohol in a high concentration at the micellar interface, displaying the most hydrophobic substrate to the enzyme (*57*).

More recently, Topakas et al. (*31*) investigated the synthetic activity of a feruloyl esterase from *F. oxysporum* in catalyzing the esterification of various phenolic acids (cinnamic, *p*-coumaric, ferulic, *p*-hydroxyphenyl acetic, and *p*-hydroxyphenyl

propionic acids) with 1-propanol using surfactantless *n*-hexane/ 1-propanol/water microemulsions (**Table 1**). This system is thermodynamically stable and serves as an appropriate medium for enzymatic catalysis, presenting some advantages: easy separation of reaction products, enzyme reuse, and good solubility of the polar phenolic acid due to the presence of a large amount of polar alcohol (1-propanol) (*58*). The enzyme presented no activity on cinnamic acid, showing the necessity of a hydroxyl group on the C-4 phenol ring. Ferulic acid yield esterification was lower than that obtained in the microemulsion system used by Giuliani et al. (*30*), but the acid solubility was better.

In summary, in a free-solvent mix, Guyot et al. (*23*) found that *C. antarctica* lipase lipophilization of ferulic acid was possible only when using fatty alcohols with eight or more carbons, obtaining octyl ferulate with a 13% yield after 15 days, and Stamatis et al. (*26, 27*) found that *R. miehei* lipase is a good option to obtain a 30% esterification yield of ferulic acid with 1-octanol. When using *C. antarctica* lipase and working on a reaction mix containing 2-methyl-2-propanol as solvent, Compton et al. (*28*) obtained a 14% yield after 13 days of reaction. Feruloyl esterases from *A. niger* (*30*) and *F. oxysporum* (*31*) can be used to lipophilize ferulic acid. The best yields of esterification (50−60%) of this acid with a fatty alcohol were obtained using ferulate esterase and working in a CTAB/hexane/ pentanol water-in-oil microemulsion (*30*). It is likely that the low solubility of ferulic acid in nonpolar solvents such as the fatty alcohols limited its enzymatic lipophilization (*23*), and the use of a more polar solvent is necessary to facilitate the reaction (*28*). The use of a ferulic acid specific esterase solubilized in a water-in-oil microemulsion is the better way for bioconversion involving a hydrophilic substrate (ferulic acid) and a hydrophobic product (pentylferulate) (*30*).

Yu et al. (*32*) synthesized esters of gallic acid and alcohols in organic solvents using a chitosan−alginate complex microencapsulated tannase (EC 3.1.1.20) from *A. niger* (**Table 1**). With this technique, the enzyme is not in direct contact with the solvent, and it is preserved from its toxicity. They obtained propyl gallate in a yield of 37.3% after 8 h of reaction, using hexane as the solvent. The microencapsulated tannase was more efficient than the free enzyme in this reaction. When a molecular sieve was added at 8 h of reaction, the yield was improved by 10%. When only 1-propanol was added (free-solvent system), a low yield (1.7%) of propyl gallate was obtained after 12 h of reaction. Among the 11 organic solvents tested, the highest yields were obtained with benzene (11.3%) and hexane (35.7%). They tested the influence of the alcohol chain length (C_1−C_{18}) on the synthesis of gallic acid esters, using benzene as solvent. Optimum synthesis was observed with alcohols ranging from C_3 to C_5. The maximum yield was obtained with 1-propanol (44.3%). More polar alcohols (methanol, ethanol) remove water from the enzyme hydration shell, inactivating it; more hydrophobic alcohols (>C_8) cannot solvate gallic acid efficiently, and their long chains cannot establish favorable interactions with the active site of tannase. The solubility of substrates and products, the intrinsic enzyme selectivity, and the enzyme stability are directly correlated to the added solvent.

Immobilized enzymes are good options to lipophilize phenolic acids, due to the facility of recovering the enzyme and the product separately. Enzymes can represent an obstacle to obtain these esters at low cost. Therefore, further research on the lipophilization of phenolic acids should be done to reduce the prolonged reaction times, to improve yields, to test the functional properties of the final product, and to verify its lack of toxicity.

The final objective is to obtain a safe and pure multifunctional efficient antioxidant, readily available, with a reproducible quality and with no adverse effect on taste.

ANTIOXIDANT ACTIVITY OF ESTERIFIED PHENOLIC ACIDS

Phenolic compounds can act as free radical scavengers due to their hydrogen-donating ability, forming aryloxyl radicals, the stabilization of which by other functional groups in the structure enhances the antioxidant activity. Monophenols are less efficient as hydrogen-donating radical scavengers than polyphenols. Addition of an *o*- or *p*-hydroxyl group or an ortho substitution with electron donor (alkyl or methoxy) groups increases the antioxidant activity (*15*). According to these authors, the presence of the CH=CH—COOH group in cinnamic acids ensures greater efficiency than the COOH group in benzoic acids.

Silva et al. (*7*) measured the antiradical activity by the DPPH• method of methyl, ethyl, and propyl esters of caffeic and dihydrocaffeic acids. They observed that the *n*-alkyl esters of both phenolic series had significant antiradical scavenger activity compared with (±)-α-tocopherol. According to Brand-Williams et al. (*59*), the antiradical power of caffeic acid is ~2.2-fold that of BHA and BHT. Thus, the esters of caffeic acid would be more efficient than these synthetic antioxidants, as Silva et al. (*7*) observed that alkyl caffeates had a higher antiradical potency when compared to the corresponding phenolic acid, in contrast to the dihydrocaffeic acid esters, which presented a lower scavenging activity. The authors concluded that the antiradical activity was independent of the alkyl chain length, as the alkyl esters of both phenolic series had similar efficacies. Later, Silva et al. (*60*) studied the oxidative stability of refined sunflower oil in the presence and in the absence of propyl caffeate (PC), propyl hydrocaffeate (PHC), propyl ferulate (PF), propyl isoferulate (PI), α-tocopherol (α-TOH), and propyl gallate (PG), using the Rancimat method and the antiradical DPPH• method. The order of antioxidant effectiveness obtained by the Rancimat method was PG > PHC > PC ≫ α-TOH > PI > PF. The antiradical efficacy order of the phenolic compounds was PG > PHC > PC > α-TOH ≫ PF > PI. The electron-donating ability was related to the degree of hydroxylation being higher for PG than for the other compounds. It was concluded that compounds having greater antioxidant efficiencies (PG, PC, PHC) show also greater antiradical activities.

Chalas et al. (*20*) studied the inhibition of copper-catalyzed low-density lipoprotein (LDL) oxidation, the radical attack of erythrocyte membranes by 2,2′-azobis(2-aminopropane)dihydrochloride (AAPH), and the scavenger potency using the DPPH• test, by phenolic acids (caffeic, ferulic, gallic, *p*-hydroxycinnamic, sinapic) and their ethyl esters. They demonstrated that ethyl esterification increased the lipophilicity of the five acids (ethyl sinapate = ethyl caffeate = ethyl *p*-hydroxycinnamate > ethyl gallate > ethyl ferulate), but no direct relationship of lipophilicity and antioxidative activity was demonstrated. The caffeate, sinapate, and ferulate ethyl esters were more potent inhibitors of apoprotein oxidation and of hydroperoxide formation than their corresponding acid forms, ethyl caffeate being the most effective. For these esters, lipophilization facilitates their incorporation into the lipid layer of the LDL particle, enhancing their antioxidant properties by acting in the true site of lipoperoxidation. Ethyl esterification of gallic and *p*-hydroxycinnamic acids had no improving effect. Phenolic acids were effective (sinapic > caffeic > *p*-hydroxy-

cinnamic > ferulic > gallic), and esterification of phenolic acids decreased (caffeic and gallic), increased (ferulic acid), or had no effect (sinapic and *p*-hydroxycinnamic acids) on inhibiting the AAPH-induced haemolysis. The five acids were effective in scavenging the DPPH radical (caffeic > sinapic > gallic > ferulic > *p*-hydroxycinnamic), but only the *p*-hydroxycinnamic acid presented a higher scavenger activity after ethyl esterification. For the other acids, no significant change was observed. Finally, they proposed that these molecules could be used as drugs to prevent atherosclerosis. Nenadis et al. (*61*) demonstrated that ethyl ferulate was more effective than ferulic acid as an antioxidant in both bulk oil and oil-in-water emulsions.

According to Neudörffer et al. (*62*), evaluating the true protective effects of antioxidants is difficult. A reliable antioxidant protocol requires the measurement of more than one property relevant to biological systems. They studied the relationship between the oxidation potential of a series of monomers of 4-hydroxycinnamic ethyl ester derivatives, together with related dehydrodimers, and their antioxidant ability to inhibit copper-catalyzed oxidation of human LDL. Both the oxidation potential and the protective effect decreased in the following order: sinapate > ferulate > *p*-coumarate ethyl esters. The noncyclized 8−8 diphenol dehydrodimers followed the same sequence order and were better antioxidants than their monomer counterparts, the most substituted dehydrodimers (i.e., ethyl sinapate and ferulic dehydrodimers) being the most attractive antioxidants. The noncyclized ethyl sinapate dehydrodimer presented a higher antioxidant ability to inhibit LDL oxidation (lipid phase), whereas the cyclized was the best radical scavenger [aqueous phase, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS•+) method].

Cuvelier et al. (*15*) found a very similar antioxidative potency of propyl gallate and gallic acid and concluded that alkyl esterification does not modify efficiency. According to Kubo et al. (*50*), dodecyl gallate, a currently permitted amphiphile antioxidant additive in food, exhibits both potent chain-breaking and preventive antioxidant activity, and the hydrophobic dodecyl group is associated with this last activity. Both gallic acid and dodecyl gallate act as radical scavengers (*49*). Dodecyl gallate was more effective than gallic acid in preventing autoxidation of linoleic acid (measured by the thiocyanate method), and it showed a dose−response. Dodecyl gallate inhibited the soybean lipoxygenase linoleic acid peroxidation; it was also effective in inhibiting the generation of superoxide anion catalyzed by xanthine oxidase.

Kikuzaki et al. (*63*) demonstrated the antioxidant properties of hydroxycinnamic acids and ferulic acid esters, as well as gallic acid and alkyl gallates, in both bulk and multiphase systems together with their radical scavenging activities. They suggested that the antioxidant activity in multiphase systems might be affected by several parameters, including the concentration of antioxidants, partitioning, and interactions with other compounds such as contaminating transition metals. They demonstrated that esterification of ferulic acid increased the radical scavenging activity when measuring it using a bulk oil system at 40 and 90 °C. In the liposome system, hexyl-, octyl-, and 2-ethyl-1-hexylferulates showed the strongest activities among the tested ferulic acid and its esters. Concerning gallic acid and its alkyl esters, lauryl gallate had the highest affinity toward liposome bilayers, being the most effective among the tested alkyl gallates, although their radical scavenging abilities were almost the same.

More recently, Ha et al. (*16*), working on octyl gallate, found that it is a competitive inhibitor of soybean lipoxygenase 1 (EC

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1.13.11.12, type I), it has free radical scavenging properties (test of DPPH•), it inhibits the tyrosinase (EC 1.14.18.1) L-DOPA oxidation, and it is more efficient than α-tocopherol in inhibiting lipid peroxidation, one of the major factors in deterioration during the storage and processing of foods.

Anselmi et al. (*64*) evaluated the antioxidant/radical scavenging activities of ferulic acid and some alkyl ferulates [*n*-octyl (*n*-C$_8$), 2-ethyl-1-hexyl (branched C$_8$), *n*-dodecyl (*n*-C$_{12}$), *n*-hexadecyl (*n*-C$_{16}$)] in acellular (DPPH•) and cellular [rat liver microsomes−thiobarbituric acid (TBA, anti-lipoperoxidant activity) tests, rat erythrocytes−cumene hydroperoxide (CuOOH) induced hemolysis] systems. It was observed from the acellular system that the quenching ability of ferulic acid was similar to that of the *n*-C$_8$ ferulate, followed by the *n*-C$_{16}$, the branched C$_8$, and the *n*-C$_{12}$ derivatives. The cellular systems give deeper insight into the protective action of the molecule, providing information about its interaction with specific receptors on membrane surface, by binding to the phospholipid bilayer and by disposing itself in a suitable position over/inside the membrane surface. From these tests, the *n*-C$_{12}$ derivative was the most potent, followed by *n*-C$_8$, *n*-C$_{16}$, and branched C$_8$, and the ferulic acid. They concluded that the alkyl ferulates act with a similar mechanism in both the membrane models and that the side chain is primarily involved in the interaction with the phospholipid bilayer of the membrane. They then realized a conformational analysis in solution by 2D NOE (NOESY) measurements, and they concluded that the straight conformation of the branched C$_8$, together with the presence of an hindering ethyl group, hampers the access of the molecule to the lipid binding sites. In the *n*-C$_8$ ferulate the chain folds, increasing the capacity of the molecule to anchor to the phospholipid pocket on the membrane/cell surface, allowing biological activity. The alkyl chain in *n*-C$_8$ and *n*-C$_{12}$ derivatives folds in an opposite way. In the *n*-C$_8$ ferulate it folds toward the aromatic ring. In *n*-C$_{12}$ ferulate, it folds toward the aromatic ring, but also on itself, similarly as in the *n*-C$_{16}$ derivative. These two molecules present a similar conformation with a more expanded and flexible lipophilic anchor chain, but in the *n*-C$_{12}$ ferulate, the anchor chain is not completely folded as in the *n*-C$_{16}$, explaining it greater activity. In 2002, Kikuzaki et al. (*63*) had already concluded from their work on the antioxidant properties of ferulic and gallic acids and their derivatives, that in addition to the radical scavenging activity of an antioxidant, both its polarity and three-dimensional interaction with lipid bilayers might participate in the exertion of the antioxidant activity toward liposome oxidation.

CONCLUSIONS

Enzymatic lipophilization of phenolic acids with fatty alcohols can be obtained using different lipases (*Candida antarctica*, *Candida cylindracae*, *Candida rugosa*, *Geotrichum candidum*, *Humicola lanuginosa*, *Mucor miehei*, *Pseudomonas* sp., *Rhizomucor miehei*, *Rhizopus*), feruloyl esterases (*Fusarium oxysporum*, *Aspergillus niger*), tannase (*Aspergillus niger*), and cutinase (*Fusarium solani*). Reactions can take place in free- or added-solvent systems in some hours or several days at temperatures ranging from 30 to 60 °C. Several authors demonstrated that the lipase-inhibiting effect of electron-donating substituents conjugated to the carboxylic groups in hydroxylated derivatives of cinnamic acid is strong, and if the lateral chain is saturated, no inhibition is observed and esterification takes place. Different factors are to be considered to obtain good esterification yields (solubility of substrates and products, enzyme selectivity and stability, solvent polarity, temperature, water content, etc.).

Lipophilization of phenolic acids with fatty alcohols can be used as a tool to alter the phenolics solubility in oil-based formulas and emulsions. These new amphiphile antioxidant molecules could be used as multifunctional emulsifiers in the food, cosmetic, and pharmaceutical industries, as they should conserve their other functional properties (UV A and UV B filters, antimicrobial, antiviral, bacteriostatic, etc.). Further investigation on the functional, application, and toxicological properties of these products should be carried out, followed by the development of an economical and good yield enzyme-catalyzed manufacture on an industrial scale, once the advantages of the biocatalyzed industrial process have been proved.

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