Carbonyl-Phenol Adducts – An Alternative Sink for Reactive and Potentially Toxic Lipid Oxidation Products

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ABSTRACT

Differently to the well-characterized function of phenolics as antioxidants, their function as lipid-derived carbonyl scavengers is mostly unknown. However, phenolics react with lipid-derived carbonyls as a function of the nucleophilicity of their reactive groups and the electronic effects and steric hindrances present in the reactive carbonyls. Furthermore, the reaction produces a wide variety of carbonyl-phenol adducts, some of which are stable, and have been isolated and characterized, but others polymerize spontaneously. This perspective updates present knowledge about the lipid-derived carbonyl trapping ability of phenolics, its competition with carbonyl-amine reactions produced in foods, and the presence of carbonyl-phenol adducts in food products.

KEYWORDS: *Carbonyl-amine adducts; Carbonyl-phenol adducts; Fried foods; Lipid oxidation; Maillard reaction; Phenols; Reactive carbonyls*

INTRODUCTION

Lipid oxidation is one of the major causes of deterioration of fat-containing foods, and particularly, of foods rich in polyunsaturated fatty acids (PUFAs).¹ PUFAs are easily degraded and this degradation initiates a cascade of reactions that is roughly shown in Figure 1. The first stage of this cascade is the oxidation of the unsaturated fatty acyl chains to form odorless and tasteless hydroperoxides.² These compounds are relatively stable. However, in the presence of metal cations, or at a relatively high temperature, they suffer decompositions to produce a myriad of secondary lipid oxidation products.³ Among them, the formation of short chain reactive carbonyls (RCOs) has a great relevance because of their sensory properties, their ability to modify food macromolecules, and their tendency to produce nonenzymatic browning.⁴ In addition, some of them have been shown to be cytotoxic and mutagenic to humans.⁵

RCOs, among other secondary oxidation products, are produced as the final step of the free radical part of the lipid oxidation process (stages 1 and 2 in Figure 1). These compounds include alkanals, 2-alkenals, malondialdehyde, 2,4-alkadienals, 4-hydroxy-2-alkenals, 4-oxo-2-alkenals, and 4,5-epoxy-2-alkenals, as well as long chain derivatives having the same functional groups. Once produced, they react with the nucleophiles present in food products. One sink for RCOs is amine compounds, including amines, amino acids, aminophospholipids (phosphatidylethanolamine and phosphatidylserine), and proteins. This reaction, which is initiated by the formation of the corresponding Schiff base, produces a wide array of carbonyl-amine adducts, also named advanced lipoxidation end products (ALEs).⁶ These compounds play a major role in the sensory properties of foods, including changes in flavor,⁷ color,⁸ and texture.⁹

These carbonyl-amine reactions compete with other carbonyl-amine reactions produced simultaneously in foods. Among them, Maillard reaction produced between reducing sugars and amine compounds is a major alternative pathway. As shown in Figure 1, this reaction follows a pathway that can also be roughly divided into three stages. Thus, the first one would be the formation of the Amadori products. This stage would be followed by the degradation of sugars and Amadori products to produce carbohydrate-derived reactive carbonyls. Finally, the last step would be the reaction of the formed reactive carbonyls with amine compounds to produce a wide array of low and high molecular advanced glycation end products (AGEs).⁶

As observed in Figure 1, both reaction pathways are parallel and very similar once reactive carbonyls are produced. Furthermore, the products of one pathway influence the other pathway and vice versa.⁴ Moreover, both reactions are so interrelated that share common intermediates and mechanisms, and some authors have suggested that they should be considered simultaneously to understand the products formed as consequence of the Maillard reaction in the presence of lipids and vice versa.⁴ Therefore, amine compounds will compete for carbonyl compounds coming from either lipids or carbohydrates and the most reactive carbonyls that are generated in the proximity of amine compounds are expected to be the most easily trapped.

In addition to amine compounds, phenolic compounds are also nucleophiles. Therefore, they might also be competing for the produced RCOs. However, and differently to the well-characterized function of phenolics as antioxidants, their function as lipid-derived RCO scavengers is mostly unknown. In an attempt to fill this gap, this perspective discusses some advances recently produced in the study of the role of phenolics on RCO trapping in food products.

PHENOLIC COMPOUNDS AS AN ALTERNATIVE SINK FOR RCOs: THE FORMATION OF CARBONYL-PHENOL ADDUCTS

Analogously to amine compounds, phenolics have also been shown to react with carbonyl compounds.¹⁰ The reaction takes place with phenolics having either aromatic carbons or hydroxyl groups with a high nucleophilicity. This occurs mainly in *m*-diphenols (or in the part containing an *m*-diphenol group in complex phenolics such as the A-ring of many flavonoids).^{11,12} The reaction produces the corresponding carbonyl-phenol adduct, which has a structure related to the structure of the involved RCO. Examples of the different structures produced, as well as the key intermediates formed in RCO/phenol reactions as a function of the structure of the RCO involved, are collected in Figure 2. The different carbonyl-phenol adducts were isolated and characterized by using model systems. The identification of some of them in food systems will be discussed in the next sections.

The reaction with alkanals (**1**) is produced between the aromatic carbon of the phenol, which is at α -position in relation to the aromatic hydroxyl group, and the carbonyl group of the aldehyde (Figure 2).¹³ The reaction is a simple addition of the aromatic carbon to the carbonyl carbon, and the formation of the corresponding hydroxyl derivative (**6**) is produced. This is usually the main reaction product. However, depending on the reaction conditions, the dehydration of this compound can be produced and the dehydrated adduct (**7**) is sometimes detected.

The reactivity observed in the reaction among the different alkanals (**1**) and phenolics depends on the electronic effects, the steric hindrances, and the collateral reactions that can be produced.¹³ Thus, in relation to the phenolic structure, the presence of groups that increased the nucleophilicity of phenolic carbons resulted in an increased carbonyl-

trapping ability of these compounds. On the other hand, the presence of groups that increased the steric hindrance of the reactive positions of the phenolic without increasing their nucleophilicity resulted in a decreased carbonyl-trapping ability of these compounds. In addition, the presence of branchings at position 2 of the aldehyde inhibited the reaction by steric hindrance.

Differently to alkanals (**1**), the reaction with 2-alkenals (**2**) is more complex.¹⁴ In this case, the number of nucleophilic and electrophilic groups involved is higher and both one aromatic phenol carbon and its continuous hydroxyl group are involved in the formation of the carbonyl-phenol adducts. As shown in Figure 2, the reaction is produced in a first step by the addition of one of the two nucleophilic groups of the phenol (hydroxyl group or aromatic carbon) to the carbon-carbon double bond of the aldehyde to produce compounds **8** and **9**, respectively. In the second step, the formed adducts react with the second nucleophilic group of the phenol to produce adducts **10** and **11**, respectively. The hemiacetal (**11**) is a stable compound. However, when adduct **10** is formed, this compound is not stable suffers a dehydration to produce a more stable olefin (**12**).

The reaction with 2,4-alkadienals (they are not shown in Figure 2) is much more complex because, in addition to carbonyl-phenol adducts, many other compounds are produced. These products include carbonyl degradation products,¹⁵ adducts between these carbonyl degradation products and the phenolics, and polymers. The corresponding structures of the carbonyl-phenol adducts produced have not been reported yet.

In the case of 4-hydroxy-2-alkenals (**3**), the only report published to date suggests that the reaction takes place similarly to that of 2-alkenals (**2**). However, the isolation and the characterization of the corresponding adduct(s) was not carried out.¹⁶ The reaction is believed to be produced by addition of the aromatic carbon to the carbon-carbon double

bond of the aldehyde to produce the adduct **13**. In a second step, the carbonyl group in the adduct **13** would be stabilized by formation of a hemiacetal ring to produce compound **14**.

The complexity of the reaction increases still more with 4-oxo-2-alkenals (**4**) because these compounds have two α,β -unsaturated carbonyl functions.¹⁷ As indicated in Figure 2, the reaction is initiated by addition of either the hydroxyl group or the aromatic carbon of the phenol to the carbon-carbon double bond of the 4-oxo-2-alkenal to produce adducts **15**, **16**, and **17**, respectively. The second step is the reaction of the formed adduct with the other nucleophilic group of the phenol. This reaction produced the new adducts **18**, **19**, and **20**, respectively. Finally, a further stabilization is obtained by formation of the dehydrated compound **21** from compound **18** and the hemiacetal **22** from compound **20**.

Finally, 4,5-epoxy-2-alkenals (**5**) also reacted very rapidly with phenolics and produced a mixture of compounds.¹⁸ In this case the reaction pathway was very different to other pathways because of the presence of the epoxy ring in the epoxyalkenal. In this case, the reaction was initiated by addition of the hydroxyl group of the phenol to both carbons of the epoxide ring to produce compounds **23** and **24**, respectively. These first adducts reacted then with the second nucleophilic group of the phenolic compound to produce adducts **25** and **26**, respectively. Finally, these adducts were stabilized by formation of the corresponding hemiacetals to produce compounds **27** and **28**.

INSTABILITY OF CARBONYL-PHENOL ADDUCTS AND THE FORMATION OF CARBONYL-PHENOL POLYMERS

Depending on the complexity of the RCO, the formed carbonyl-phenol adducts can involve, or not, all reactive groups of the aldehyde. Thus, the simplest aldehydes (alkanals) produced adducts stable enough to be isolated and characterized, and adducts between saturated aldehydes (mainly glyoxal and methylglyoxal) and phenolics have been either isolated or detected in different studies.¹⁹ On the other hand, RCOs having two oxygenated functions always produced unstable adducts because the phenolic compound did not block all reactive functions of the RCO. The isolation and characterization of these last adducts always implied their stabilization either by reducing the second carbonyl compound or by blocking reactive groups using acetylation.^{17,18}

When the reactive groups of the RCOs were not stabilized during formation of carbonyl-phenol adducts, the formation and disappearance of these adducts was observed. This happened with the olefin (**12**) produced in the reaction with 2-alkenals,¹⁴ and with all carbonyl-phenol adducts produced in the reactions of phenolics with 4-oxo-2-alkenals¹⁷ and 4,5-epoxy-2-alkenals,¹⁸ which are supposed to polymerize unless their reactive groups are blocked. At present, neither potential structures of the produced polymers nor polymerization pathways have been proposed. The only isolated compound that was produced by reaction of an adduct with an additional molecule of aldehyde was the dihydropyrano[3,2-*g*]chromene isolated in the reaction between 2-methylresorcinol or 2,5-dimethylresorcinol and 2-pentenal.¹⁴ Its formation pathway is shown in Figure 3. Although they have not been identified so far, the formation of mixed adducts involving one molecule of phenol and two different aldehydes can be speculated.

COMPETITION BETWEEN AMINES AND PHENOLS FOR RCOs IN BOTH MODEL SYSTEMS AND FOODS

Reactions of reactive carbonyls with both phenolics and amine compounds are produced under similar reaction conditions. Therefore, competition between both nucleophiles should be expected. This competition was analyzed recently when the disappearance of phenylalanine was studied in the presence of both RCOs and phenolics.²⁰ Thus, phenylalanine disappeared rapidly in the presence of epoxyalkenals. However, when catechin or epicatechin were present, the disappearance of the amino acid was not produced. This protection of the amino acid was explained by a rapid scavenging of epoxyalkenals by catechin and epicatechin, and this reaction was produced more rapidly than the reaction between the epoxyalkenals and the amino acid. When other phenolics were assayed, amino acid disappearance depended on the structure of the phenolic involved, and was related to the rate of the carbonyl-phenol reaction. Thus, when the carbonyl-phenol reaction occurred slowly, the epoxyalkenal had time to react with the amino acid. The effectivity of phenolic compounds for protecting phenylalanine, which is related to their epoxyalkenal-trapping ability, increased in the order 2,6-dihydroxybenzoic acid $\approx$ quercetin < myricetin $\approx$ resveratrol $\approx$ morin < 2,5-dimethylresorcinol $\approx$ resorcinol $\approx$ 2-methylresorcinol < catechin $\approx$ epicatechin.

The fact that phenolics can compete efficiently with amine compounds for RCOs means that the identification of carbonyl-phenol adducts in foods where phenolics and amine compounds are simultaneously present should be possible. In fact, the presence of carbonyl-phenols adducts produced between quercetin and 2-alkenals (acrolein, crotonaldehyde, and 2-pentenal) was recently detected in both onions fried in the laboratory and commercially crispy fried onions.²¹ Curiously, the main carbonyl-phenol adducts found were different when onions were fried in the laboratory or when they were fried at an industrial scale. Thus, in the laboratory, onions were fried in a fresh oil that was spiked with the three 2-alkenals (acrolein, crotonaldehyde, and 2-pentenal). In this

case, the main adduct present was produced with the 2-alkenal of the higher boiling point (2-pentenal), most likely because it was the less volatile aldehyde and was evaporated to a lower extent (the spiked oil was heated before adding the onions). At industrial scale, the main adduct found was that produced with acrolein. The reason was probably a consequence of the different ways of frying. Thus, industry employs continuous frying, the employed oils were likely more oxidized than those used in the laboratory, and acrolein should be easily produced at the same time that the food was being fried.^{22,23}

CHALLENGES AND PERSPECTIVES

Phenolics have been suggested to protect foods against the consequences of lipid oxidation in several ways: chelating the metals responsible for the formation of the first radicals and for the breakage of lipid hydroperoxides; scavenging the produced free radicals and avoiding in this way the broadcasting of the free radical damage to other food components; and trapping the produced RCOs that are responsible for the carbonyl-amine reactions produced as a consequence of lipid oxidation.²⁴

The trapping of RCOs by phenolics implies the formation of carbonyl-phenol adducts. As described above, some characteristics of these reactions have been clarified and first conclusions have been obtained: adducts are produced with all at present studied RCOs; the structures of the formed adducts depend on the structures of the RCO and the phenolic compound involved; electronic and steric effects will determine the adducts produced and their reaction kinetics; etc. However, many questions arise and food chemists will have to confront many challenges for a full understanding of these reactions. Some of them are discussed below.

204 Carbonyl-phenol adducts are expected to accumulate in foods. However, their
205 determination will be a challenge for food chemists because of their tendency to
206 polymerize. Will be possible the detection/determination of the carbonyl-phenol adducts
207 produced with the most reactive carbonyls? Is the formation of carbonyl-phenol adducts
208 a sink for these toxic aldehydes? Will the quality of frying oils improve if phenolics-rich
209 foods are occasionally fried to remove most reactive and toxic RCOs?

210 What is the fate of carbonyl-phenol adducts upon digestion? Are RCOs definitively
211 removed from foods upon formation of carbonyl-phenol adducts or can they be recovered
212 during the digestive process as a function of the stability of the produced adduct? Is the
213 stability of carbonyl-phenol adducts related to the reactivity of the involved aldehydes?

214 Carbonyl-phenol adducts of complex phenolics still maintain an unchanged part of the
215 initial phenol after reaction with the carbonyl compound.²¹ In flavonoids, this unreacted
216 part corresponds to the portion with a higher free radical scavenging ability (usually their
217 B-ring). Does this phenolic portion maintain its original function or has it been modulated
218 by the presence of the adduct? Furthermore, some aldehydes introduce a relatively long
219 hydrocarbon chain. Will it contribute to convert mostly hydrophilic phenolics into
220 partially lipophilic derivatives with improved properties? Will these adducts be stable
221 enough to be employed as alternative antioxidants?

222 Phenolics are nowadays mainly assessed in relation to their antioxidant properties.
223 However, antioxidant function is only one of phenolic functions. The different studies
224 discussed in this perspective suggest that carbonyl trapping should also to be considered
225 when studying phenolics' properties. In fact, this ability seems to be playing a major role
226 in the mitigation of lipid oxidation consequences. However, its importance in relation to
227 other phenolic functions remains to be investigated.

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236 **Notes**

237 The authors declare no competing financial interest.

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FIGURE CAPTIONS

Figure 1. Comparative formation of carbonyl-amine adducts formed in the lipid oxidation pathway and in the Maillard reaction.

Figure 2. Structures of carbonyl-phenol adducts identified in the reactions of phenolics with lipid-derived reactive carbonyls as well as key intermediates produced in carbonyl-phenol reactions. Only the portion of the phenolic compound that takes part in the reaction has been drawn.

Figure 3. Proposed reaction pathway for the formation of a complex carbonyl-phenol adduct between one molecule of phenol and two molecules of a lipid-derived reactive carbonyl. Only the portion of the phenolic compound that takes part in the reaction has been drawn.

Figure 1

Figure 2

Figure 3

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