Postprint of Journal of Agricultural and Food Chemistry 64 (27): 5583–5589 (2016)
DOI: 10.1021/acs.jafc.6b02165

Toxicologically Relevant Aldehydes Produced during Frying Process are Trapped by Food Phenolics

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ABSTRACT

The lipid-derived carbonyl trapping ability of phenolic compounds under common food processing conditions was studied by determining the presence of carbonyl-phenol adducts in both onions fried in the laboratory and commercially crispy fried onions. Four carbonyl-phenol adducts produced between quercetin and acrolein, crotonaldehyde, or (*E*)-2-pentenal were prepared and characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and high performance liquid chromatography coupled to high resolution mass spectrometry (HPLC-HRMS). The synthesized compounds were 2-(3,4-dihydroxyphenyl)-3,5,8-trihydroxy-9,10-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**4**), 2-(3,4-dihydroxyphenyl)-3,5,8-trihydroxy-10-methyl-9,10-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**5**), 2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-8-methyl-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**9**), and 2-(3,4-dihydroxyphenyl)-8-ethyl-3,5-dihydroxy-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**10**). When onions were fried in fresh rapeseed oil spiked with acrolein, crotonaldehyde, and (*E*)-2-pentenal (2.7 μmol/g of oil), adduct **10** was the major compound produced and trace amounts of adducts **4** and **5**, but not of adduct **9**, were also detected. In contrast, compound **4** was the major adduct present in commercially crispy fried onions. Compound **10** was also present to a lower extent, and trace amounts of compound **5**, but not of compound **9**, were also detected. These data suggested that lipid-derived carbonyl-phenol adducts are formed in food products under standard cooking conditions. They also pointed out to a possible protective role of food polyphenols, which might contribute to the removal of toxicologically relevant aldehydes produced during deep-frying, assuming that the formed products are stable during food consumption in the human organism.

KEYWORDS: *Aldehydes, Carbonyl-phenol reactions, Deep-frying, Fried onions, Lipid oxidation, Quercetin, Reactive carbonyls*

41

INTRODUCTION

Lipid oxidation is a major concern during food production because it causes potential safety problems and consumer rejection as a consequence of the changes in flavor, texture, appearance, and nutritional quality of food products.[1–3] All these changes are based on numerous complex interrelated reactions induced by oxygen in the presence of initiators.[4] Although these reactions also occur at low temperatures, reaction rates increase with reaction times and temperatures. For that reason, the nature and concentrations of the compounds present in frying oils as a consequence of its thermal degradation during heating have been a matter of great interest for a long time, as some of these compounds are toxicologically relevant and can be ingested directly from the degraded oil or via the fried food.[5,6] To this respect, recent studies of Granvogl et al. found significant amounts of short chain toxicologically relevant aldehydes, namely 2-propenal and (*E*)-2-butenal (acrolein and crotonaldehyde, respectively) in both frying oils and fried foods. Acrolein was present to a higher extent compared to crotonaldehyde and the amounts of these compounds were much higher in the frying oils than in the fried foods.[7–9] Thus, for example, in rapeseed oil heated at 180 ºC, up to 63.2 mg of acrolein/kg of oil and 27.5 mg of crotonaldehyde/kg of oil were found, whereas the amounts of acrolein and crotonaldehyde present in potato chips ranged from 23 to 26 µg/kg and from 12 to 25 µg/kg, respectively, depending on the oil used.[8]

The fact that the amount of lipid-derived aldehydes in fried foods is reduced compared to the content of these aldehydes in the frying oils might be the consequence of a limited absorption of these compounds by the foods, but also of a reaction of the generated aldehydes with other food components. Among these components, the formation of carbonyl-phenol adducts has recently been suggested,[10] which would imply a mostly

unknown protective role of phenolic compounds in the elimination of these toxicologically relevant aldehydes, assuming the stability of the formed adducts.

In an attempt to investigate whether the formed aldehydes in frying oils are incorporated into food products in the form of carbonyl-phenol adducts, this study aimed at the identification of this kind of adducts in onions submitted to frying in rapeseed oil containing acrolein, crotonaldehyde, and (*E*)-2-pentenal as well as in commercially crispy fried onions. These aldehydes were selected because of both their confirmed formation in oils submitted to frying[7–9] and the fact that the reaction mechanism of (*E*)-2-alkenals with phenolic compounds is now well understood.[10] In addition, onion was chosen as a model food because its flavonoid composition and content is well known.[11–15] Thus, total flavonoids in red onions are about 140 mg/100 g of fresh weight from which quercetin derivatives are about 85% of total flavonoids. The main quercetin derivative is quercetin 3,4'-O-diglucoside (77.1 mg/100 g) followed by quercetin 4'-O-glucoside (33.8 mg/100 g), whereas the quercetin content is much lower (about 1.31 mg/100 g). However, diglucosides can be hydrolyzed upon maceration, among other processes, and the amount of monoglucosides and free quercetin increases. During the frying process, the quercetin content is reduced by about 20%.[12,13]

MATERIALS AND METHODS

Safety. Acrolein and crotonaldehyde are hazardous and should be handled carefully.

Food Samples. Rapeseed oil, red onions (*Allium cepa* L.), and commercially crispy fried onions were purchased at local supermarkets.

Chemicals. Acrolein, crotonaldehyde, (*E*)-2-pentenal, and quercetin were obtained from Sigma-Aldrich (St. Louis, MO) and were of the highest available quality. Sephadex LH-20 was obtained from GE Healthcare Europe (Freiburg, Germany). All other

chemicals were purchased from Sigma-Aldrich, Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany).

Preparation of Aldehyde-Quercetin Adducts as Reference Compounds. Keeping in mind that quercetin is the major flavonoid present in onions,[12,13] the corresponding adducts between quercetin and acrolein, crotonaldehyde, or (*E*)-2-pentenal were synthesized and characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and high performance liquid chromatography coupled to high resolution mass spectrometry (HPLC-HRMS). The chemical structures of the isolated compounds are illustrated in **Figure 1**. These compounds were prepared by dissolving quercetin (200 µmol) and the corresponding aldehyde (400 µmol) in methanol (2 mL; containing 290 µmol of triethylamine). The solution was heated under nitrogen at 100 ºC for 3.5 h (acrolein), 10 h (crotonaldehyde), or 96 h ((*E*)-2-pentenal), respectively. At the end of the heating time, the reaction mixture was fractionated on a Sephadex LH-20 column using methanol/water (80/20, v/v) as eluent at a flow rate of 15 mL/h. Eluted products were detected by mass spectrometry using direct injection.

2-(3,4-Dihydroxyphenyl)-3,5,8-trihydroxy-9,10-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**4**). HRMS, *m/z* 357.06154 ($M^+ - 1$), error 0.2 ppm. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.97 (br, 2, H9), 2.88 (br, 2, H10), 3.38 (br, OH), 5.58 (br, 1, H8), 6.18 s (s, 1, H6), 6.90 (d, 1, *J* = 8.5 Hz, H5'), 7.61 (dd, 1, *J* = 2.1 Hz, *J* = 8.5 Hz, H6'), 7.73 (d, 1, *J* = 2.1 Hz, H2'), 9.47 (br, OH), 12.30 (br, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 14.85 (C10), 26.87 (C9), 93.29 (C8), 98.89 (C6), 101.26 (C13), 104.20 (C11), 115.32 (C2'), 116.18 (C5'), 120.43 (C6'), 122.64 (C1'), 136.56 (C3), 145.64 (C3'), 147.26 (C2'), 148.27 (C4'), 153.10 (C14), 158.59 (C12), 158.78 (C5), 176.51 (C4).

2-(3,4-Dihydroxyphenyl)-3,5,8-trihydroxy-10-methyl-9,10-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**5**). HRMS, *m/z* 371.07763 ($M^+ - 1$), error 0.9 ppm. ^{1}H

NMR (500 MHz, DMSO-d_6): δ 1.38 (d, 3, *J* = 7.0 Hz, H1”), 1.94 (br, 2, H9), 3.38 (br, OH), 3.41 (br, 1, H10), 5.50 (br, d, 1, *J* = 7.9 Hz, H8), 6.18 s (s, 1, H6), 6.91 (d, 1, *J* = 8.5 Hz, H5’), 7.60 (dd, 1, *J* = 2.2 Hz, *J* = 8.5 Hz, H6’), 7.73 (d, 1, *J* = 2.2 Hz, H2’), 9.52 (br, OH), 12.38 (br, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 19.02 (C10), 21.74 (C1’), 56.49 (C9), 92.76 (C8), 98.64 (C6), 104.38 (C11), 105.74 (C13), 115.27 (C2’), 116.20 (C5’), 120.25 (C6’), 122.61 (C1’), 136.56 (C3), 145.71 (C3’), 147.31 (C2), 148.27 (C4’), 153.44 (C14), 158.88 (C12), 159.16 (C5), 176.51 (C4).

2-(3,4-Dihydroxyphenyl)-3,5-dihydroxy-8-methyl-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**9**). HRMS, *m/z* 353.0625 ($M^+ - 1$), error 4.8 ppm. ^{1}H NMR (500 MHz, DMSO-d_6): 1.42 (d, 3, *J* = 6.6 Hz, H1”), 3.36 (br, OH), 5.15 (m, 1, H8), 5.83 (dd, 1, *J* = 3.2 Hz, *J* = 10.0 Hz, H9), 6.23 s (s, 1, H6), 6.87 (dd, 1, *J* = 1.1 Hz, *J* = 10.0 Hz, H10), 6.91 (d, 1, *J* = 8.5 Hz, H5’), 7.62 (dd, 1, *J* = 2.2 Hz, *J* = 8.5 Hz, H6’), 7.73 (d, 1, *J* = 2.2 Hz, H2’), 9.52 (br, OH), 12.49 (br, OH), 12.65 (br, OH), 12.99 (br, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 21.56 (C1”), 72.88 (C8), 98.83 (C6), 101.63 (C13), 104.48 (C11), 115.21 (C2’), 116.00 (C10), 116.25 (C5’), 120.58 (C6’), 122.36 (C1’), 124.55 (C9), 136.61 (C3), 145.68 (C3’), 147.57 (C2), 148.39 (C4’), 150.47 (C14), 159.24 (C12), 160.71 (C5), 176.57 (C4).

2-(3,4-Dihydroxyphenyl)-8-ethyl-3,5-dihydroxy-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one (**10**). HRMS, *m/z* 367.0810 ($M^+ - 1$), error 3.6 ppm. ^{1}H NMR (500 MHz, DMSO-d_6): 0.97 (t, 3, *J* = 7.3 Hz, H2”), 1.74 (qu, 2, *J* = 7.3 Hz, H1”), 3.36 (br, OH), 4.98 (m, 1, H8), 5.83 (dd, 1, *J* = 3.4 Hz, *J* = 10.1 Hz, H9), 6.23 s (s, 1, H6), 6.89 (dd, 1, *J* = 1.1 Hz, *J* = 10.1 Hz, H10), 6.91 (d, 1, *J* = 8.5 Hz, H5’), 7.61 (dd, 1, *J* = 2.2 Hz, *J* = 8.5 Hz, H6’), 7.72 (d, 1, *J* = 2.2 Hz, H2’), 9.52 (br, OH), 12.49 (br, OH), 12.65 (br, OH), 12.99 (br, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 9.23 (C2”), 28.39 (C1”), 77.49 (C8), 98.75 (C6), 101.69 (C13), 104.39 (C11), 115.21 (C2’), 116.24 (C5’), 116.40 (C10), 120.59 (C6’),

122.37 (C1'), 123.20 (C9), 136.52 (C3), 145.66 (C3'), 147.39 (C2), 148.35 (C4'), 150.42 (C14), 159.47 (C12), 160.69 (C5), 176.52 (C4).

Deep-Frying Experiments. Deep-frying experiments were carried out on rapeseed oil (100 g) containing acrolein, crotonaldehyde, and (*E*)-2-pentenal (each 2.7 μmol/g of oil), which were added before heating the oil. The oil was firstly pre-heated for 9 min to achieve 160 ºC and then heated in the presence, or absence, of thin slices of onions (5 g) for further 3 min. At the end of the heating time, the heated oils were analyzed for aldehyde contents and the fried onions were analyzed to identify aldehyde-quercetin adducts.

Determination of Aldehydes in Heated Oils. Aldehydes were determined by ^{1}H NMR spectroscopy analogously to the analysis of oil components developed by Sopelana et al.[16] Briefly, oil samples (200 mg) were diluted with $CDCl_3$ (400 μL), which contained 0.2% of non-deuterated chloroform, and their spectra were obtained by a Bruker Advance III spectrometer (Karlsruhe, Germany) operating at 500 MHz. Quantitation of the three aldehydes was carried out by considering the area of the $CHCl_3$ signal (at δ 7.29 ppm) as internal standard. The proton of the aldehyde group appeared as a doublet that was independent for each one of the three analyzed aldehydes and appeared far from other signals in the spectrum. The signals of the aldehydic proton appeared at δ 9.55 (d, J = 7.4 Hz) for acrolein, 9.47 (d, J = 7.9 Hz) for crotonaldehyde, and 9.49 (d, J = 7.7 Hz) for (*E*)-2-pentenal. Four samples were analyzed for each heated oil and each sample was measured three times. Therefore, the aldehyde content for each analyzed oil was the mean value of twelve determinations.

Determination of Aldehyde-Quercetin Adducts in Fried Onions. Onions fried in the laboratory and commercially crispy fried onions (5 g each) were homogenized in water (20 mL) and hexane (50 mL). The resulting mixture was centrifuged at 7500 *g* for

10 min at room temperature. The organic layer was discarded and other 50 mL of hexane were added. The mixture was homogenized again and centrifuged at 7500 *g* for 10 min. The organic layer was discarded and methanol (80 mL) was added. The mixture was newly homogenized and centrifuged at 7500 *g* for 10 min. The supernatant was collected and the solid was extracted with methanol/water (80/20, v/v; 100 mL). The resulting mixture was centrifuged at 7500 *g* for 10 min and the supernatant was collected. Both supernatants were combined, concentrated to about 4 mL using a rotatory evaporator (35 ºC, 16 mbar) and fractionated on a Sephadex LH-20 column using methanol/water (80/20, v/v) as eluent at a flow rate of 15 mL/h. Fractions obtained were analyzed by HPLC-HRMS.

NMR Spectroscopy. All NMR spectra were obtained by a Bruker Advance III spectrometer operating at 500 MHz for protons. For ^{1}H spectra, acquisition parameters were: spectral width 10000 Hz, relaxation delay 1 s, number of scans 16, acquisition time 3.277 s, and pulse width 90º, with a total acquisition time of 1 min 17 s. For ^{13}C spectra, acquisition parameters were: spectral width 27500 Hz, relaxation delay 2 s, acquisition time 1.188 s, and number of pulses depended on the concentration of the sample. All experiments were performed at 23 ºC. For structural determinations, COSY, HMQC and HMBC experiments were carried out.

Low Resolution MS. To control carbonyl-phenol reactions and the eluates from the LH-20 column during the isolation of reference compounds, a triple quadrupole API 2000 mass spectrometer (Applied Biosystems, Foster City, CA) was employed by using an electrospray ionization interface in the negative ionization mode (ESI^-). The nebulizer gas and the curtain gas were set at 19 and 10 (arbitrary units), respectively. The electrospray capillary voltage was set to –4.5 kV, the declustering potential was –50 V, the focusing potential was –400 V and the entrance potential was –10 V.

HPLC-HRMS. The HPLC-ESI-MS system consisted of a Dionex Ultimate 3000RS U-HPLC (Thermo Fisher Scientific, Waltham, MA) coupled to a micrOTOF-QII ultra high resolution time-of-flight mass spectrometer (UHR-TOF) with q-TOF geometry (Bruker Daltonics, Bremen, Germany). Chromatographic separation was performed on a Zorbax Eclipse XDB-C18 column (15 cm × 0.46 cm i. d., 5 µm) from Agilent (Santa Clara, CA). As eluent A, a mixture of acetonitrile containing 0.2% formic acid and 4 mM ammonium formiate (30/70, v/v) was used. As eluent B, acetonitrile containing 0.2% formic acid was employed. The flow rate was 0.5 mL/min in linear gradient mode: 0-13 min 7% B, 13-20 min from 7 to 60% B, 20-30 min 60% B, 30-32 min from 60 to 90% B, 32-42 min 90% B, 42-45 min from 90 to 7% B. A split post-column with a flow rate of 0.25 mL/min was inserted directly into the mass spectrometer ESI source. The scan range applied was *m/z* 50-1500 and mass resolving power was always over 18,000 ($m/\Delta m$). The instrument was operated in the negative ion mode. Mass spectra and data were obtained by broadband Collision Induced Dissociation (bbCID) mode, providing MS and MS/MS spectra simultaneously. Collision energy was estimated dynamically based on appropriate values for the mass and stepped across a ±10% magnitude range to ensure good quality fragmentation spectra. The instrument control was performed using Compass 1.3 for micrOTOF-Q II + Focus Option Version 3.0 (Bruker Daltonics).

Statistical Analysis. Statistical differences among the amounts of aldehydes remaining in the oils after heating in the presence or in the absence of onions were evaluated by the Student *t*-test.[17] These comparisons were carried out using Origin, version 7.0 (OriginLab Corporation, Northampton, MA). Significance level was $p < 0.05$.

RESULTS AND DISCUSSION

Formation of Carbonyl-Phenol Adducts Between Acrolein, Crotonaldehyde, or (*E*)-2-Pentenal and Quercetin. The reaction between (*E*)-2-alkenals and phenols is

complex and different single compounds as well as polymers are produced.[10] When the reaction was carried out with a flavonoid as phenol, namely quercetin, the complexity of the reaction increased because this phenol has a higher number of reactive positions. However, the reactivity of some positions was shown to be higher than others and, thus, only a limited number of adducts was produced. The reaction between (*E*)-2-alkenals and quercetin took place as indicated in **Figure 2**. The first step is the addition of a carbon or an oxygen atom with a high electronegativity in the phenol to the olefinic carbon at β-position of the aldehyde. Quercetin (**1**) has different atoms susceptible for this addition: the carbons at positions 6 and 8, and all the hydroxyl groups. If the reaction takes place with an aromatic carbon of quercetin, an adduct similar to compound **2** is produced. If the reaction takes place with a hydroxyl group, an adduct similar to compound **3** is formed. The stabilization is different for compounds **2** and **3** and involves the other reactive group that did not react in the first step. This first step is reversible and only the adducts that can later be stabilized are able to be isolated.

Adducts **2** are stabilized by reacting with the contiguous hydroxyl group and forming the corresponding hemiacetals **4-6**. These compounds **4-6** have a molecular weight resulting from the addition of the molecular weights of quercetin and the respective carbonyl compounds. Theoretically, three isomers can be produced for each aldehyde, which would involve carbon 8 and hydroxyl 7, carbon 6 and hydroxyl 7, and carbon 6 and hydroxyl 5 of quercetin, respectively.

Adducts **3** are stabilized by addition of an aromatic carbon of quercetin to the carbonyl carbon of the aldehyde resulting in the cyclic structure **7**, which is later dehydrated to yield the conjugated olefins **8-10**. These compounds **8-10** have a molecular weight resulting from the addition of the molecular weights of quercetin and the respective carbonyl compounds minus one molecule of water. Analogously to compounds **4-6**, three

isomers can be produced for each aldehyde, which would involve carbon 8 and hydroxyl 7, carbon 6 and hydroxyl 7, and carbon 6 and hydroxyl 5 of quercetin, respectively.

When the reaction between the aldehydes and quercetin was carried out, the presence of both kinds of products (the hemiacetal and the conjugated olefin) could be observed. However, the ratio among them depended on the involved aldehyde. **Figure 3** shows the mass spectra of the reaction mixtures obtained for the three assayed aldehydes. Acrolein mostly produced the hemiacetal **4** [*m/z* 357 ($M^+ - 1$)] and only small amounts of the conjugated olefin **8** [*m/z* 339 ($M^+ - 1$)] (**Figure 3A**). In addition, an adduct involving 2 molecules of acrolein and 1 molecule of quercetin was also observed [*m/z* 413 ($M^+ - 1$)]. This is likely a consequence of the high reactivity of this aldehyde, which is able to react with several positions of the phenolic compound. For 1:1 adducts, the hemiacetal is a more stable compound than the conjugated olefin. This last compound is susceptible to polymerize because of the presence of the additional double bond.[10] Besides, the conjugated olefin suffers the addition of methanol to produce a further adduct [*m/z* 371 ($M^+ - 1$)].

Differently to acrolein, the two 1:1 adducts were produced for crotonaldehyde (**Figure 3B**), although the hemiacetal [*m/z* 371 ($M^+ - 1$)] seemed to be formed to a higher extent than the conjugated olefin [*m/z* 353 ($M^+ - 1$)]. Because of the reactivity of this olefin, the corresponding methanol adduct was also present in the reaction mixture [*m/z* 385 ($M^+ - 1$)].

The introduction of a further methylene group into the aldehyde shifted the reaction towards the formation of the conjugated olefin as the main product [*m/z* 367 ($M^+ - 1$)], although the hemiacetal [*m/z* 385 ($M^+ - 1$)] was also formed to a significant extent

(**Figure 3C**). As observed for the other conjugated olefins, the corresponding methanol adduct was also present in the reaction mixture [*m/z* 399 ($M^+ - 1$)].

The main adducts formed in the three reaction mixtures were isolated by column chromatography on Sephadex LH-20 and characterized by ^1H and ^{13}C NMR spectroscopy and HPLC-HRMS. These compounds were the hemiacetals produced with acrolein and crotonaldehyde (compounds **4** and **5**, respectively) and the conjugated olefins produced with crotonaldehyde and (*E*)-2-pentenal (compounds **9** and **10**, respectively). Chemical structures for all these compounds are shown in **Figure 1**. Long distance NMR couplings were determined by HMBC experiments and allowed the unequivocal characterization of the formed structures. Observed HMBC couplings for the two kinds of produced adducts are also shown in **Figure 1**. These adducts always involved carbon 8 and hydroxyl 7. The reason for the lower reactivity of position 6 is likely a higher steric hindrance. In addition, hydroxyl 5 is likely involved in an intramolecular hydrogen bond with the carbonyl group at position 4.

Fate of Toxicologically Relevant Carbonyls during Thermal Heating of Oils in the Presence and in the Absence of Added Food. During the frying process, toxicologically relevant aldehydes are formed as a consequence of oxidation, but short chain aldehydes have a boiling point lower than the employed frying temperature and might be evaporated. Therefore, and independently of the presence or absence of food, the amounts of aldehydes present in the spiked oil (2.7 μmol/g of oil) at the end of the frying process were much reduced. Thus, the initial amount of aldehyde was lowered by 35% in the case of acrolein, but by more than 90% in the case of crotonaldehyde and (*E*)-2-pentenal (**Figure 4**). This surprising result (the boiling point of acrolein is lower than that of crotonaldehyde and (*E*)-2-pentenal) is likely a consequence of the higher formation of this aldehyde by oil oxidation in comparison to the other assayed aldehydes.[8]

When the oil was heated in the presence of onions, only slight decreases in the aldehyde concentrations were observed compared to the aldehyde concentrations in the oils heated in the absence of onions (**Figure 4**). However, these decreases were significant ($p < 0.05$) for acrolein and crotonaldehyde, but they were not for (*E*)-2-pentenal.

Formation of Aldehyde-Phenol Adducts in Fried Onions. When the oil was heated in the absence of food, the above described decrease of aldehydes was a consequence of their evaporation. In fact, these aldehydes are considered as environmental pollutants and they have been detected, among others, in the exhaust of kitchens and thermally processed oils.[7–8,18–20] However, when a food is present, in addition to aldehyde evaporation, the absorption of the aldehydes in the food and the reaction of these aldehydes with food components might also occur. In fact, trace amounts of these aldehydes have already been found in fried foods.[8] When the onions fried in the laboratory were analyzed for detection of the four adducts previously synthesized and characterized, adduct **10** produced between (*E*)-2-pentenal and quercetin was present in all analyzed samples (**Figure 5**). This compound was unequivocally identified by its retention time and HRMS (error < 3 ppm). In addition, some samples showed the presence of compound **4** and/or compound **5**, which were also identified analogously (the sample shown in **Figure 5** exhibited trace amounts of compound **5**, but not of compounds **4** or **9**). These compounds were present to a much lower extent than compound **10**. Compound **9** was not detected in any of the analyzed samples.

The major presence of the adduct derived from (*E*)-2-pentenal in comparison to that derived from acrolein and crotonaldehyde might be a consequence of both the higher boiling point of (*E*)-2-pentenal and its lower reactivity. Oils were heated for 9 min before adding the onions, and the lower the boiling point of the aldehyde, the easier the evaporation of the aldehyde is expected. In addition, the higher reactivity of acrolein and

crotonaldehyde might lead to their faster reaction with other food components than with food phenols.

Identification of Aldehyde-Phenol Adducts in Commercially Crispy Fried Onions. Differently to the frying carried out in the laboratory, in which non-oxidized oils spiked with selected aldehydes were used and these oils were only heated for 9 min before adding the onions, the history of the oils employed as well as the process followed in the preparation of the commercially crispy fried onions is unknown. But in this case, all available aldehydes, which were able to react with onion phenols, must originate from the frying oil. Interestingly, several carbonyl-quercetin adducts were present in the analyzed samples (**Figure 6**). Compound **4** was the main adduct detected, followed by compound **10**. As can be observed in the figure, only trace amounts of compound **5** could be found and compound **9** was absent in the different assayed samples. These compounds were identified on the basis of their retention times and HRMS (errors always <5 ppm).

The difference in the amounts of adduct **4** found between the onions fried in the laboratory (only present in trace amounts in some samples) and those found in commercially crispy fried onions (the main carbonyl-phenol adduct) might be a consequence of the quality of the employed oils. In the laboratory, fresh non-oxidized oil was used (although spiked with a small amount of acrolein) and most acrolein was likely evaporated during oil heating before adding the onions. On the other hand, the oil employed for preparing the commercially crispy fried onions was likely more oxidized and acrolein was produced at the same time that the onions were fried.

Phenolic Compounds as Carbonyl Scavengers: an Additional Destiny for the Toxicologically Relevant Aldehydes Produced as a Consequence of Lipid Oxidation. The lipid-derived carbonyl trapping ability of amino compounds has been known for a

long time as well as their contribution to browning development in food products.[21–23] On the other hand, the lipid-derived carbonyl trapping ability of phenolic compounds is less known, although the trapping of acrolein, 4-hydroxy-(*E*)-2-nonenal, (*E*)-2-pentenal and (*E*)-2-octenal by different phenols has been previously described.[10,24] Nevertheless, these last studies neither were carried out with food products nor demonstrated that carbonyl-phenol adducts could be formed during common food cooking conditions. To the best of our knowledge, the results found in this study demonstrate for the first time both the formation of lipid-derived carbonyl-phenol adducts in food products under standard cooking conditions and the presence of this kind of compounds in processed foods. In addition, they point out to an additional protective role of food polyphenols in the lipid oxidation pathway in addition to their well-known functions as free radical scavengers and chelators: the scavenging of toxicologically relevant carbonyl compounds that are produced as a consequence of lipid oxidation (**Figure 7**). This figure also shows a previously unknown competition for the removal of these aldehydes between amino and phenolic compounds, which is expected to be shifted towards the formation of either carbonyl-amine adducts or carbonyl-phenol adducts depending on the involved compounds and reaction conditions. The importance of the formation of carbonyl-amine adducts on food properties has been the objective of numerous studies for the last century.[25] However, the role of these carbonyl-phenol adducts remains to be elucidated, although they are expected to contribute to food browning as well as changes in food flavors as a consequence of a potential selective sequestering of significant flavor components in processed foods.[26]

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Funding

This study was supported in part by the European Union (FEDER funds) and the Plan Nacional de I + D of the Ministerio de Economía y Competitividad of Spain (project AGL2015-68186-R). Dr. Michael Granvogl thanks the Bayerische Forschungsallianz (Bavarian Research Alliance) for the financial support for his stay in Seville, Spain.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are indebted to José L. Navarro for technical assistance and José J. Ríos for the HPLC-HRMS analyses.

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FIGURE CAPTIONS

Figure 1. Chemical structures of the compounds synthesized in this study. HMBC couplings exhibited by compounds **4** and **9** are also shown. These couplings were similar for compounds **5** and **10**, respectively.

Figure 2. Formation mechanism of aldehyde-quercetin adducts. The aldehydes employed in this study were acrolein (R = H), crotonaldehyde (R = CH_3), and (*E*)-2-pentenal (R = CH_2CH_3).

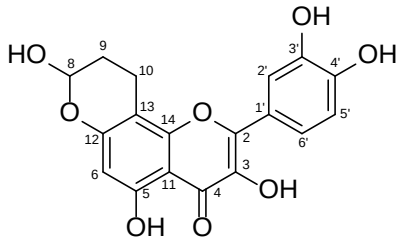
Figure 3. Mass spectra of the reaction mixtures between quercetin and: A) acrolein; B), crotonaldehyde; and C), (*E*)-2-pentenal obtained by direct injection.

Figure 4. Aldehydes recovered from the oil after the frying process in the absence (stripped bars) or in the presence (open bars) of onions. Bars for each aldehyde having different letters are significantly ($p < 0.05$) different. Abbreviations: ACR, acrolein; CRO, crotonaldehyde; PEN, (*E*)-2-pentenal.

Figure 5. Trace chromatograms obtained by LC-HRMS of (A) a mixture of the four carbonyl-phenol adducts prepared in this study, and (B-E) the extract of an onion sample fried in the laboratory for 3 min at 160 ºC in a rapeseed oil containing acrolein, crotonaldehyde, and (*E*)-2-pentenal (2.7 µmol/g of oil). The traces correspond to the exact mases ($M^+ - 1$) of (B) compound **4**; (C) compound **5**; (D) compound **9**; and (E) compound **10**.

Figure 6. Trace chromatograms obtained by LC-HRMS of (A) a mixture of the four carbonyl-phenol adducts prepared in this study, and (B-E) the extract of a commercial crispy fried onion sample. The traces correspond to the exact mases ($M^+ - 1$) of (B) compound **4**; (C) compound **5**; (D) compound **9**; and (E) compound **10**.

Figure 7. Protective roles of phenolic compounds as inhibitors of the lipid oxidation process and as scavengers of the aldehydes produced as a consequence of lipid oxidation. Additional carbonyl-scavenging ability of amino compounds is also indicated.



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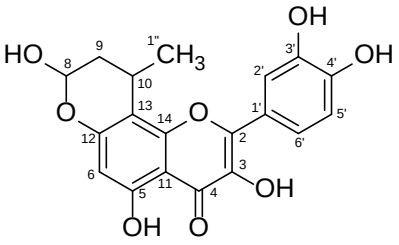

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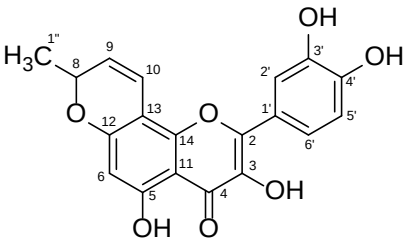

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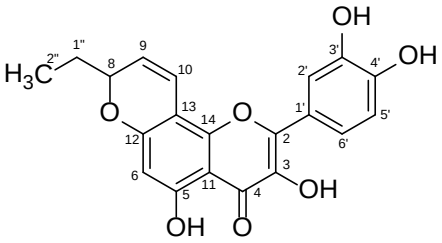

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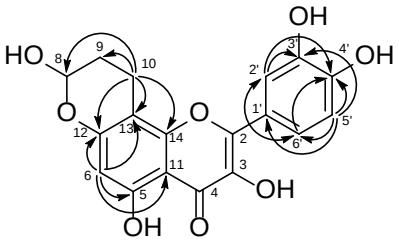

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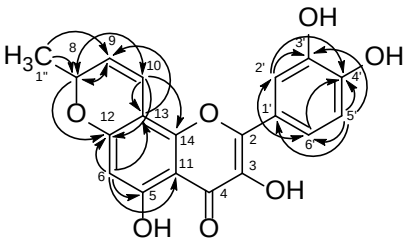

(**9**)

Figure 1

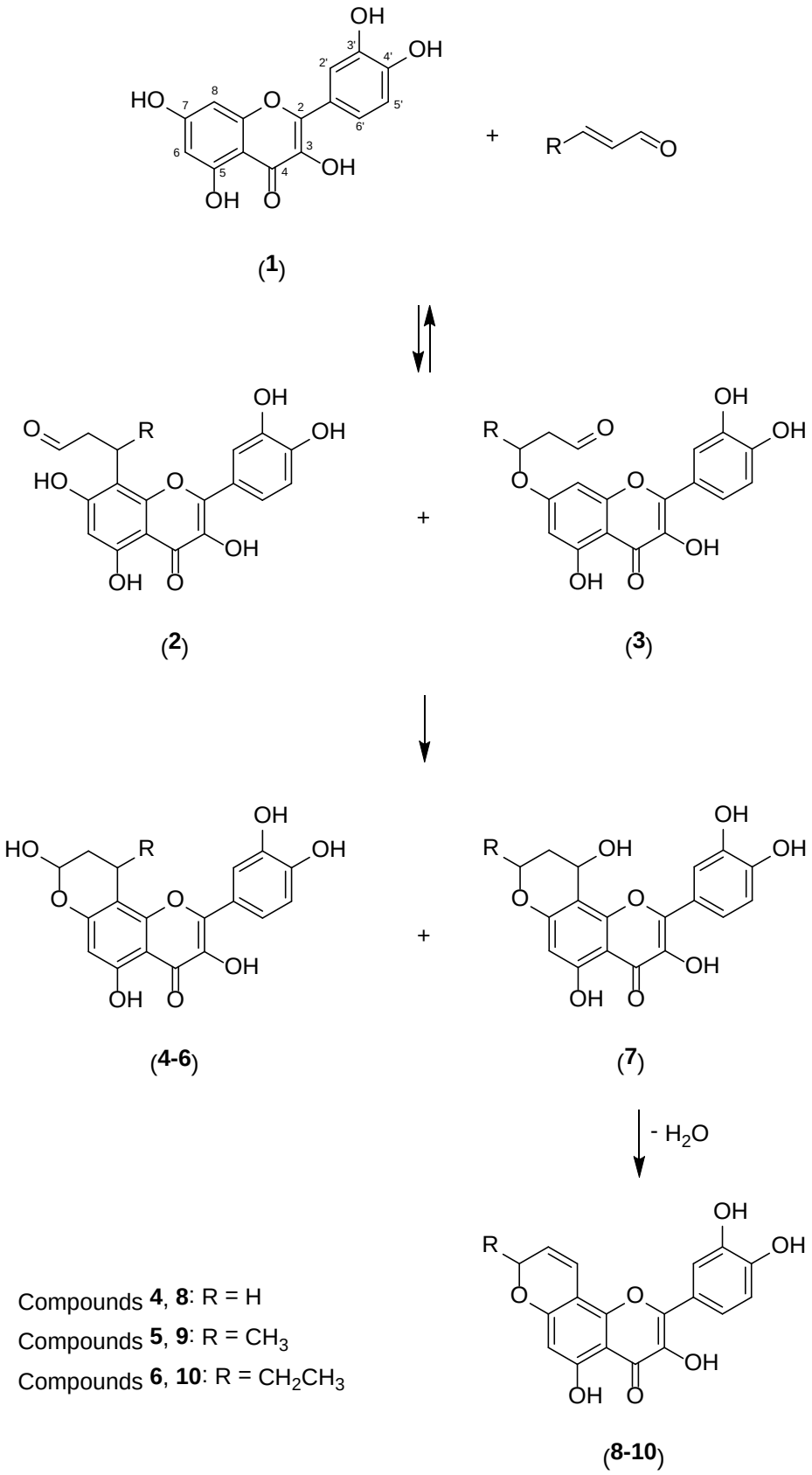
OH
3'
4'
OH
2'
HO
7
8
O
2
5'
6'
6
3
OH
5
4
OH
O
+
R
O
(1)
OH
OH
O
R
HO
O
OH
OH
O
+
R
O
OH
OH
O
O
OH
OH
O
(2)
(3)
OH
OH
HO
R
O
O
OH
OH
O
+
R
OH
OH
O
O
OH
OH
O
(4-6)
(7)
- H2O
R
OH
OH
O
O
OH
OH
O
(8-10)
Compounds 4, 8: R = H
Compounds 5, 9: R = CH3
Compounds 6, 10: R = CH2CH3

Figure 2

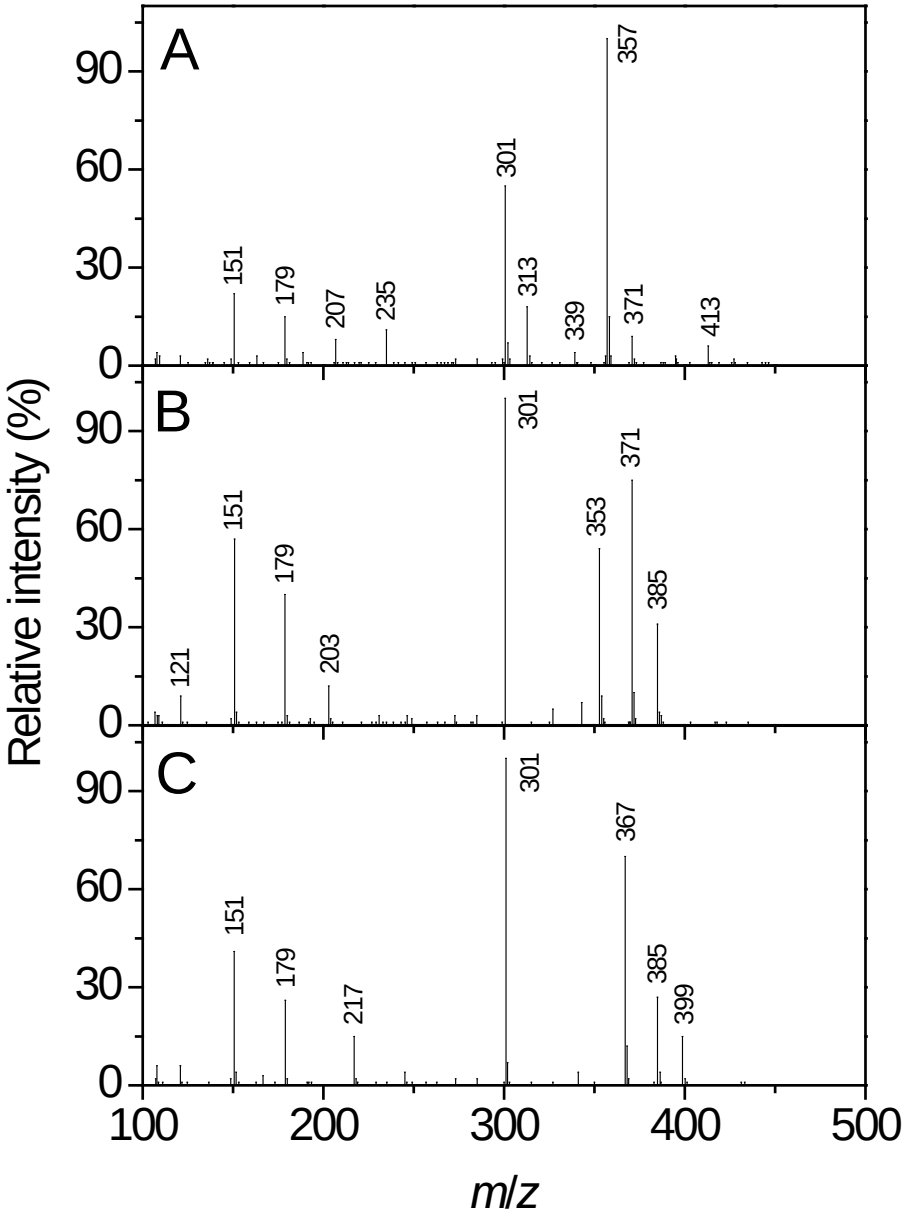
A
B
C
Relative intensity (%)
m/z
0
30
60
90
100
200
300
400
500
151
179
207
235
301
313
339
357
371
413
121
203
353
385
217
367
399

Figure 3

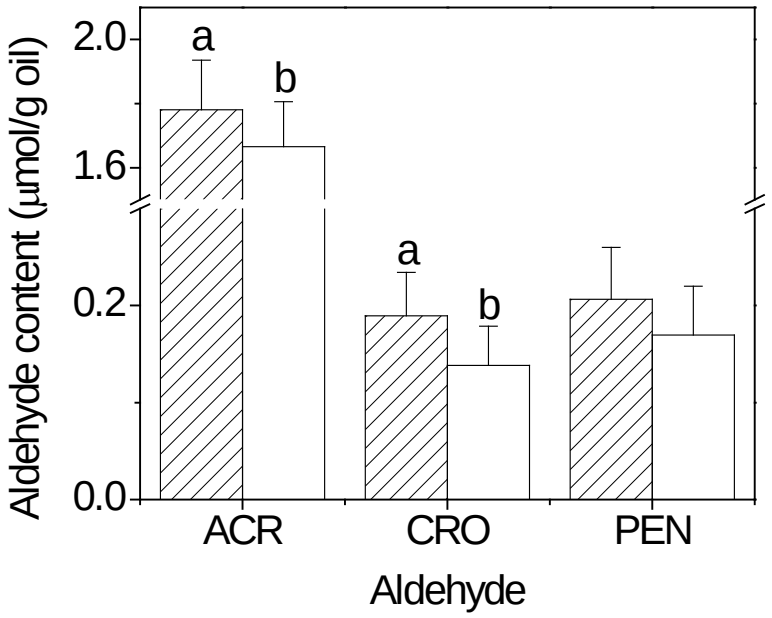
Aldehyde content (µmol/g oil)
2.0
1.6
0.2
0.0
a
b
a
b
ACR
CRO
PEN
Aldehyde

Figure 4

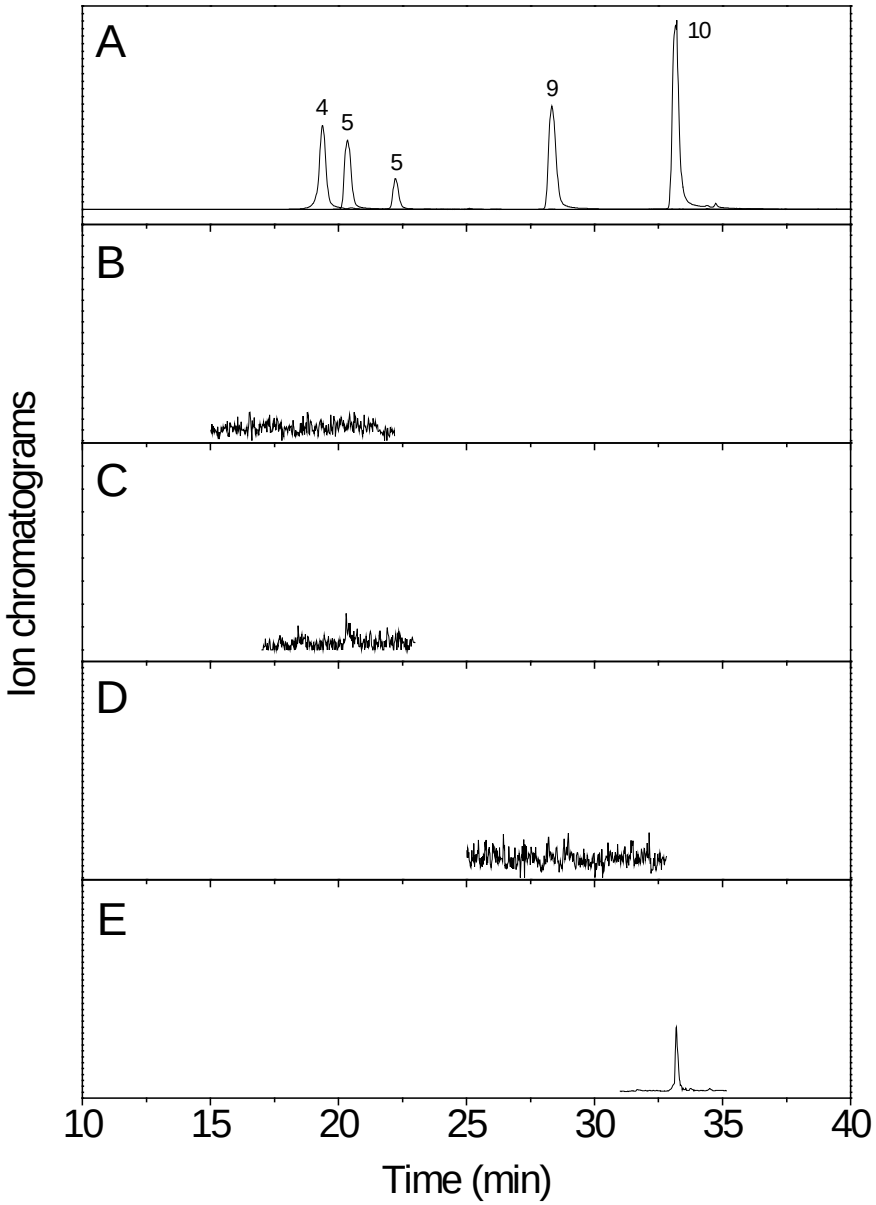

Figure 5

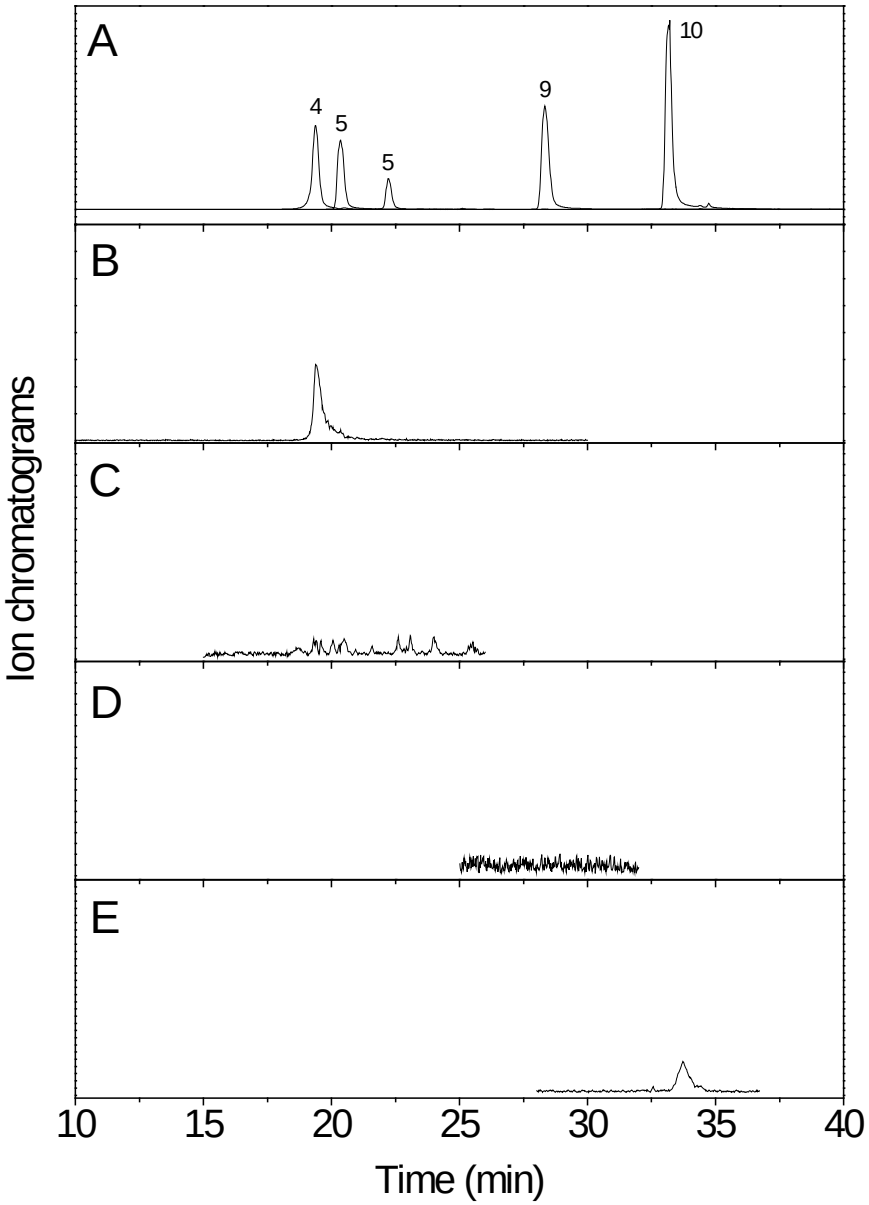

Figure 6

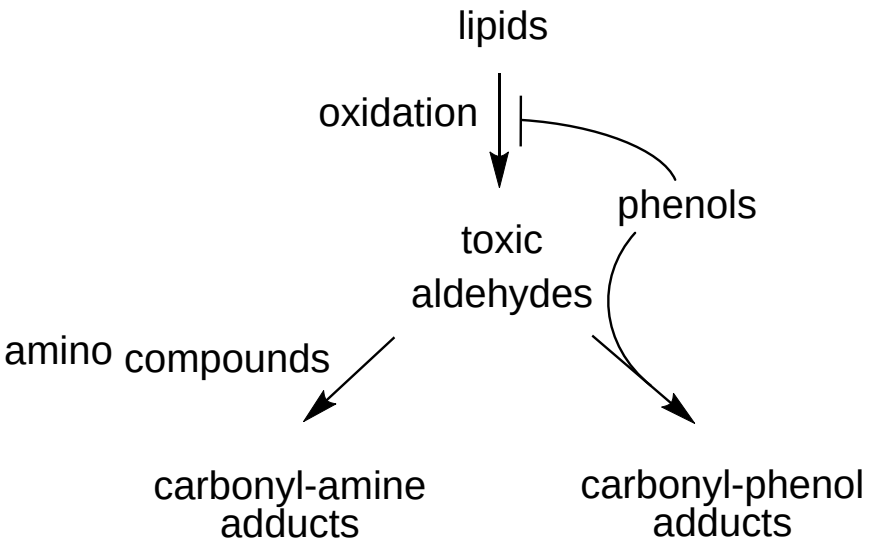
lipids
oxidation
phenols
toxic
aldehydes
amino compounds
carbonyl-amine adducts
carbonyl-phenol adducts

Figure 7

GRAPHIC FOR TABLE OF CONTENTS