JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Food and Beverage Chemistry/Biochemistry

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J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.8b04158 • Publication Date (Web): 08 Oct 2018

Formation of Phenolic Compounds from D-Galacturonic Acid

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ABSTRACT

Aqueous D-galacturonic acid (D-GalA) model systems treated at 130 °C at different pH values show an intense color formation, whereas other reducing sugars, like D-galactose (D-Gal), scarcely react. GC-MS measurements revealed the presence of several phenolic compounds e.g. 3,8-dihydroxy-2-methyl-*4H*-chromen-4-one (chromone) and 2,3-dihydroxybenzaldehyde (2,3-DHBA). These phenolic compounds, especially 2,3-DHBA, possess an intense browning potential and cannot be found within heated model solutions of reducing sugars. Investigations regarding the formation of these substances show that α-ketoglutaraldehyde plays an important role as an intermediate product. In addition, MS analysis of model systems of norfuraneol in combination with 2,3-DHBA showed the formation of oligomers that could also be detected in D-GalA model systems, leading to the assumption that, besides reductic acid, these compounds are jointly responsible for the strong color formation during the heat treatment of D-GalA.

KEYWORDS

D-galacturonic acid, 2,3-dihydroxybenzaldehyde, 3,8 dihydroxy-2-methyl-*4H*-chromen-4-one, D-galactose furfural, polygalacturonic acid, pectin, polyphenol, catechol, methylcatechol, α-ketoglutaraldehyde

INTRODUCTION

Since the beginning of food processing, the thermal formation of reaction products formed via non-enzymatic browning has increasingly become of interest. Depending on the application, these mechanisms are either desirable or not. Uronic acids are abundant in nature and consequently play a very important role in the formation of browning and volatile products in groceries. For D-galacturonic acid (D-GalA), which is present in the backbone of the pectin molecule, a drastic influence on the color formation was already reported in several studies, e.g. Hodge et al.[1-3] Uronic acids in general show a stronger browning potential than reducing sugars, such as D-galactose (D-Gal).[2,4-6] It was observed that one of the major chromophore developing substances in model systems of these sugar acids is reductic acid. It is formed after decarboxylation of D-GalA and further dehydration reactions that lead to the reactive α-ketoglutaraldehyde. Bornik succeeded in verifying this important intermediate through a derivatization reaction with dinitrophenylhydrazine and subsequent MS analysis.[7] This intermediate then cyclizes into carbocyclic pentenones, such as reductic acid.[6] In model reaction systems, the pure substance showed the development of reddish-brown polymers.[8] This color drift into red could also be observed in D-galacturonic acid model systems. Still, the intense browning formation of heat-treated D-GalA model systems without amine catalysis cannot solely be explained through the formation of reductic acid.

Another interesting pathway for the formation of browning precursors could be observed by Popoff and Theander. They were able to prove that several polyphenolic reaction products were formed during the heat treatment of reducing sugars and uronic acid.[5] They assumed that the polyphenolic compounds may have an influence on the formation of color within the model systems but did not explore the matter further. Later, the same group, postulated that the phenolic compounds were intermediates of the temperature

induced degradation of uronic acids and that unsaturated aldehydes take part in their formation mechanism.[9]

The aim of the present paper is to attempt a more in-depth analysis of the reaction variety of uronic acids. GC-MS measurements were conducted to reveal the product spectrum and kinetics during the heat treatment of D-GalA and oligo- or polymer representatives, such as pectin or polygalacturonic acid, at different reaction conditions. The analysis of the degradation products focuses primarily on polyphenolic compounds in combination with the formation of color. Aqueous model systems of D-GalA were therefore heated at temperatures between 130 °C to 160 °C and pH values between pH 3 and 8. Single compounds that appeared to possess a high browning potential were individually heated and characterized using various analytical methods. Furthermore, the formation mechanism of these compounds was reconstructed through the reaction of known intermediates.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: D-galacturonic acid monohydrate, furfural, norfuraneol, 2,3-dihydroxybenzaldehyde, D-glucuronic acid, scopoletin, L-arabinose and the solvents (HPLC- or GC ultra-grade) were purchased from Sigma-Aldrich (Steinheim, Germany); L-alanine, D-galactose, D-xylose, and sodium acetate were purchased from Merck (Darmstadt, Germany), MN Polyamid SC 6 was purchased from Macherey-Nagel (Düren, Germany)

Syntheses.

3,8-dihydroxy-2-methyl-4H-chromen-4-one. 3,8-dihydroxy-2-methyl-*4H*-chromen-4-one (chromone) was synthesized using a slightly modified method of Lindgren and Pernemalm 1980.[10] 5.0 g of D-xylose were dissolved in 200 mL of an acetate buffer (0.5 mol/L) solution and the pH was adjusted to 4.5. The solution was heated for 48 hours under reflux and, after cooling down, extracted with ethyl acetate (3x100 mL). The combined extract was dried with anhydrous sodium sulfate and the solvent completely removed under a nitrogen stream. Clean-up was done using a SPE phase. The residue was then resolved in water and added onto the polyamide cartridge (MN Polyamid SC 6, particle size 0.05-0,16 mm, Macherey-Nagel, Düren, Germany). The cartridge was rinsed with 10 mL of distilled water, after which the product was eluted with a mixture of methanol/water/acetic acid (90:5:5, v:v:v). The organic solvent was removed by vacuum distillation and the residual was freeze-dried. Purity was determined via HPLC-RI and GC-MS detection. The product was yielded in 92 % purity. GC-MS t_R = 32.11 min. *m/z* 192 [M^+, 100 %], 163 (15 %), 146 (10 %), 137 (42 %), 121 (38 %), 108 (7 %), 79 (9 %), 65 (11 %)

Model Reaction. *D-galacturonic acid.* To investigate the formation of volatile and potential chromophore-building substances, D-galacturonic acid (0.25 mol/L), pectin (0.25 mol/L D-GalA equivalent) and polygalacturonic acid (PGA) (0.25 mol/L D-GalA

equivalent) were dissolved in water and the pH was adjusted with NaOH and HCl to 3.0, 6.0 and 8.0 (to prevent the formation of lumps the polymer compounds were added to a stirred solution). The use of buffering solutions was avoided, as they are known to change the reaction process. The thusly prepared solutions were filled into headspace vials and heated in heating blocks at 130 °C for varying lengths of time, up to a maximum of 4 hours. The solutions were cooled down and spiked with an internal standard and subsequently extracted three times with 10 mL dichloromethane. The solvent was removed under a nitrogen stream and 250 µL of the residue dissolved in dichloromethane was analyzed by GC-MS (Shimadzu, QP 2010, Duisburg, Germany). The browning intensity of the heated solutions was measured with a UV/VIS spectrometer at 420 nm and converted into CIELab colors (Specord® 40, Analytik Jena, Jena, Germany). The change in the pH value was measured after cooling down to room temperature with a pH-electrode (pH-Meter 761 Calimatic, Knick). Furthermore, the formation of potential browning polymers and the degradation of the pectin molecule were measured with HPLC-SEC-UV-VIS/RI (size exclusion chromatography) at 420 nm and a refractometric index detector, as described previously by Wegener *et a*l.[11]

2,3-dihydroxybenzaldehyde. The concentration of model reaction systems containing 2,3-dihydroxybenzaldehyde (6) was 0.025 mol/L. Spiked model systems contained the doped substance in concentrations that were also found in D-galacturonic acid or pectin systems.

Qualification and Quantitation.

To ensure a correct assignment of the peaks in the GC chromatogram, standard substances were measured and compared with the sample. Quantitation was done using an internal standard (scopoletin) and response factors were determined with reference substances. A three-fold determination was performed.

Gas Chromatography−Mass Spectrometry

GC-MS investigations were performed on a Shimadzu GC-QP 2010, using a capillary DP-5 column (60 m, 0.25 mm, 0.25 µm, Supelco SLB-5MS, Bellefonte, PA, USA). The measurements of the samples were performed via split injection (1:5), using helium as carrier gas, with a flow rate of 2 mL/min. The injection temperature was set to 270 °C and the initial temperature, for the temperature gradient, to 30 °C. After 3 minutes, the temperature was raised to 120 °C at a rate of 5 °C/min. Subsequently, the temperature was increased to 200 °C, at a rate of 20 °C/min, and held for 5 minutes. Again, the temperature was raised to 250 °C, at a rate of 20 °C/min. This temperature was held for 5 minutes, before it was ultimately increased to 320 °C, at a rate of 20 °C/min, and held for another 15 minutes. To ensure that the all molecules remain in the gas phase the interface temperature was adjusted to 270 °C and the ion source to 200 °C. An ionization energy of 70 eV was used and a mass range of 35-400 *m/z* was scanned. The solvent cut time was set to 5 minutes.

Electrospray Ionization-Mass Spectrometry

ESI-MS investigations were performed on a LTQ Orbitrap XL (Thermo Fischer Scientific, Bremen, Germany), between a mass range of 150-1000 *m/z*. The source voltage was set to 3.54 kV and a current of 1.01 uA. The capillary temperature was 300 °C. The samples were diluted with methanol/water to 1 mg/mL and directly injected to the spectrometer with a syringe pump at a flow rate of 20 µL/min. Measurements were performed using a negative electrospray ionization mode.

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

Parameters used for the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) were as follows. All experiments were performed on a Bruker Daltonics Autoflex III smartbeam MALDI TOF mass spectrometer (Bruker

135 Daltonics, Bremen) equipped with a 50 Hz pulsed N2 laser (wavelength 337 nm). The ion
136 source was set to a voltage of 19.00 kV, lens and reflector had a voltage of 8.50 and
137 20.00 kV. Laser intensity was set to 60 %. The MALDI matrix was prepared by dissolving
138 25 mg/mL 2,5-dihydroxybenzoic acid in acetonitrile/water (30/70, v/v) and 0.1 %
139 trifluoroacetic acid. Samples were dissolved in methanol and diluted to 1 mg/mL. 0.5 µL
140 matrix were spread on a MALDI plate and mixed with 0.5 µL of sample. The solution could
141 dry at room temperature and build a layer of crystals. The scanning range was set from
142 400-2000 *m/z.*

RESULTS and DISCUSSION

Polyphenol formation during the degradation of D-galacturonic acid

Uronic acids, in this case D-galacturonic acid, as well as reducing sugars, such as D-galactose, are structurally very similar. Both substances have of a pyranoid backbone and a carbonyl group at C-1 that is next to the C-4 OH function included in the glycosidic bond of oligo- and polymers. Sugar acids consist furthermore of a carboxylic group located at C-6 that influences the reactivity of the uronic acids. Earlier works have already shown that D-GalA reacts far more drastically than D-Gal although they are structurally related. The heat treatment of aqueous model systems of D-GalA, for example, showed a ten times higher browning compared to that of D-Gal and L-Ara at temperatures of 130 °C and a heating time of 240 minutes (figure 1).[8] In addition to products, such as furfural and norfuraneol that are also generated during the degradation of D-Gal and D-Ara, 2-furoic acid, 5-formyl-2-furoic acid and reductic acid are specific degradation products for uronic acids that may influence the color development. Alongside these already known degradation products (e.g. Bornik (2013)[7], Madson (1979)[2], Isbell (1944))[6], Popoff and Theander were able to determine the formation of phenolic reaction products during the reaction of sugars and uronic acids in aqueous solutions under reflux at a temperature of 96 °C and a heating time of 48 hours. Based on these experiments, one can see that the use alone of D-glucuronic acid under basic conditions forms phenolic compounds. Reducing sugars react to form phenolic compounds under Maillard-Reaction (glycine) conditions. Without amino acid catalysis, only traces of phenolic products can be detected. The reaction products found include catechol (**1**), methylcatechol (**2**), 3,8-dihydroxy-2-methyl-4*H*-chromen-4-one (chromone, **3**), 5,6,7,8-tetrahydro-3,5-dihydroxy-2-methyl-8-oxobenzo-pyrene (**4**), trihydroxybenzaldehyde, 2,3-dihydroxybenzoic acid and 2,3-dihydroxyacetophenone.[5]

In the present study, it was examined what kind of reaction products are formed throughout the degradation of D-galacturonic acid (0.25 mol/L) during heat treatment at 130 °C, in acidic and basic conditions, in comparison to D-Gal model reaction systems. Buffering solutions were not used to prevent a possible influence of these substances on the reaction. In accordance with other publications, furfural (3.5 mmol/L, pH 3, 130°C, 2 h) was found to be one of the main products produced in model systems in an acidic milieu, followed by norfuraneol (0.02 mmol/L, pH 3, 130°C, 2 h)[8, 12]. In addition to these already very well-known oxygen heterocycles, degradation products, such as short chain aldehydes, e.g. glycolaldehyde amounting to 1 % or acetaldehyde, amounting to 25 % of the analyzed aldehydes, could be detected. These aldehydes may contribute to the formation of phenolic compounds. Along with these well-known sugar degradation products, several phenolic compounds were also able to be identified [5,13-16]. Besides the already described phenolic compounds, such as **1, 2, 3** and **4,** we could detect 2,3-dihydroxybenzaldehyde (**6**) and 3,4-dihydroxyacetophenone (**5**) (figure 2). These additional phenolic substances could be identified via comparison with reference substances and interpretation of EI-MS and ESI-MS/MS spectra. The present objective is to examine whether, and to what extent, these phenolic compounds can form chromophoric polymers that influence the color of the D-GalA model system. It is known that phenolic compounds show a strong tendency to polymerize due to oxidative coupling reactions, resulting in colored compounds. For instance, an aqueous solution of **6** gains up to 100 times more color than the D-GalA solution.

Given the fact that **6** and high concentrations of **3** are detected in aqueous D-GalA model systems (table 1), they are of special interest and deserving of further investigations concerning their reactivity.

Alongside D-GalA model-solutions, **6** could also be detected in di-, tri- and polygalacturonic acid systems, as well as in pectin. The calculated concentrations

detected in these model-solutions are listed in table 1. One can see that the concentration of **6** found in pectin is more than twice as high as in the PGA system. This is mainly because pectin consists of neutral sugars, primarily pentoses (L-arabinose, D-xylose) that may also degrade into furfural and smaller amounts of α-ketoglutaraldehyde and thus enhance the possibility for the formation of **6**. In heated model systems of D-Gal, no polyphenols could be detected. The assumption that neutral sugars positively influence the formation of polyphenols could be confirmed through the introduction of L-Ara or D-Gal to polygalaturonic acid model systems. As figure 3 shows, this addition leads to a significant increase in the concentration of **6**. Especially the use of pentoses, shown here for L-Ara, lead to a drastic enhancement of **6** after only 120 minutes. Along with an increasing of polyphenol concentration, an increase of the furfural concentration, a precursor molecule of **6,** could also be registered. This is because furfural is the main degradation product of pentoses. The comparison of color formed through D-GalA polymers, such as polygalacturonic acid, shows that the introduction of neutral sugars to the pure substance not only leads to an increase in the concentration of **6**, but it also causes an increase of the color formation. This rise of a factor of 2.3 with the addition of L-arabinose goes hand in hand with an increase in the concentration of **6** and furfural.

Analyses regarding the involvement of degradation products in the formation of polyphenols and their color-characteristic

a) 2,3-DHBA

To gain further insights into the browning potential of **6**, model systems of D-GalA were conducted in combination with **6**, in concentrations found in the sugar acid systems. Although **6** was only added in µM concentrations, the increase in color intensity compared to D-GalA on its own is clearly visible (figure 1). In addition, model reaction systems containing only **6** in higher concentrations were performed at 130 °C for up to 4 hours.

After 60 minutes in a slightly acidic milieu, the solution started to develop a brownish color that drastically intensified after 2 hours and turned into a nearly black solution after 4 hours of heating. This effect even increases in a mild alkaline milieu. Investigations of the model reaction systems show that these reactions may, as well, take place during the processing of food (storage, drying) and may lead to an undesired color formation. If one compares the temperature induced browning of the polyphenols, e.g. **6,** with other browning precursor molecules, such as furfural (0.025 M, 130 °C, 4 h, pH 3, absorption at 420 nm: 0.10) or norfuraneol (0.025 M, 130 °C, 4 h, pH 3, absorption at 420 nm: 0.54), during caramelization, they are unable to reach the browning values at 420 nm of **6**. Similar results occur during the investigation of D-GalA and L-Ara model systems, where ten times higher browning values (420 nm) are found in D-GalA samples. Furfural, the main degradation product of L-Ara, only exhibits a very low browning potential.

In literature, several methods of coupling phenolic compounds have already been described, e.g. oxidative coupling reactions.[17,18] These could lead to the formation of high molecular weight products that potentially have an influence on the browning of the reaction solution. To examine the products, samples of pure heated solutions of **6** were investigated with LC-MS. The results reveal the presence of polymerization products, such as the dimer. High molecular weight products could only be detected with SEC-UV-VIS/RI (figure 4) (7 to 20 min, >400 to 10 kDa). This is probably due to the diversity of reaction pathways that may take place and that lead to a multitude of different polymers in individual low concentrations. Nevertheless, not only phenolic compounds may react together. Within heat treated sugar acid model systems, the reaction of phenolic compounds with degradation products, such as heterocyclic or short chained molecules formed through D-GalA, is very likely as well and may even enhance the diversity. To prove that polymerization reactions also take place within mixtures of polyphenoles and *O*-heterocyles, norfuraneol was mixed with **6** and heated for 4 hours at 130 °C and pH 3.

HR-ESI-MS measurements of these model systems revealed that a condensation reaction between these two substances takes place, leading to the condensation product with a mass of 233.05 *m/z* (figure 6, right corner). This mass could also be found within ESI-MS investigations of D-GalA model systems. In addition, further products of polymerization could be observed.

To compare the different molecular weight domains of polymers formed in the polyphenol and uronic acid model system, size exclusion chromatography was performed in combination with a refractometric index and UV-detector. The D-GalA model system shows a broad peak at 20 min that splits into three molecular weight domains. High molecular weight fractions were not observed. Model systems of **6** show, in addition to the 2,3-DHBA peak, oligomeric (20 to 25 min,10 to 5 kDa) and, in minor concentrations, polymeric polyphenolic compounds (around 12 min, 7 to 20 min, >400 to 10 kDa) (figure 4, left). The investigations of D-GalA and 2,3-DHBA model reaction systems after heat treatment showed similar molecular weight domains in the low (25 to 35 min, 5 to <1 kDa) to intermediate molecular weight fractions (figure 4, left). Analyzed with an UV-detector (figure 4, right) at 420 nm, the same samples show a peak at 22.3 minutes. The intense signal leads to the prediction that the substance responsible for this peak is formed in D-GalA, as well as in model systems of **6,** and has a high browning potential. The polymeric substances of **6** also lead to a signal at a detection wavelength of 420 nm, indicating that these compounds also contribute to the color of the polyphenolic system. They could not be detected within the sugar acid solution, most likely due to their low concentration.

b) 3,8-dihydroxy-2-methyl-*4H*-chomen-4-one

For its investigations, **3** was synthesized using a slightly modified method of Lindgren and Pernemalm 1980.[10] GC/MS measurements were used to confirm its identity. This provided a reliable identification and enabled subsequent quantitation in model systems

to be performed. As already described above, **3** belongs to the major polyphenolic degradation products in heated aqueous D-GalA model systems (130 °C, pH 3, figure 5, left). Model reactions systems performed at 160 °C, at pH 3, show a great increase up to 60 minutes, in comparison to 130 °C, where **3** could not be determined up to this point. After this point in time, the concentrations start to decline, due to degradation and polymerization reactions. A similar course could also be registered for furfural. Furthermore, the formation of 5,6,7,8-Tetrahydro-3,5-dihydroxy-2-methyl-8-oxobenzo-pyren as a precursor molecule of **3** was measured.

To have a closer look at the characteristics of **3**, tests concerning its browning potential and possible formation mechanisms were conducted. Model systems of pure **3** (aqueous solutions of **3** are slightly yellow) under different conditions displayed the formation of brown polymers of medium molecular weight (figure 5, right). Nevertheless, the browning intensity of model systems of **6,** conducted under the same conditions, could not be reached. One explanation could be that **3** does not have the same polymerization potential as **6** and partly accumulates within the system.

Formation pathways of the investigated polyphenols

a) 2,3-DHBA

Popoff and Theander (1972)[5] already described the formation of **1** and **2** in uronic acid model systems, albeit using different temperatures, time intervals and pH values. The formation of **6** in D-GalA systems has not yet been described. To elucidate possible formation mechanisms, the formation out of smaller degradation products was considered in a first step. The results demonstrate that furfural is a key component. One pathway already described for the formation of polyphenolic products is the ring opening of *O*-heterocyclic compounds (e.g 5-hydroxymethylfurfural) under hydrothermal conditions. This ring-opening is followed by an addition reaction.[19] Since HMF is not a degradation

296 product of D-GalA, the main product furfural was used in combination with short-chained
297 aldehydes (glycolaldehyde, acetaldehyde, glyoxal and formaldehyde) that may be
298 possible reaction partners in the D-GalA model reaction system.
299 Furthermore, the reactions were carried out under more moderate temperatures, since
300 the formation of phenolic compounds in D-GalA model systems could already be
301 observed at 130 °C and small concentrations could be measured even in systems heated
302 at 100 °C. During the investigations of furfural with glycolaldehyde and acetaldehyde,
303 small amounts of **6** could be measured. Since only small concentrations of **6** are formed
304 within the heterocyclic systems, it was concluded that pathway A cannot be the favored
305 course for the formation of 2,3-DHBA, since an acid catalyzed ring opening must take
306 place. If D-GalA itself is used, pathway B can occur, which leads through the formation of
307 α-ketoglutaraldehyde. The occurrence of α-ketoglutaraldehyde was postulated in 1944
308 by Isbell[6] and proven by Bornik[7,8]. Accordingly, higher concentrations of **6** can be
309 measured in uronic acid model systems. This assumption is supported by considerations
310 of Ahmad *et al*., (1993), who postulated a possible formation mechanism for
311 2,3-dihydroxyacetophenone in which an unsaturated aldehyde reacts with acetol[9]. Both
312 products occur during the degradation of D-GalA.
313 To exclude that **6** degrades itself from a higher molecular weight product, examinations
314 with chromone-like structures were conducted. One of the major products found in the
315 D-GalA model systems is **3** and could be an intermediate product for the formation of **6**.
316 To demonstrate this reaction mechanism, **3** was firstly synthesized[10] and then heated in
317 a model system at 130 °C. GC-MS investigations that have been used to identify the
318 phenolic compounds within the model systems did not provide any trace of **6**. Thus, the
319 respective formation mechanism was no longer considered and the reaction pathway
320 through furfural and α-ketoglutaraldehyde under acidic conditions should be the preferred
321 one (pathway A and B, figure 6).

b) 3,8-dihydroxy-2-methyl-4*H*-chromen-4-one

M/z of 702, 815, 928 and 1024 were detected within MALDI-TOF-MS experiments, which could belong to the medium molecular weight substances found within the chromone system during SEC measurements. The same *m/z* were registered in model systems of norfuraneol in combination with furfural, which leads to the assumption that a possible formation of **3** originates from these two *O*-heterocycles. GC-MS investigations of the model systems of norfuraneol and furfural confirmed this hypothesis, since **3** could be detected. According to model reaction systems of furfural and glycolaldehyde used for the formation mechanism of **6**, only small concentrations of **3** were registered, leading again to the conclusion that precursor molecules of furfural and norfuraneol are responsible for the formation of chromone and further chromone-like structures (figure 6, pathway A). Again, this suggests that precursor molecules of these two compounds are responsible for the formation of **3.** α-ketoglutaraldehyde, in particular, moves into focus, since it is one of the compounds that is not found during the degradation of hexoses and only in very small concentrations in pentoses.[7] Ahmad *et al.* (1993), postulated a formation pathway that uses an unsaturated aldehyde and an enediol as educts[9], similar to those of **6**. These degradation intermediates of D-GalA can also be found in the postulated formation mechanisms of norfuraneol and furfural, which underscores the above-mentioned assumption.

To investigate temperature and pH dependency of the formation of **3** from norfuraneol and furfural, the reaction partners were heated to 150 °C, at pH-values of 1, 2, 3, 5. The lowering of the pH-value to 1 should enhance the ring opening and raise the yield. Consequently, the oxygen atom located within the ring should become protonated and the attack of an oxonium ion at the adjacent carbon atom should be facilitated. However, the conducted experiments show a rapid and strong degradation of norfuraneol, only allowing a formation of **3** to occur in trace amounts. During the reaction at pH 2, the

concentration of **3** doubles in relation to pH 3, which is a sign that the catalytic ring opening occurs (without norfuraneol reacting with itself). The enhancement to a pH value of 5 does not lead to any further changes in the concentration of **3**. Along with the pH value, the temperature of 150 °C also has an impact on the formation of **3**. Furthermore, model systems heated at this temperature and a pH value of 2, show more than a twofold increase in the yield of the product in comparison to 130 °C. The increase to a temperature of 160 °C does not further enhance the formation. Under these conditions, an enforced degradation can be registered, most likely attributable to polymerization. Moreover, norfuraneol could not be detected anymore after 2 hours of heating, so that further formation of **3** was no longer possible.

In addition to the variation of reaction conditions for the norfuraneol, furfural model systems, the addition of different compound classes such as alcohols, aldehydes and carboxylic acids, were also investigated. At a pH value of 3 and a temperature of 130 °C (standard conditions), the addition of acetic acid (pH was adjusted to 3 again after the addition) leads to an increase in concentration of **3** of 50 %**,** whereas no influence can be registered during the addition of the other compound classes.

In summary, the temperature and pH dependent degradation of D-GalA leads first of all to the formation of typical carbohydrate reaction products, such as furfural, and norfuraneol or short chain aldehydes to a broad substance spectrum, from which different phenolic compounds can be formed. 2,3-DHBA was detected for the first time and plays an important role. Although only found in small amounts within the heated D-GalA model systems, it possesses a high browning potential. 3,8-dihydroxy-2-methyl-4*H*-chromen-4-one, on the other hand, possesses a comparatively low browning potential and partly accumulates within the system, thus becoming one of the main components found within the D-GalA model system. Conducted experiments with the pure substances show the formation of medium to high molecular weight compounds that proportionally contribute

to the total browning of the D-GalA system. According to current knowledge, the formation of both substances passes through the intermediate stage of α-ketoglutaraldehyde. Two different reaction pathways derive from this intermediate, leading, on the one hand, to furfural and, on the other, to reductic acid through the elimination of water. Through addition reactions, a chain elongation is possible. Depending on the reaction partner, acetaldehyde or norfuraneol, the compounds 2,3-dihydroxybenzaldehyde or rather 3,8-dihydroxy-2-methyl-4*H*-chromen-4-one are formed. In both cases, α-ketoglutaraldehyde reacts as the key component of the D-GalA degradation. Given that reducing sugars, such as D-Gal or D-Glc, do not degrade into α-ketoglutaraldehyde, the participation of phenolic compounds in color formation remains limited to the reaction of uronic acids.

ABBREVIATIONS USED

2,3-DHBA, 2,3-dihydroxybenzaldehyde; L-Ara, L-arabinose; chromone, 3,8-dihydroxy-2-methyl-*4H*-chromen-4-one; DAD, diode array detector; ESI, electrospray-ionization; Fur, furfural; D-Gal, D-galactose; D-Glc, D-glucose D-GalA, D-galacturonic acid; GC, gas chromatography; HPLC, high performance liquid chromatography; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MS, mass spectrometry; RI, refractometric index detector SEC, size-exclusion chromatography,

ACKNOWLEDGEMENT

The authors are grateful for the financial support of the Forschungsgemeinschaft (DFG-project “Structure-depending degradation reactions of pectins and their impact on non-enzymatic browning and technological functionality”) (DR 806/4-1).

Thanks are also extended to Luzia Hammer and Isabell Buggisch for the support of the sample analyses.

SUPPORTING INFORMATION

Figure 1: ESI mass spectrum of a heated 2,3-DHBA model system.

Figure 2: ESI mass spectrum of a heated model system of a mixture of 2,3-DHBA and norfuraneol

Figure 3: MALDI-TOF spectrum of 2,5-dihydroxybenzoic acid (matrix)

Figure 4: MALDI-TOF spectrum of a heated model system of chromone

Figure 5: GC-MS spectrum of 3,8-dihydroxy-2-methyl-*4H*-chomen-4-on

Table 1: chromone concentration in various model systems of furfural and norfuraneol performed at different temperature and pH values.

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FIGURE CAPTIONS

Figure 1: Color (absorption at 420 nm) of aqueous model reaction systems (0.015 mol/L) consisting of D-GalA, 2,3-DHBA, L-Ara, D-Gal and D-GalA doped with 2,3-DHBA heated at 130 °C and a pH of 6. Samples were taken after 0, 120 and 240 minutes.

Figure 2: Polyphenolic compounds that are formed during the heat treatment of D-Gal. catechol (1); methylcatechol (2); 3,8-dihydroxy-2-methyl-4*H*-chromen-4-one (chromone) (3); 5,6,7,8-tetrahydro-3,5-dihydroxy-2-methyl-8-oxobenzo-pyrene (4); 3,4-dihydroxy-acetophenone (5); 2,3-dihydroxybenzaldehyde (2,3-DHBA) (6)

Figure 3: Formation of furfural and 2,3-DHBA in pure polygalacturonic acid and with addition of the sugars D-Gal and L-Ara at 130 °C and pH 3.

Figure 4: Molecular weight distribution of formed degradation products of D-GalA and 2,3-DHBA solutions heated at 130°C and a pH of 8. The measurements were performed using the size exclusion chromatography in combination with a RI and a parallel detection at a wavelength of 420 nm.

Figure 5: a) Concentrations of chromone and furfural, found in D-GalA model systems at 130 °C and a pH of 3 (GC-MS). b) SEC investigation of a heated chromone model system, showing an intense signal at 420 nm at around 18 min.

Figure 6: Formation scheme of 2,3-DHBA and chromone, originating, on the one hand, from furfural (pathway A) and, on the other hand, from D-GalA (pathway B).

Table 1: Concentrations of 2,3-DHBA, catechol, methylcatechol and chromone found in different model systems heated for 120 minutes at 130 °C and a pH of 3. NQ: not quantitated

concentration [µmol/L]	D-GalA
2,3-DHBA (6)	196.2 ± 4.3
catechol (1)	1.8*
methyl-catechol (2)	NQ
3,4-dihydroxy-acetophenone (5)	0.3 ± 0.01
chromone (3)	1573.4 ± 0.14

concentration [µmol/L]	2,3-DHBA
D-GalA	196.2 ± 4.3
DiGalA	6.60 ± 0.1
TriGalA	4.40 ± 0.2
PGA	1.32 ± 0.1
Pektin	47.40 ± 1.8

* Only detected in one sample

Figure captions

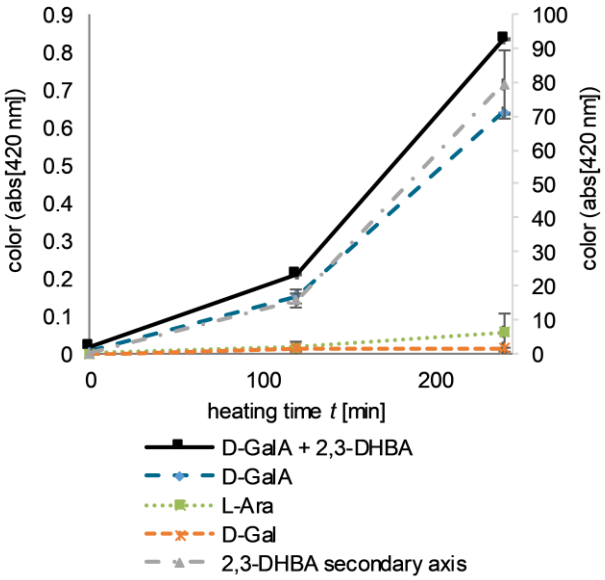

Figure 1

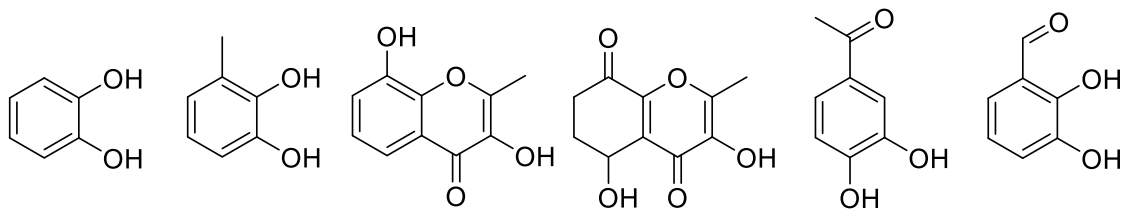

Figure 2

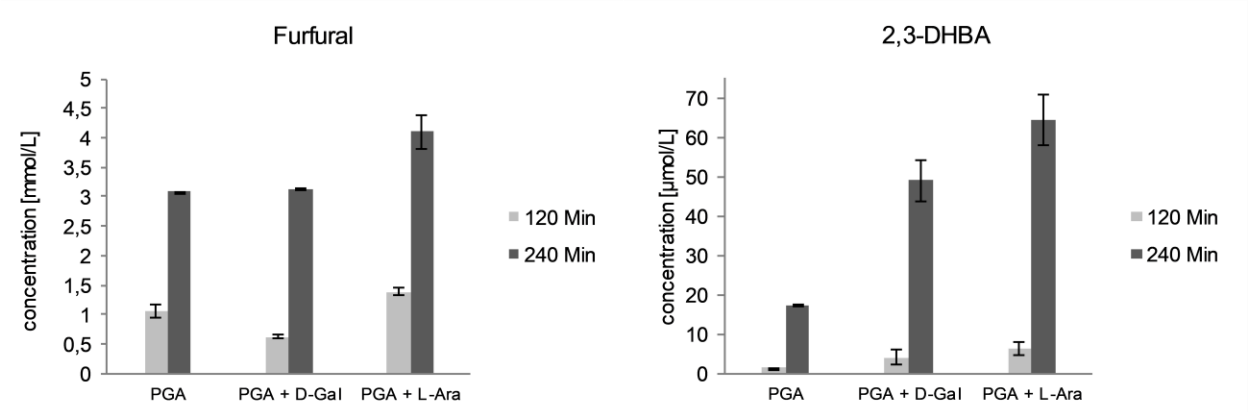
Furfural
concentration [mmol/L]
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
PGA
PGA + D-Gal
PGA + L-Ara
120 Min
240 Min
2,3-DHBA
concentration [µmol/L]
70
60
50
40
30
20
10
0
PGA
PGA + D-Gal
PGA + L-Ara
120 Min
240 Min

Figure 3

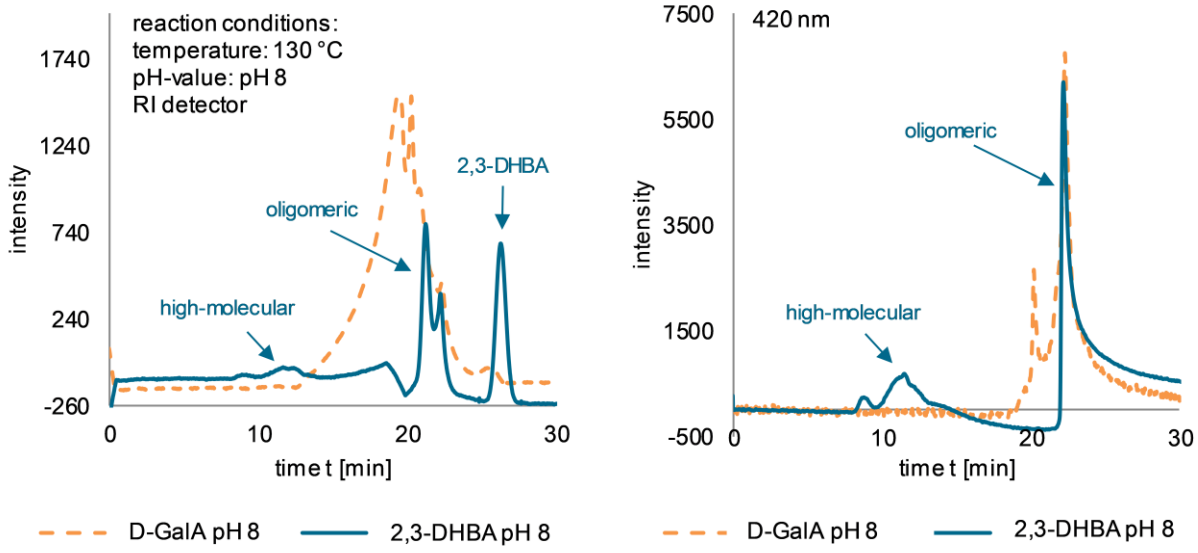
reaction conditions:
temperature: 130 °C
pH-value: pH 8
RI detector
1740
1240
740
240
-260
intensity
oligomeric
2,3-DHBA
high-molecular
0
10
20
30
time t [min]
D-GalA pH 8
2,3-DHBA pH 8
420 nm
7500
5500
3500
1500
-500
intensity
oligomeric
high-molecular
0
10
20
30
time t [min]
D-GalA pH 8
2,3-DHBA pH 8

Figure 4

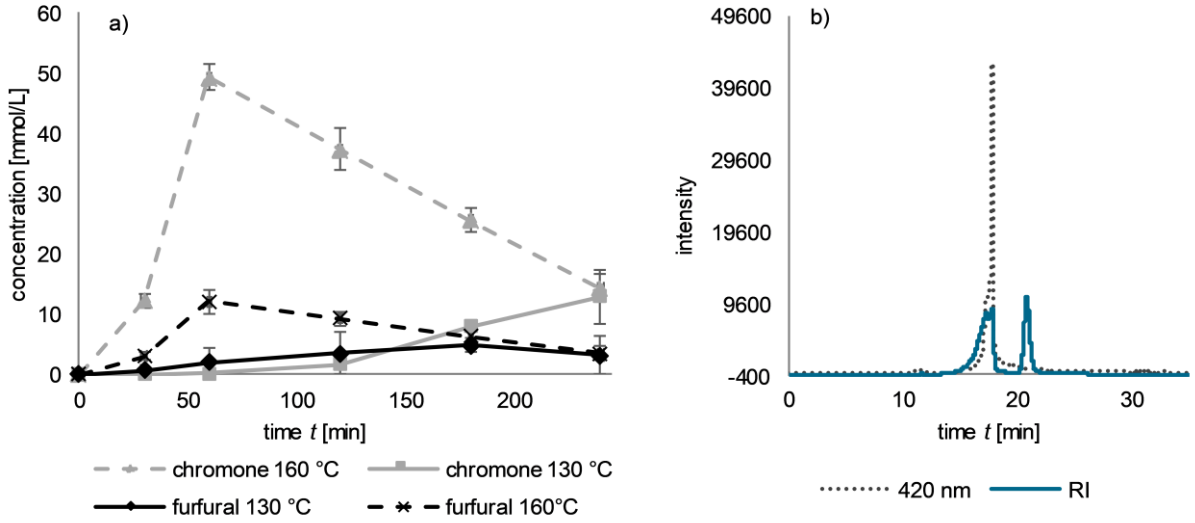
a)
concentration [mmol/L]
60
50
40
30
20
10
0
0
50
100
150
200
time t [min]
chromone 160 °C
chromone 130 °C
furfural 130 °C
furfural 160°C
b)
intensity
49600
39600
29600
19600
9600
-400
0
10
20
30
time t [min]
420 nm
RI

Figure 5

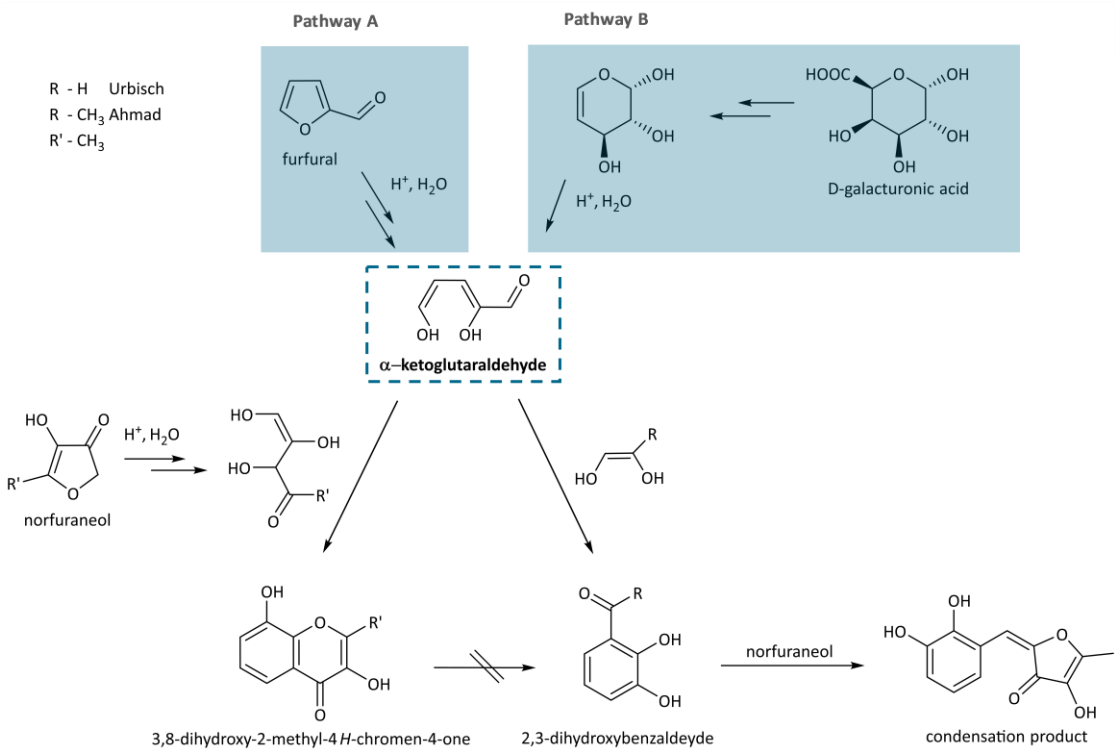
Pathway A
Pathway B
R - H Urbisch
R - CH3 Ahmad
R' - CH3
furfural
H+, H2O
H+, H2O
D-galacturonic acid
α–ketoglutaraldehyde
norfuraneol
H+, H2O
norfuraneol
3,8-dihydroxy-2-methyl-4 H-chromen-4-one
2,3-dihydroxybenzaldeyde
condensation product

Figure 6

TOC graphic