HYDROXYCINNAMIC ACID-MAILLARD REACTIONS: INSIGHTS INTO FLAVOUR DEVELOPMENT OF WHOLE GRAIN FOODS

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Abstract

The flavour properties of cereal food products have been reported to be negatively influenced when formulated with whole grain versus refined flour. The objective of this study was to investigate the reactivity of the phenolic compounds in whole grain on the mechanisms of the Maillard reaction in low moisture high temperature simulated roast model systems. Using isotope labelling techniques, the hydroxycinnamic acids were reported to be reactive with key transient Maillard reaction products, such as hexose sugar fragments. The structure of one ferulic acid-Maillard reaction product was characterised by NMR and MS and identified as 6-(4-hydroxy-3-methoxyphenyl)-5-(hydroxymethyl)-8-oxabicyclo[3.2.1]-oct-3-en-2-one. Based on retrosynthesis, this reaction product was suggested to be generated by a [5+2] cycloaddition reaction between a dehydrated/cyclised degradation product of 3-deoxy-2-hexosulose and a decarboxylated ferulic acid. In summary, ferulic acid was reported to influence Maillard chemistry, a key mechanism of flavour generation.

Introduction

With the increasing scientific evidence supporting a positive connection between the consumption of whole grain foods and health promotion, the food industry has responded by reformulating traditionally refined flour based products into 'whole grain' foods. Although these whole grain products have a perceived health benefit among consumers, changes in the flavour properties can be viewed negatively and ultimately influence product choice. For example, Bakke and Vickers (1) reported people preferred refined bread to whole grain bread when both were made with equivalent ingredients (refined versus whole grain flour). The Maillard reaction is well known to be a key mechanism of flavour development in cereal based foods. We have previously reported that the hydroxycinnamic acids suppressed the generation of Maillard-type aroma compounds in a hard red spring whole wheat bread model system (2). In the current study, we investigate the reactivity of ferulic acid, the most abundant phenolic in wheat, on the mechanisms of the Maillard reaction to suggest how these food phenolics may alter flavour development of a whole grain food in comparison to a refined flour-based product.

Experimental

Model reaction systems. Five reaction mixtures, (a) glucose + glycine, (b) glucose + glycine + ferulic acid, (c) 1:1 $^{13}C_6$:$^{12}C_6$ Glucose + Glycine + Ferulic acid (d) Glucose + 1:1 $^{13}C_2$,^{15}N:$^{13}C_2$,^{14}N Gly + Ferulic acid and (e) 3-deoxy-2-hexosulose + ferulic acid; each at 3 mmol (except 3-deoxy-2-hexosulose was at 3 µmol) were heated in 15 g

quartz sand with 10% moisture for 15 min at 200ºC. The reaction mixtures were extracted with methanol, further purified and concentrated by solid phase extraction for further analysis.

Liquid Chromatography/Mass Spectrometry (LC/MS). Samples were analysed in electrospray ionization (ESI) mode on a Shimadzu HPLC system (Shimadzu, Columbia, MD) equipped with a degasser (DGU-14A), two pumps (LC-10ADvp), an autosampler (SIL-10vp), and a Waters column heater (model TCM; Waters, Milford, MA) with an altra aqueous C18 column (particle size 5 µm, 250 x 2 mm I.D.; Restek, Bellefonte, PA) interfaced to a Quattro Micro triple quadrupole mass spectrometer (Waters) as previously described (3). In brevity, the mobile phase consisted of a linear concentration gradient of two solvents (A and B). Solvent A was 0.1% formic acid and solvent B was methanol. The mobile phase consisted of a series of linear gradients of B in A starting at 10% B in A (0-2 min), increasing to 90% B in A (2-30 min), and then held at 90% for 5 min (30-35 min).

Nuclear Magnetic Resonance (NMR). The isolate was further fractionated by LC-MS (instrument configuration was the same as described above) utilizing the FractionLynx software (Waters Corp.) and a Waters Fraction Collector (III). The purified target analyte was freeze dried, dissolved in deuterated methanol and analysed by a Bruker DRX-400 NMR. The chemical shifts (δ values) were referenced to the ^{1}H or ^{13}C chemical shifts of the internal standard trimethylsilane. ^{1}H, ^{13}C, gradient selected ^{1}H-^{1}H correlation spectroscopy (COSY), gradient selected heteronuclear multiple quantum coherence (HMQC), and gradient selected heteronuclear multiple bond coherence (HMBC) spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C. ^{1}H-^{1}H COSY, HMBC, and HMQC two-dimensional (2D) NMR techniques were used to assign correlations between ^{1}H and ^{13}C signals.

Results

Food phenolics, such as the flavan-3-ols, have been previously reported to alter the mechanisms of Maillard-type flavour development in model and food systems (*3-8*). Based on a similar analytical approach utilized for the flavan-3-ols, the reactivity of hydroxycinnamic acids were investigated in simple glucose-glycine Maillard model systems. Figure 1 (top) illustrates the MS spectrum for an analyte with the predicted molecular weight of 276 that was only detected when ferulic acid was added to a glucose-glycine model reaction. To further define the composition of this reaction product, a CAMOLA reaction (9) where glucose was added as a 1:1 $^{12}C_6$ to $^{13}C_6$ glucose mixture as well as a AMMOLA reaction (5), glycine added as a 1:1 $^{13}C_2$,^{15}N:$^{13}C_2$,^{14}N glycine, were subsequently analysed. An MW+6 isotopomer for this compound with a MW of 276 was reported for the CAMOLA reaction (see Fig. 1 bottom) whereas no isotopomers were identified for the AMMOLA reaction. This indicated this unknown compound consisted of an intact C_6 sugar moiety but nothing from glycine. Because it is unlikely the C_6 sugar moiety would have a molecular weight of 276, a ferulic acid moiety was consisted a component of this analyte. Further fractionation/purification and NMR analysis of this unknown analyte revealed the molecular structure which is shown in Figure 2, or 6-(4-hydroxy-3-methoxyphenyl)-5-(hydroxymethyl)-8-oxabicyclo[3.2.1]-oct-3-en-2-one. Based on retrosynthesis, the suggested pathway for the formation for this compound is illustrated in Figure 3. The Maillard reaction product, 3-deoxy-2-hexosulose, was proposed to generate an oxypyrylium zwitterion by dehydration and cyclization which reacted with decarboxylated ferulic acid, by a less common [5+2] cycloaddition. For a

[5+2] cycloaddition reaction, a more electron rich dienophile would be more reactive (*i.e.* decarboxylated ferulic acid, 2-methoxy-4-vinylphenol). This reaction product could be easily generated by directly reacting 3-deoxy-2-hexosulose with either ferulic acid or 2-methoxy-4-vinylphenol. Interestingly, this cyclo-adduct compound was not generated by reacting glucose and ferulic acid but required glycine, suggesting only negligible amounts of 3-deoxy-2-hexosulose were generated via caramelisation reactions in these model systems.

The phenolic-Maillard interactions identified provide a new food chemistry reaction that may explain, in part, the lower flavour preference associated with whole grain foods. The negative flavour properties of whole grain foods have been previously related to the bitter taste properties of the phenolic compounds and perhaps due to increased generation of lipid oxidation products. However, phenolic induced changes in Maillard-type flavour development may also influence product acceptability. Additionally, the cyclo-adduct generated by our model phenolic-Maillard reactions (Figure 2) has been previously identified as a potential bioactive in ancient Chinese medicine thought to promote blood circulation (11). Related Maillard-phenolic reaction products may provide further insights into health benefits of whole grain foods and likewise strategies for process optimization of the product quality.

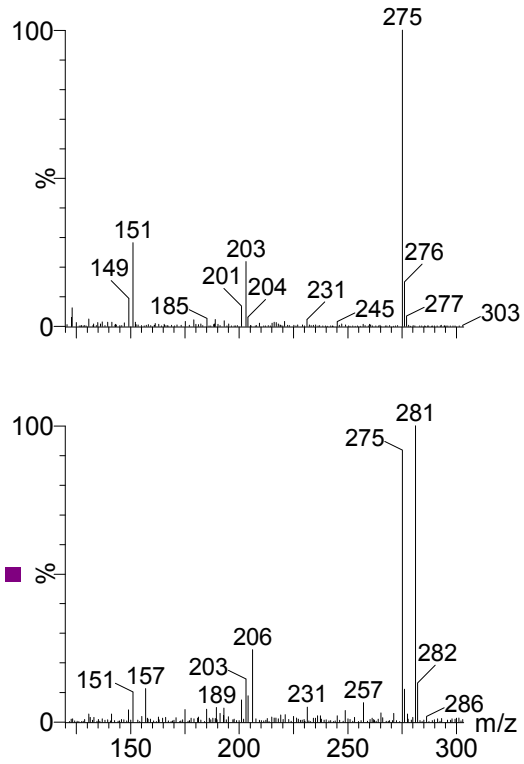

Figure 1. *LC/MS-ESI (-ve ion mode) measured isotopomers of a select analyte (M-1, 275) from glucose + glycine + ferulic acid (top) and 1:1 $^{13}C_6$:$^{12}C_6$ glucose + glycine + ferulic acid (bottom); at equivalent retention time.*

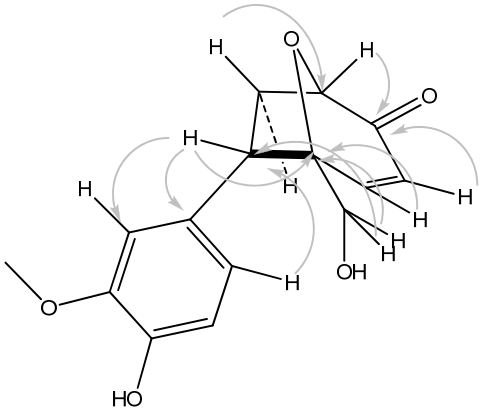

Figure 2. *Stucture of ferulic acid-Maillard adduct, M-1 of 275; illustrating significant HMBC correlations (→).*

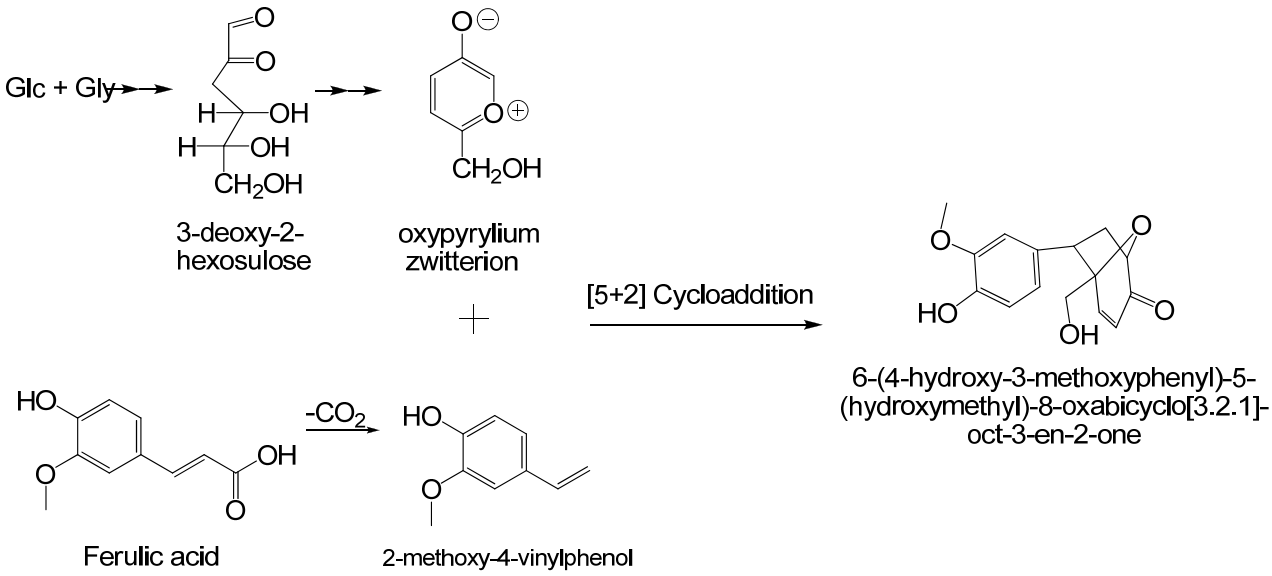

Figure 3. *Proposed reaction mechanism for a select reaction product generated in a ferulic acid-Maillard model system; adapted from (10)*

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