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Research Article

Vinylogous Amadori rearrangement: Implications in food and biological systems

Varoujan A. Yaylayan and Carolina P. Locas

Department of Food Science and Agricultural Chemistry, McGill University, Quebec, Canada

The 4-hydroxy-alkenals are important lipid peroxidation products and are known to play a major role both in the development of degenerative diseases in biological systems and off-flavors, or rancidity in food systems. The 4-hydroxy-alkenals can also be formed in nonlipid systems from 2-deoxy-sugar moieties such as 2-deoxy-ribose. FTIR spectroscopic evidence was provided for such a transformation catalyzed by amino acids through monitoring the decrease in intensity of the aldehydic band centered at 1716 cm^{-1} of the open form of 2-deoxy-ribose and increase in the intensity of the formed conjugated aldehydic band centered at 1672 cm^{-1}. Furthermore, 4-hydroxy-alkenals can react with nitrogen nucleophiles such as amino acids and proteins to form Schiff base adducts that are able to undergo vinylogous Amadori rearrangement (vARP) and subsequently cyclize to generate a pyrrole moiety. This cyclization is prevented in the case of secondary amino acids such as proline to form a stable vinylogous Amadori rearrangement product (vARP). Monitoring this reaction of proline with 4-hydroxy-2-nonenal (HNE) has indicated that within 15 min at 28°C the 1685 cm^{-1} band of HNE completely disappears and that at 50°C, vARP is formed within 5 min, as indicated by the formation of a characteristic band at 1709 cm^{-1}.

Keywords: Amadori rearrangement / 2-Deoxy-sugars / FTIR / 4-Hydroxy-2-alkenals / Protein damage

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1 Introduction

In the past two decades, nonenzymatic molecular transformations in living systems occurring outside the framework of normal metabolic processes and generating toxic by-products have gained considerable importance [1, 2]. The so called "Amadori rearrangement" (AR) of reducing sugars [3] is one of several such transformations that include Pictet–Spengler (P–S) condensation, Michael addition of sulfur nucleophiles to 4-hydroxy-2-alkenals – important lipid oxidation products (see Fig. 1), and modifications of biomolecules with oxygen centered free radicals. Interestingly, all these nonenzymatic transformations have their counterparts in food systems [4]. AR initiates the well known Maillard reaction sequence responsible for the desirable aromas and colors in food and similarly, it is also responsible for the glycation of many biologically relevant proteins that leads to the pathogenesis of different age-related disorders [5, 6]. This rearrangement of reducing sugars can occur with any amino acid without any structural restrictions. On the other hand, P–S condensation (see Fig. 1), is specific to β-arylethylamines such as L-DOPA (levodopa), histamine, tyrosine, tryptamine, dopamine, tryptophan, *etc*. and similar to AR, is initiated by a carbonyl–amine interaction and formation of Schiff base. This reaction was discovered around the same time as the Maillard reaction. Factors that govern the competition between the two reactions were studied by Manini *et al*. [7]. Glycation reactions of reducing sugars through AR leads to the formation of a wide range of chemical structures including acyclic, monocyclic, fused bicyclic, and other potentially toxic compounds that can inflict irreparable damage to biological systems [5]. On the other hand, P–S condensations can generate mainly two moieties, tetrahydroisoquinoline and β-carboline. The drug L-DOPA, extensively used to treat Parkinson's disease, eventually induces dyskinesias in the patients after prolonged administration due to the generation of neurotoxic tetrahydroisoquinoline derivatives through P–S condensation (see Fig. 1). Similarly, the amino acid tryptophan can generate β-carboline derivatives both in food and biological systems [8]. These compounds have been demonstrated to

Correspondence: Professor Varoujan A. Yaylayan, Department of Food Science and Agricultural Chemistry, McGill University, 21111 Lakeshore, Ste. Anne de Bellevue, Quebec, Canada
E-mail: varoujan.yaylayan@mcgill.ca
Fax: +1-514-398-7977

Abbreviations: AR, Amadori rearrangement; **HNE,** 4-hydroxy-2-nonenal; **P–S,** Pictet–Spengler; **vARP,** vinylogous Amadori rearrangement product

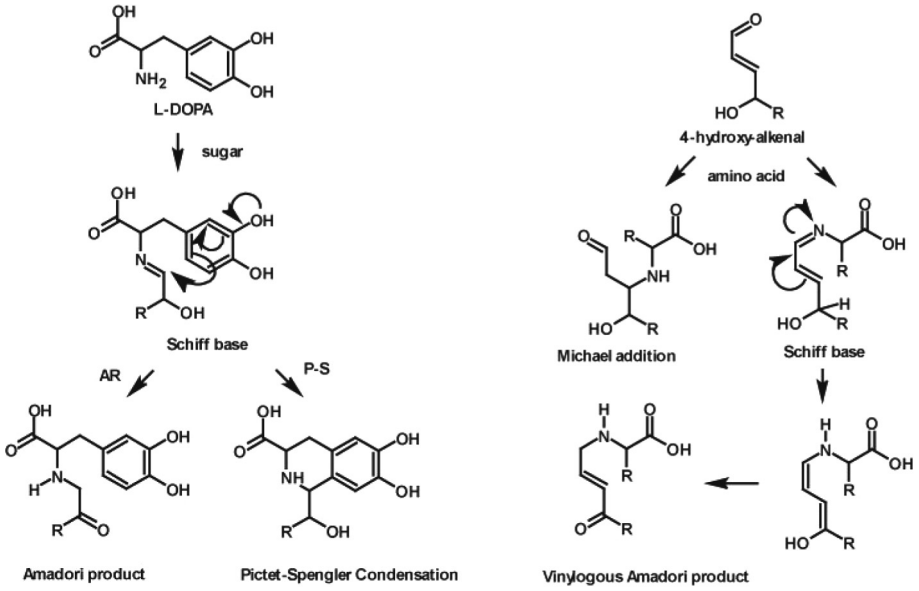

Figure 1. Comparison of Amadori rearrangement (AR) with Pictet – Spengler (P – S) condensation and Michael addition with vinylogous Amadori rearrangement.

possess antioxidant activities and to inhibit platelet aggregation, monoamine oxidase, and binding to benzodiazepine receptors.

In addition to reducing sugars, 4-hydroxy-2-alkenals are also known to undergo metabolic side-reactions with amino acids (see Fig. 1). They are generated through lipid oxidation [9] and are considered to be toxic intermediates due to their reaction with amino acids and proteins. Although 4-hydroxy-alkenals can undergo both Michael addition and Schiff base formation with amino acids, the latter can lead to the formation of vinylogous Amadori rearrangement product (vARP). This pathway has not been characterized in detail. This report provides spectroscopic evidence for such rearrangement and explores the possible impact in food and biological systems.

2 Materials and methods

All reagents and chemicals were purchased from Aldrich (Milwaukee, WI) and used without further purification. HNE (in ethanol) was purchased from Cayman Chemical Co. (Ann Arbor Michigan).

2.1 Sample preparation

All samples were dissolved in ethanol (total volume 10 μL) at a concentration of 10 mg/mL. Reaction mixtures were equimolar.

2.2 FTIR analysis

Infrared spectra were recorded on a Nicolet 8210 spectrometer equipped with a temperature controlled single-bounce ATR sample accessory. A total of 64 scans at 4 cm^{-1} resolution were added together. Processing of the FTIR data was performed using GRAMS/32 AI (ThermoGalactic) software. Second-order derivatization was performed using Savitsky – Golay function (30 points) to enhance closely absorbing peaks. Peak assignments were performed according to the standard procedures [10].

2.3 Temperature studies

Sample solutions (10 μL) were placed on the ATR crystal and the solvent was allowed to evaporate before data acquisition at the specified temperature and/or time. The time of the initial mixing of the reactants was considered as time zero. Infrared spectra were recorded as described in Section 2.2.

3 Results and discussion

Similar to biological glycation and Maillard reactions in food, oxidative degradation of PUFAs and subsequent formation of lipid peroxides are known to play a major role both in the development of degenerative diseases in biological systems [11] and off-flavors or rancidity in food systems [12]. In addition, many of the dicarbonyl intermediates produced by Maillard reaction, such as glyoxal and methylglyoxal, have also been shown to be generated from lipid oxidation [9]. Not only the products generated by lipid oxidation and Maillard reactions are similar in structure, but some, such as 4-hydroxy-2-alkenals and 4,5-epoxy-2-alkenals can also undergo Strecker-type degradation [13, 14]. These alkenals result from the subsequent homolytic cleavages of PUFA hydroperoxides, catalyzed by transition

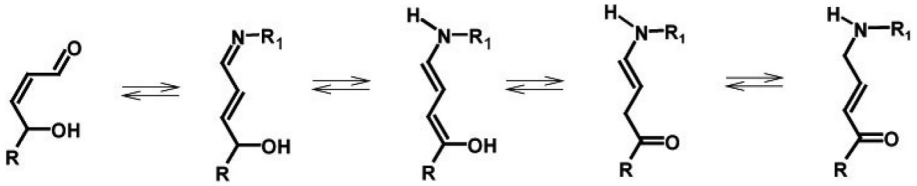

Figure 2. Vinylogous Amadori rearrangement of 4-hydroxy-2-alkenal moiety with amino acids. R_1 = −CH(R)COOH.

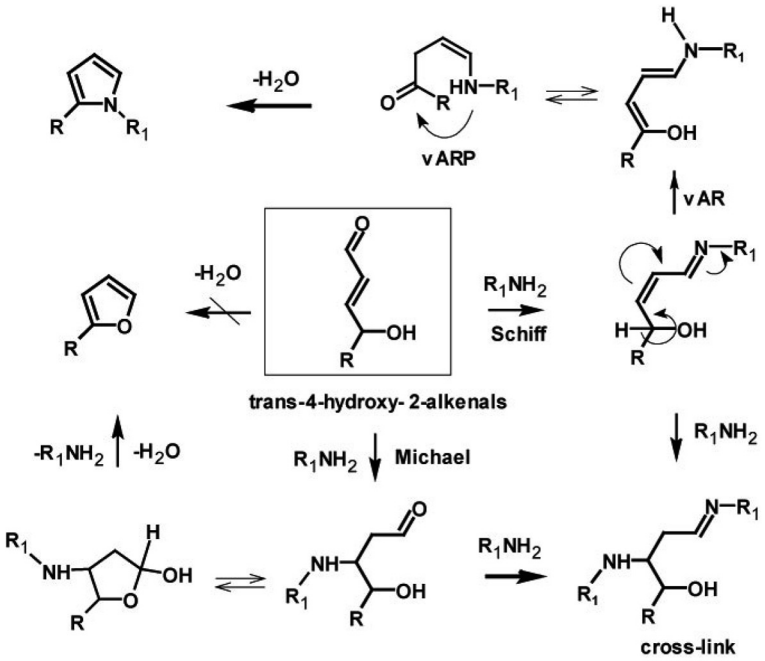

Figure 3. Various reactions of 4-hydroxy-2-alkenals with amino acids. vARP, vinylogous Amadori rearrangement product; vAR, vinylogous Amadori rearrangement.

metal ions [11]. Similar to reducing sugars, these highly cytotoxic 4-hydroxy-2-alkenals, such as 4-hydroxy-2-nonenal (HNE), are capable of modification of proteins, DNA, and LDL through either Michael addition and/or Schiff base formation which can undergo vinylogous AR (see Fig. 2).

3.1 FTIR monitoring of HNE reaction with amino acid

The reactions of HNE with amino acids and proteins have been investigated extensively. The type of product formed, whether Schiff base or Michael adduct, is dependent on the reaction conditions as well as on the properties of the nucleophile [15]. For example, 2,4-dinitrophenylhydrazine has been shown to react exclusively *via* Schiff base to form 2,4-dinitrophenylhydrazone with a strong yellow color that absorbs at λ_{max} 360−380 nm [16]. On the other hand, sulfur nucleophiles (glutathione, cysteine, *etc*) are known to react with HNE exclusively through Michael reaction [15]. The Michael adducts are usually in equilibrium with their corresponding acetal forms as shown in Fig. 3. HNE can react with different amino acids at different rates, sequentially forming first a Schiff base followed by Michael adduct or forming Michael adduct first followed by Schiff base generating identical crosslink structure (see Fig. 3).

Although the sequence of the addition of amino acids on to HNE will make no difference in the structure of the crosslink, however, the initial Michael adduct is prone to acetal formation due to the disruption of the *trans* geometry that prevents cyclization and makes it much more stable than the corresponding *cis* isomer [16]. The acetal can undergo dehydration and deamination to produce 2-pentylfuran, a product that has been identified mainly in food systems. On the other hand, the initial Schiff adduct can undergo vinylogous AR, followed by cyclization to produce N-substituted 2-pentypyrrole as shown in Fig. 5. Such pyrroles were isolated from HNE and primary amine reaction mixtures and characterized by independent synthesis [17]. Furthermore, immunochemical evidence supporting the formation of 2-pentylpyrrole on proteins exposed to HNE was also later provided by Sayre *et al*. [18]. Sayre *et al*. [17] have proposed vinylogous AR as a mechanism for the formation of 2-pentylpyrroles from the interaction of HNE with amines, without naming it as such. However, Wondrak *et al.* [19] characterized the reaction mechanism that leads to the formation of pyrroles as a vinylogous AR in amino acid/2-deoxy-ribose model system. Consequently, it can be concluded that, vARPs can only be isolated when the product is prevented from cyclization to form pyrroles. Hidalgo *et al.* [14] inadvertently, isolated such a product (*m*/*z* 277 in Fig. 4) in the model system of phenylalanine and HNE.

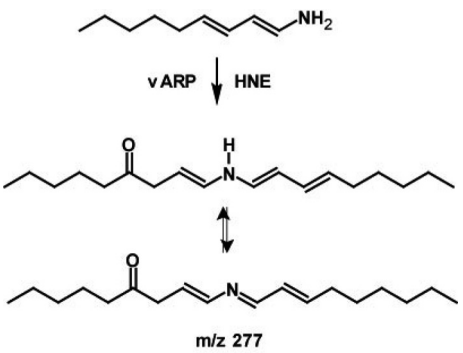

Figure 4. Vinylogous Amadori rearrangement (vAR) of nonadiene-amine with HNE (based on [14]).

Although the authors have provided mass spectral data consistent with our proposed structure shown in Fig. 4, they did not characterize the structure as a vARP. Due to the isomerization of the double bonds in the structure of the amine (a degradation product in the model system), the ability of the amine to undergo nucleophilic attack and form a pyrrole ring is considerably diminished and hence the vARP survives in the reaction mixture to be detected by GC/MS. Furthermore, they have also identified the vARP of HNE with phenylalanine itself, after its decarboxylation and cyclization into a pyrrole moiety. Again, the authors have not characterized the product as arising from a vinylogous AR. On the other hand, the reaction of proline with HNE should also produce relatively stable vARP due to the formation of a tertiary amine unable to cyclize into a pyrrole ring and this reaction can be conveniently monitored by FTIR. Figure 5 shows the FTIR spectrum of HNE and Fig. 6 shows time dependant spectra of HNE incubated with proline at 28°C. According to this figure, the carbonyl band disappears within 15 min at 28°C forming the Schiff base. When temperature was increased to 50°C, a new carbonyl band appeared at 1708 cm^{-1} consistent with the formation of vARP (see Fig. 7).

3.2 Precursors of 4-hydroxy-2-alkenal moieties in nonlipid systems: 2-Deoxy-sugars

The main precursor of 4-hydroxy-alkenals in nonlipid systems is the 2-deoxy-sugar moiety that can be formed during Maillard reaction through several pathways [20] and eventually can be dehydrated to produce 4-hydroxy-2-alkenal (see Fig. 8). Hydrolysis of DNA can also provide 2-deoxyribose as a precursor. Studies performed on model systems using pyrolysis-GC/MS analysis and ^{13}C-labeled sugars and amino acids [20] have indicated that certain amino acids such as serine and cysteine can degrade and produce acetaldehyde and glycolaldehyde that can undergo aldol condensation to produce 2-deoxy-aldotetrose moiety. Other amino acids such as aspartic acid, threonine and α-alanine can degrade and produce only acetaldehyde and thus need sugars as a source of glycolaldehyde to generate similar 2-deoxy-sugars. On the other hand, monosaccharides are also known to undergo degradation to produce both acetal-

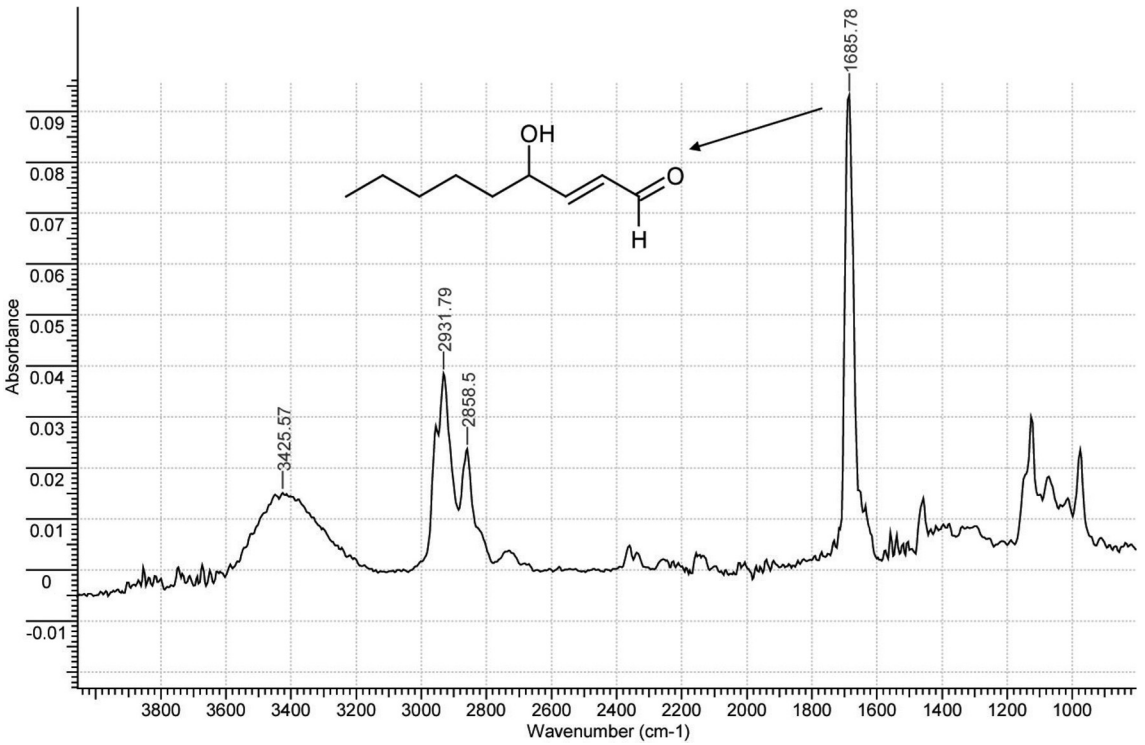

Figure 5. FTIR spectrum of HNE.

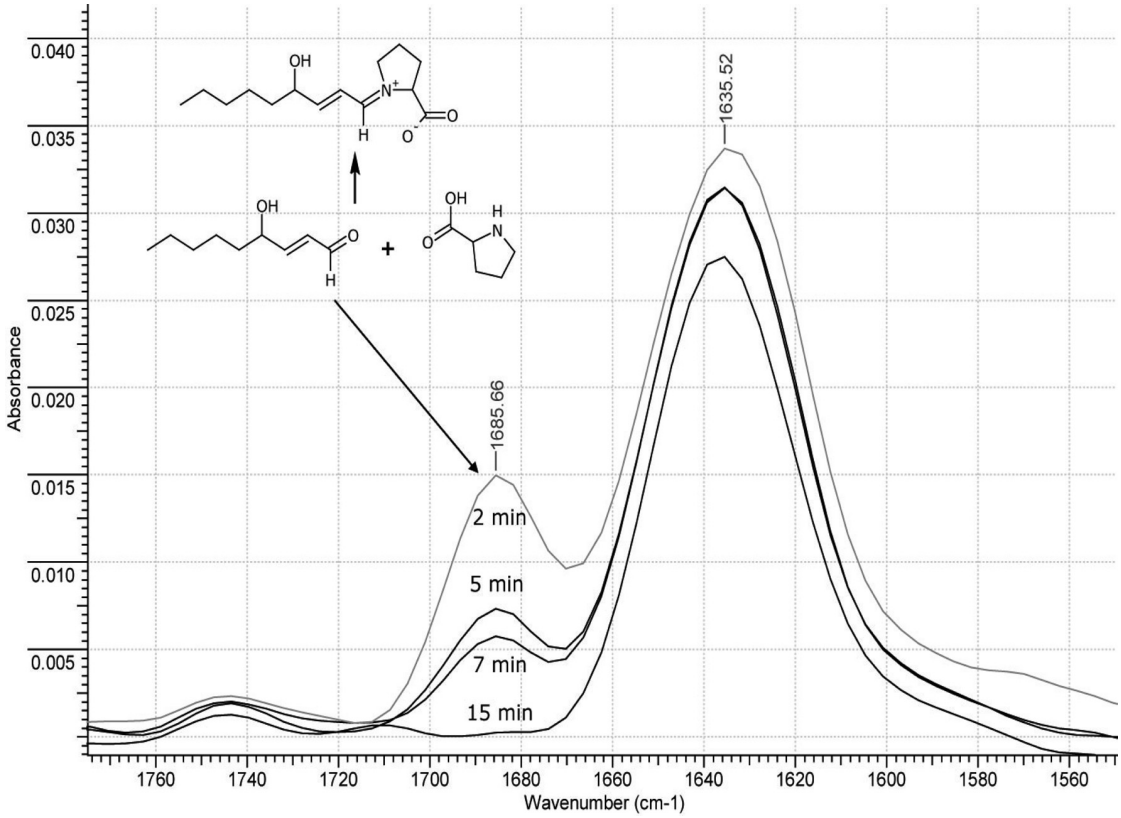

Figure 6. Time-dependent spectra of HNE incubated with proline at 28°C.

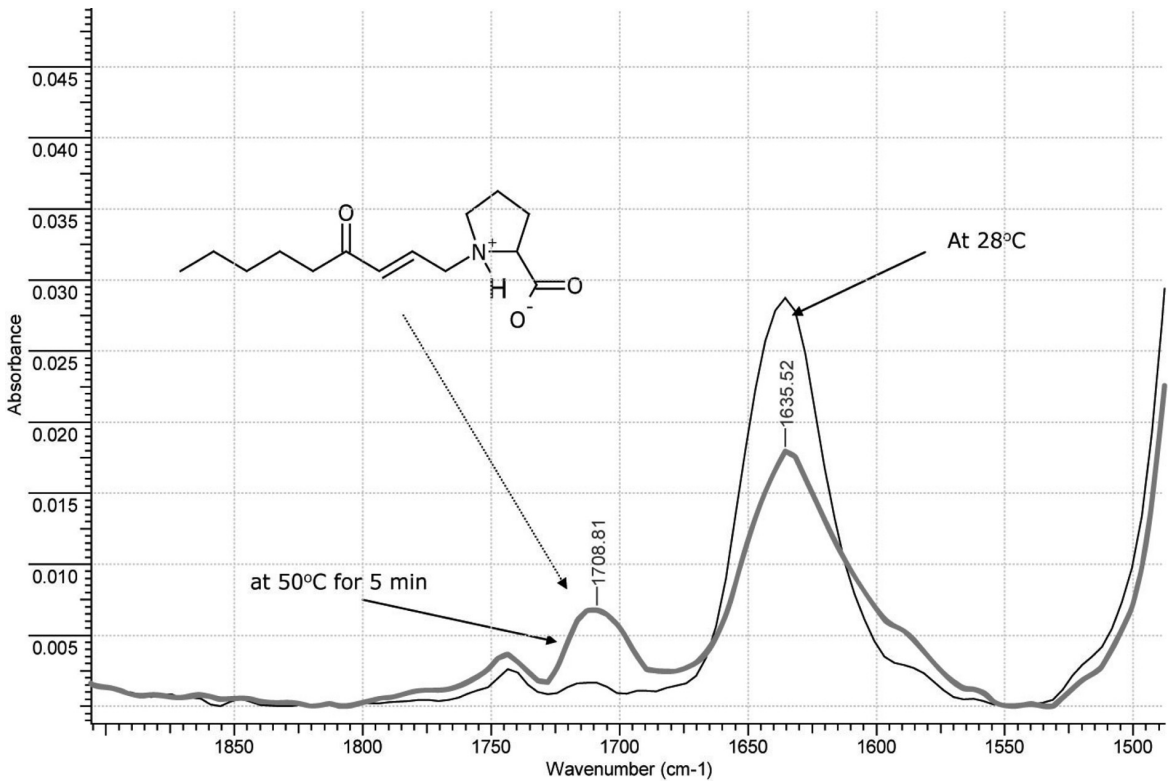

Figure 7. FTIR spectrum of HNE incubated with proline for 5 min at 50°C.

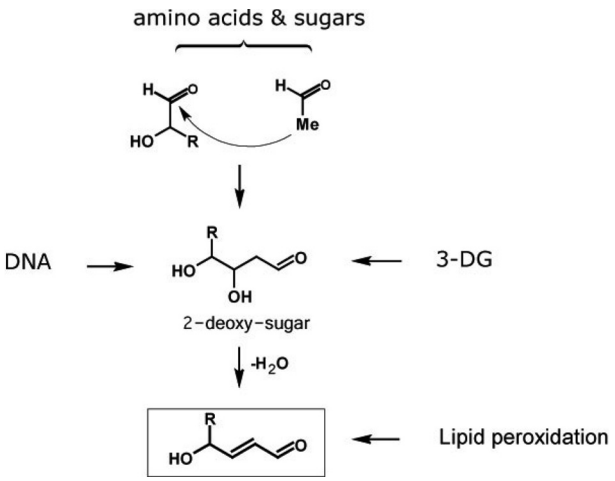

Figure 8. Origin of 4-hydroxy-2-alkenal moiety in nonlipid systems. 3-DG = 3-deoxy-glucosone. (2-deoxy-sugars include 2-deoxy-ribose from DNA, 2-deoxy-glucose from 3-deoxy-glucosone and 2-deoxy-tetrose from amino acid and sugar degradation).

dehyde and glycolaldehyde, in addition to 2-deoxy-sugar moieties. Studies performed using ^{13}C-labeling have also revealed that glucose can degrade through the formation of 3-deoxy-glucosone (3-DG) and produce 2-deoxy-aldotetrose moiety incorporating the C2–C3–C4–C5 carbon chain of glucose. To provide evidence in support of the formation of 4-hydroxy-2-alkenal from 2-deoxy-sugars, 2-deoxy-ribose was selected as a model using Fourier transform infrared spectroscopy (FTIR) to monitor the dehydration reaction. Such transformations of an isolated aldehyde into a conjugated system can be easily detected due to a shift of the carbonyl absorption band from 1716 cm^{-1} (open form 2-deoxy-ribose) to a lower frequency such as 1686 cm^{-1} as in HNE. When 2-deoxy-ribose was incubated at different temperatures in the presence of proline or 5-amino valeric acid in methanol and the carbonyl region was monitored by FTIR, the intensity of the band centered at 1716 cm^{-1} diminished over time and new band appeared around 1672 cm^{-1}, indicating the formation of a conjugated system (see Fig. 9). Such amino acid-catalyzed dehydration of 2-deoxy-ribose into 4-hydroxy-2-pentenal had also been observed previously by Nelsestuen [21]. Furthermore, the second derivative spectrum of proline model system (Fig. 10) also indicated the presence of an absorption band centered at 1708 cm^{-1} indicating the formation of a stable vARP.

4 Concluding remarks

Reactive 4-hydroxy-2-alkenals can be generated not only in lipid containing systems but also through the dehydration

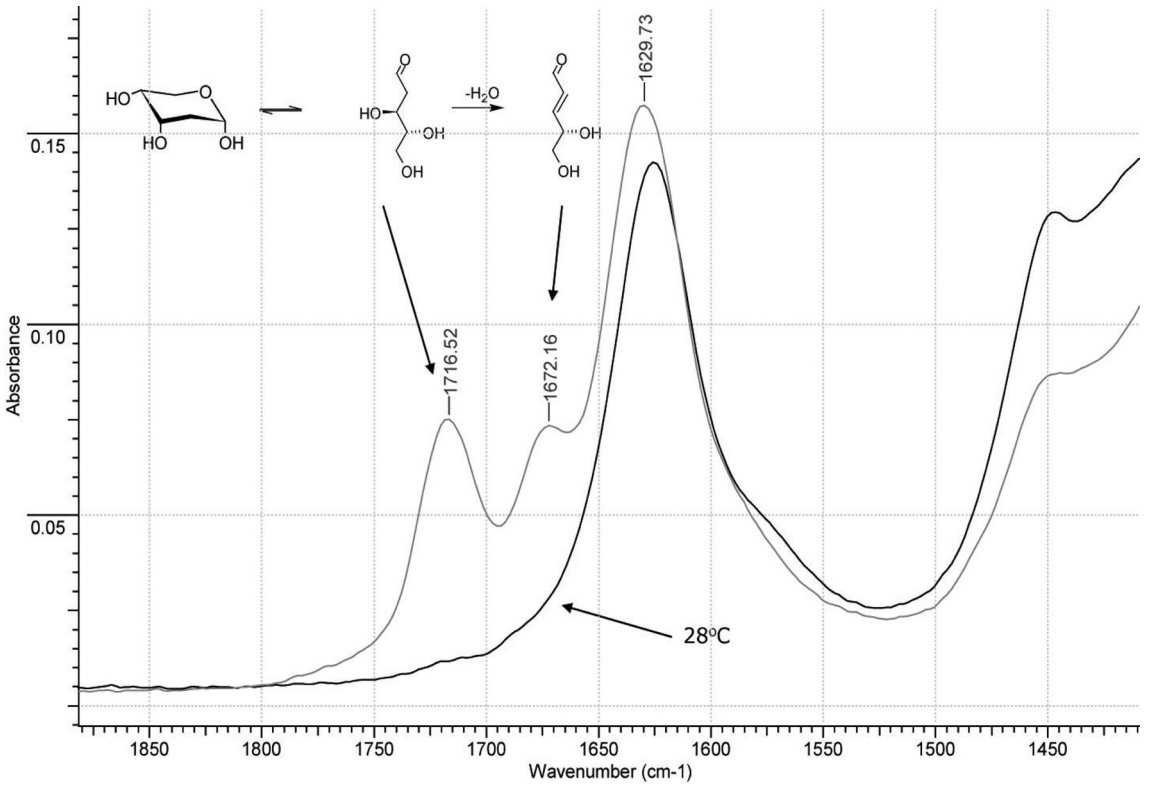

Figure 9. FTIR spectrum of 2-deoxy-ribose incubated with proline for 15 min at 50°C.

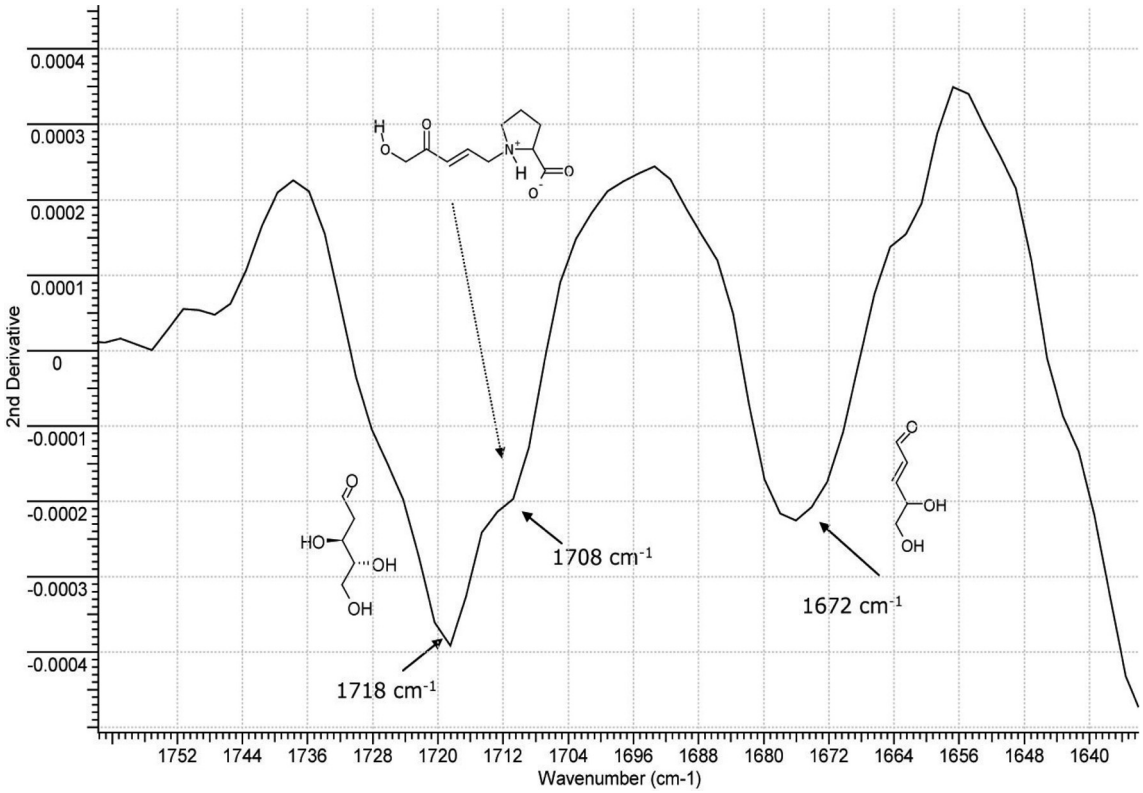

Figure 10. Second derivative spectrum of 2-deoxy-ribose incubated with proline for 15 min at 50°C.

of 2-deoxy-sugars. Most likely, the latter pathway will generate a *cis*/*trans* mixture. The *cis* isomers can readily cyclize into furans as recently detected in different food systems [22]. On the other hand, the *trans* isomer is more prone to react with different biological nucleophiles and form crosslinks or pyrrole moieties through vinylogous AR, causing the accumulation of considerable damage on important body proteins.

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