Antioxidation Capacity of Maillard Systems with Carbonyl Products of Sugar Fragmentation

K. CEJPEK, L. JAROLÍMOVÁ and J. VELÍŠEK*

*Department of Food Chemistry and Analysis, Institute of Chemical Technology, Prague, Czech Republic, *E-mail: cejpekk@vscht.cz*

Abstract: A role of reactive C_2-C_5 α-dicarbonyl and α-hydroxycarbonyl products of sugar fragmentation in the development of antioxidative activity (AOA) was investigated after heating in 0.5M binary aqueous mixtures with amino acids. Several kinetic and activity parameters related to the development of reducing power in the systems with different carbonyl fragments as well as glucose were evaluated and compared. The formation of electrochemically active compounds was correlated with colour development. To assess the antioxidation effects more properly, several different methods were used for AOA evaluation of the systems tested in addition to the HPLC-ECD method. Tests with scavenging of radicals or assays evaluating oxidation in different media (hydrophobic, hydrophilic, emulsions) were employed. Biacetyl was found as by far the most powerful precursor of reducing activity among the fragments tested. The major products possessing reducing power were isolated and characterized revealing dihydroxy dimethylbenzene structures. Based on the comparison of the same concentrations of BHA and a parent carbonyl compound, AOA found by different methods in the most powerful systems is fully comparable with BHA efficiency.

Keywords: Maillard reaction; α-dicarbonyl compounds; α-hydroxycarbonyl compounds; antioxidants

INTRODUCTION

Maillard reaction is a synoptic name for a complex set of parallel and consecutive reactions of a mixture of carbonyl and amino compounds, which occur during thermal treatment and storage of food. As a result of the reactions, numerous coloured, flavour-active, health-promoting as well as harmful products are formed, so that a quality of many foodstuffs is significantly affected. The Maillard reaction is known as an efficient source of reactive α-dicarbonyl and α-hydroxycarbonyl products of sugar fragmentation, such as glyoxal, methylglyoxal glycolaldehyde and biacetyl. These intermediates, known to form Maillard reaction products with higher rates than parent hexoses and pentoses, are among the key intermediates of the Maillard reaction. They are formed during retroaldolization and/or β-elimination, sometimes after preceding oxidation of parent sugars, (deoxy)glycosuloses, glycosamines and Amadori rearrangement products [1].

Numerous heated Maillard reaction systems have been reported to affect the oxidative stability of foodstuffs. Several studies demonstrated that various α-dicarbonyl and α-hydroxycarbonyl compounds formed from sugars undergo more intense formation of products with antioxidative activity than the parent saccharides [2]. The exact nature of the antioxidants formed is not yet well known, but multiple mechanisms are supposed. The techniques used for the assessment of AOA comprise many methods including direct evaluation of radical scavenging efficiency and redox potential of the prospective antioxidants and measurements of changes in the oxidation rate of lipids in the presence of active compounds [3].

The experimental work was focused on the assessment of the ability of important α-dicarbonyl and α-hydroxycarbonyl products of sugar fragmentation to form antioxidants using several methods for the determination of antioxidative capacity.

EXPERIMENTAL

Model systems. Aqueous binary equimolar 0.5M mixtures of glyoxal (Glx), methylglyoxal (Meglx), biacetyl (Biac), pentane-2,3-dione, glycolaldehyde (Gla), hydroxyacetone, glyceraldehyde, acetoin and glucose (Glc), and valine (Val) were heated for 10 min 40 h in closed system at 95°C. Analogous reaction mixtures of Biac with other amino acids were prepared. Reaction systems containing insoluble melanoidins were filtered (0.45 μm), the filtrates were ultrafiltered (1 kDa). Non-fractionated mixtures and fractions of insoluble pigments were dissolved in MeOH or buffer of pH 10 just before analysis.

HPLC analyses. Reaction systems and fractions were separated (Atlantis C_{18}, 150 × 3.9 mm × 3 μm, Waters) with binary gradient elution (pH 6.5/MeCN with 5mM NaCl (for ECD) or 10 mM ammonium formiate/MeCN (for MS), f = 0.7 ml/min) using electrochemical detector (ECD 2465, flow-cell 3 mm Gold WE, Hy-Ref, 0.29 μl, Waters), photodiode array detector (PDA 996, Waters) and mass spectrometer (Q-TOF – ESI, APCI, Micromass).

AOA analyses. The fractions prepared were dissolved (or dispersed) in lard. A vessel with the sample was put into the Oxipres apparatus with oxygen pressure of 0.5 MPa at 100°C. Changes in the pressure were recorded, induction period (IP) was read from the curve obtained and protection factor (PF) was calculated. 2(3)-*tert*-Butyl-4-hydroxyanisole (BHA) was used as a reference. DPPH method and β-carotene-linoleate assay were performed after the procedures described elsewhere [8, 9].

RESULTS AND DISCUSSION

The reaction of α-dicarbonyls with α-amino acids is primarily a series of oxidative decarboxylation reactions known as Strecker degradation in which amino acid is oxidized to aldehyde with one less carbon atom than parent amino acid while the molecule of α-dicarbonyl is reduced to α-hydroxycarbonyl or its amino analogue. Identical intermediates (i.e. α-aminoketones) to those of Strecker degradation of amino acid with corresponding α-dicarbonyl compounds are produced also by Amadori and Heyns rearrangements of free ammonia with α-hydroxycarbonyl compounds. Analogous reactions with amino acids give decarboxylated α-(amino acid)-carbonyl compounds. Subsequent transformations of the reactive intermediates yield a variety of mainly heterocyclic reaction products [4]. The competitive aldolization, autooxidation and Cannizzaro reaction of some α-dicarbonyl compounds also take place. The α-aminocarbonyl derivatives seems to be involved very intensely in the formation of Maillard reaction products, while the other transformation routes of a carbonyl without participation of an amino acid give only poor yields of active compounds. For example, the electrochemical activity (EA) of heated Biac solution amounts only about 2% compared to the system of Biac with Val prepared under the same conditions.

The ability of several C_2-C_5 α-dicarbonyl and α-hydroxycarbonyl fragments as well as Glc to form antioxidants was investigated separately in binary 0.5 M aqueous mixtures with amino compound, chiefly valine, heated at 95°C without pH control. The transformation rate of carbonyls and Glc in binary systems with valine decreased in the order Gla ($t_{1/2}$ = 9 min) > Meglx (14 min) > Biac (20 min) > Glx (25 min) >> Glc (290 min). All the investigated systems inclusive of Glc containing models and their particular fractions are sources of many compounds possessing reduction properties. The products appeared after a relatively short induction time, for example, the period prior to reduction power development in Biac, Glx and Glc systems with Val took 30, 45 and 90 min of heating, respectively. The ratio of initial rates for the activity formation was 9:4:1.

Comparing the reducing activity of the carbonyls tested, Biac is by far the best precursor of reductive compounds; its efficiency is by a factor 10 higher than the others. After 8 h of heating, relative electrochemical activity to BHA response under conditions used (REA_{BHA}) was 259% for Biac-derived products and 22% (acetoin) – 39% (Meglx) for the other carbonyls. α-Dicarbonyl fragments are usually presented to be more active in formation of EA compounds than reducing sugars [2]. These observations reflect the slower rate of the transformation of reducing saccharides in general. While the reduction power has reached its maximum around 6–10 h of heating for the mixtures containing carbonyl fragments and Val, the EA of Glc system has been rising over the whole observation time (40 h) and surpassed the EA of Meglx models after approx. 20 h of heating. The reducing power of Glc system is by factor of 1.4 higher compared to that of Meglx and is still rising in 40 h of heating.

In contrast to the other carbonyl systems, the shapes of total EA development in early stages of Glx and Glc containing model systems are similar, including the temporary detection of reducing power in initial stage of the reaction. This activity has been generally assigned to the formation of free radicals (e.g.,1,4-dialkylpyrazinium radicals) [5], but other factors can be also involved. The curvilinear similarity invokes formation of the same types of products responsible for the early reducing activity in both Glx and Glc systems. However, the evaluation of HPLC-ECD and HPLC-PDA data revealed quite different chromatographic profiles and UV-VIS characteristics of EA compounds. The presence of Biac together with some other fragments in the mixture results in more intense formation of EA compounds, with the major products spectrally similar to the principal products of binary mixture of Biac and Val. Besides Biac containing binary model, it is evident, that higher reducing capacity comes from more complex systems with variety of carbonyl precursors.

Comparing the ability of the fragments to form coloured products, glycolaldehyde, in contrast to its reducing power activity, revealed the highest colour activity, as presumed [6]. The relative initial rates of colour development were in the ratio of 50 (Gla):30 (Glx):20 (Meglx):3 (Biac):1 (Glc) in the progressive phase. While most of the fragments achieve roughly the same level of colour intensity (A_{420}) during 40 h of heating, biacetyl is a weak colour precursor being able to produce only about 35% of colour comparing to the other three fragments in binary model systems. Colour formation precedes the reducing power development in the Glx, Gla as well as Meglx systems, while in the Glc systems, the induction periods of the two Maillard attributes take nearly the same time. Therefore the early colour formation cannot indicate the presence of reducing products in the systems of carbonyl fragments. On the other hand, the EA developments follow the colour intensity curve very closely after two hours of heating.

The use of HPLC with PDA and ECD allows the finding of factual products responsible for colour formation or reducing power. Several dominant products with significant EA from Biac and Glx systems were characterized with UV-VIS and MS spectra and molecular weights were assigned. For example, the structure of the principal electrochemically active compound (~ 40% activity) as well as chromophore of the non-polar low-molecular fraction of Glx-Val reaction system was proposed as a derivative of pyridooxazinone. The principal heat-induced reducing products of Biac systems were formed independently on amino acid (peptide) structure and were present in most binary systems tested with exceptions of cysteine and tyrosine containing models [7]. Electrochemical and spectral characteristics of the products were determined and their identification as dihydroxyderivatives of dimethylbenzene was performed after isolation. For example, in Biac – Val (0.5M, 8 h, 95°C), two of them possessing the highest response were responsible for 87% of electrochemical activity with the yield about 4% (mol/mol Biac). The study of all particular fractions revealed that the overall EA was focused in low-molecular fraction. For example, more than 98% of EA in Biac system was caused by small products (< 1 kDa). The principal low-molecular end-products are generally stable and do not overly enter further reactions including oxidation.

To find out the rate of redox reactions in particular environment, additional methods for AOA evaluation were applied in addition to the electrochemical method. Three other methods, including antiradical activity determination by DPPH (in aqueous media), β-carotene-linoleate assay (in emulsion) and determination of protection factors in a lipidic matrix using Oxipres method, were employed for the assessment of AOA in some reaction systems, fractions and isolated 2,5-dimethylhydroquinone (DMHQ). Relatively high AOA was found for the reaction mixtures, fractions as well as isolated products also in these redox systems. Total soluble and low-molecular fractions of the studied systems show practically the same antioxidative activity using any method. Less polar insoluble products participate more significantly than solubles on AOA in emulsions using β-carotene bleaching method, as indicate, for example, 40% *vs.* 14% of the activity of BHA in the two fractions of Val – Biac reaction mixture. Some other systems, for example, the products of histidine and Biac, show even higher activity than Val-derived products in emulsions and aqueous solution using β-carotene bleaching and DPPH radical method, respectively, but not in the Oxipres test. Pure DMHQ has only a slightly lower antiradical activity comparing to BHA both in emulsions and aqueous media, namely 77% and 88%, respectively.

Owing to different mechanisms of action and various reaction media of the method used, some-

what different results were obtained in some cases. Our experiments revealed the differences in AOA found by particular method for different systems reflecting different character and localization of the active compounds from various reaction mixtures or fractions. Moreover, the time dependent developments of AOA measured by the methods affecting also prooxidative activity do not follow the reducing power growth until α-dicarbonyl precursors with prooxidative character disappears from the mixture.

CONCLUSIONS

Considering that formation of the fragments precedes reducing power development in the glucose systems with amino compounds and sufficiently fast utilization of the fragments in the formation of reducing products occurs, it can be stated that the products of sugar fragmentation play an important role in reducing power development during the Maillard transformation of saccharides. Biacetyl was found as the key precursor of compounds with AOA among products of the fragmentation of saccharides. The principal Biac-derived active products are nitrogen-free derivatives of dihydroxy dimethylbenzene. Glc comprises larger potential to form reducing compounds than fragments studied in fact (with the exception of Biac), but formation of the products is much slower compared to the fragments in the systems with amino compounds.

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