Factors Affecting the Formation of Reducing Compounds from Biacetyl

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Abstract: Antioxidative properties go with principal attributes of the Maillard reaction. Biacetyl, a reactive carbonyl intermediate formed in the initial stage of sugar transformations in the presence of amino acids, was found as an efficient precursor of several compounds with significant reducing activity which can act as antioxidants in real food systems. In this work, simple model systems of biacetyl were investigated under different conditions (amino compound involved, concentration, concentration ratio, reaction time, temperature and pH) to gain maximum yield of reducing power. Distribution of reducing power in different fractions of the reaction mixtures characterised by polarity and molecular size was evaluated. The fractionation procedure and HPLC separation step was developed to enable isolation of the major reducing products. Their characterisation was accomplished by use of photodiode-array (UV-VIS spectra) and electrochemical detection (half-wave potentials). The structures of the isolated products having reducing activity were identified by series of MS measurements and by comparison with synthesised standards.

Keywords: biacetyl; antioxidative properties; Maillard reaction; electrochemical detection

INTRODUCTION

Maillard reaction belongs to the most important chemical processes that take place during the storage and processing of foods. Primary reaction is the addition of an amino compound (protein, amino acid or amine) to the carbonyl group of a reducing saccharide. A number of reactive carbonyl and aminocarbonyl compounds are formed during the Maillard reaction. They are transformed in several parallel steps to give low-molecular and high-molecular weight final products, namely the brown pigments (melanoidins), aromatic compounds and substances with antioxidant effect. To study the Maillard reaction, more simple systems than foods are used, mainly model systems containing only one carbonyl and one amino compound.

Inhibitors of oxidation (antioxidants) are compounds that extend the durability of foods by protecting them from spoiling caused by oxidation [1]. The antioxidant characteristics of the products of the Maillard reaction are highly dependent on physical and chemical properties of the system. Main factors affecting the antioxidant activity are type, ratio and concentration of reactants and initial pH value, temperature and time of heating. Products formed from dicarbonyl sugars fragments posses higher antioxidant activity then products of sugars [2]. The influence of amino acids on antioxidative ability is not described uniformly in the literature, however, it is assumed that it grows in the order acidic < neutral < basic amino acids [3]. The environment with higher initial pH is more favourable to form melanoidins with antioxidant activity.

A number of compounds was identified in the reaction systems of biacetyl with amino acids. These products include derivates of hydroquinone, oxazole, pyrazine, furanone, quinoxaline, pyrrole or pyridine [4].

This work resumes previous experiments focused on the antioxidant effects of the products arising in binary systems containing a product of sugar fragmentation (glyoxal, methylglyoxal, biacetyl, glycolaldehyde) and valine. Biacetyl in a binary mixture with valine is by far the best precursor among the fragments tested (13–17 times) [5]. The effect of reaction parameters (pH, temperature, time of reaction, type and concentration of reactants) on the extent of formation of the substances with antioxidant activity was monitored in systems with biacetyl. The most significant electrochemically active compounds were separated in the investigated models using HPLC methods and were characterised by use of photodiode-array and electrochemical detection.

EXPERIMENTAL

Model systems. Reaction mixtures of biacetyl (Fluka) with various amino acids were prepared in 25 ml of demineralised water or buffer with pH value of 3, 5, 7, 9 or 11. Reaction mixtures of biacetyl with valine were heated according to the monitored parameter for 0 min, 20 min, 40 min, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h, 32 h or 40 h at the temperature of 23, 55, 75 or 95°C in a closed system. To evaluate the influence of the present amino acid on the antioxidative properties of reaction system, 0.5 M of the prepared biacetyl with amino acid mixtures were heated in water for 8 h at 95°C. The prepared reaction mixtures were filtered and the filtrates were fractionated with ultrafiltration (cellulose membranes, 1000 Da). The ultrafiltrates were extracted with diethyl ether.

HPLC method. The prepared reactionary mixtures were analysed (Atlantis C_{18}, 150 × 3.9 mm × 3 μm) by the binary gradient elution (phosphate buffer pH 6.5/acetonitril with 5 mM NaCl, f = 0.7 ml/min) with serial connection of the detector of the fotodiode field (PDA, 996, Waters) and the electrochemical detector (ECD, 2465, Waters).

GC-MS method. GC/MS analysis was carried out on an Agilent Technologies 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a Series 5973 quadrupole mass selective detector Agilent 5973 MSD (70 eV) and data processing system (MSD ChemStation, G1701CA version C.00.00). Gas chromatography was performed on a capillary column HP-5 (30 m × 0.25 mm i.d., 0.25 μm thickness, Hewlett-Packard). The injector was held at 260°C (splittles), the column temperature was programmed from 50°C (2 min) to 280°C (7 min) at a rate of 8°C/min. Helium at a flow rate of 0.7 ml/min was used as the carrier gas, 1 μl sample was injected.

RESULTS AND DISCUSSION

The measurement of electrochemical activity of the fractions and the whole reaction mixture by means of the HPLC-ECD method (E_C = +0.8 V) was chosen for the assessment of antioxidant capacity of the binary model systems of biacetyl with amino acids. All possible primary antioxidants with reducing ability should be oxidised at this working potential [6].

The effect of reaction conditions on the electrochemical activity was monitored in the biacetyl-valine model system. First, the effect of different values of the initial pH of the reaction mixture after 8 hours at 95°C was evaluated. The total electrochemical activity of buffered reaction mixtures grows with increasing value of the pH. Slightly lower activity in the non-buffered system can be explained by the absence of phosphate anions with catalytic effect on the Maillard reaction [7]. However, when use final pH value of reaction system for this dependence, which is more relevant for the reaction than initial pH, the effect of the buffer anions is not significant.

The temperature used in the preparation of reaction mixture is another important parameter affecting the production of the active compounds. During 8 hours of heating of half molar binary mixture, the development of electrochemical activity is qualified by the temperature higher than 55°C.

The growth of total electrochemical activity of reaction mixtures of biacetyl-valine in water at 95°C was monitored for 0–40 h. The fast development of reducing activity takes approximately the first 8 h of the heating at 95°C with transformation of 90% of biacetyl in the first hour and more than 99% after 8 h of heating.

Electrochemical activity of the biacetyl-valine system also depends on the concentration of the reactants. The results of monitoring the activity of three equimolare systems of biacetyl-valine with different concentrations of the starting compounds (0.5M, 0.05M, 0.005M) indicate the linear decrease of electrochemical activity together with decreasing concentration of the starting compounds. The total electrochemical activity of 0.05M system came to 9.2% of the electrochemical activity of 0.5M reaction mixtures.

Distribution of electrochemical activity of the biacetyl-valine mixture into the fractions (total reactionary mixture, filtrate and ultrafiltrate) shows that electrochemical activity of the reaction mix-

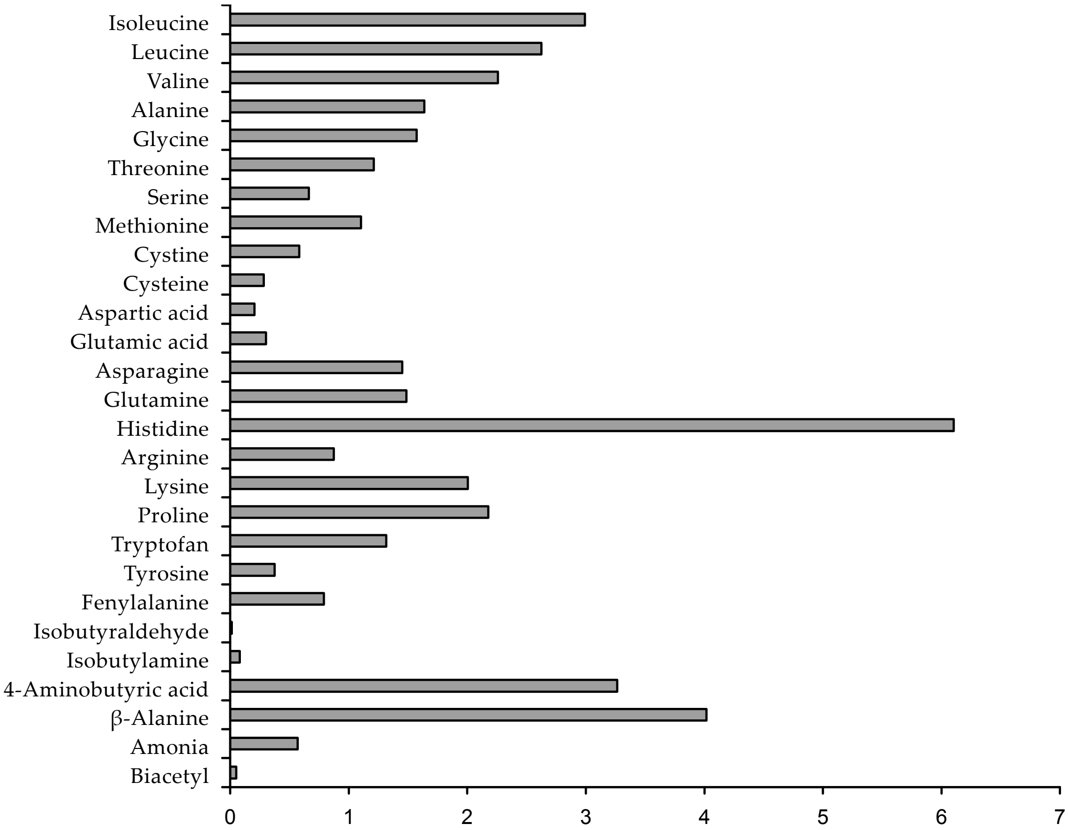

Figure 1. Effect of a reaction partner on electrochemical activity of systems with biacetyl (0.5M, 8 h, 95°C)

ture is almost entirely (< 98%) concentrated in low-molecular compounds (< 1000 Da). The chromatographic profile of the total biacetyl-valine reaction mixture is qualitatively almost identical with the chromatographic profile of filtrate and ultrafiltrate. It differs in presence of non-polar compounds with $t_R > 20$ min, which form less than 3% of the total electrochemical activity.

The effect of a particular amino acid on formation of electrochemically active substances in the presence of biacetyl was monitored in filtrate of the 0.5M mixture of an amino acid and biacetyl in water, which were heated for 8 hours at 95°C (Figure 1). When the solution of only biacetyl in water was heated (0.5M, 8 h, 95°C), the total electrochemical activity came to 2% of the electrochemical activity of the reaction mixture of biacetyl-valine (0.5M, 8 h, 95°C). The total electrochemical activity of unsubstituted aliphatic amino acids including valine grows with the increasing hydrophobicity of amino acids in the order glycine < alanine < valine < leucine < isoleucine. The total electrochemical activity of the system of biacetyl with histidine surpasses the activities of all the other reaction mixtures (it is 2.7 times higher than the activity of the system of biacetyl-valine). Almost no electrochemical activity was found in the systems containing cysteine and tyrosine. During the experiment, the influence not only of α-amino acids but also β- and γ-amino acids (β-alanine, 4-aminobutyric acid) on the generation of electrochemically active substances in reaction with biacetyl was found. Electrochemical activity of the system of biacetyl-β-alanine is 2.5 times higher than that of the mixture of biacetyl with α-alanine. The same principal products are arising in reactionaty mixtures of biacetyl with the most amino acid.

Two main active products were found to be responsible for 87% of the antioxidant activity in the system of biacetyl-valine (0.5M, 8 h, 95°C). They were spectrally characterised by means of the HPLC method with the PDA and ECD detection. Further, the value of their half-wave potential (0.63 V, 0.71 V, respectively) and their molecular weight (both 138 Da) was measured. The compounds were identified by the comparison with the synthesised standards as 2,5-dimethyl-1,4-hydroxybenzene and 4,5-dimethyl-1,2-dihydroxybenzene.

Results obtained analysing the mixtures of biacetyl with various amino acids show that the amino acid serves as a catalyst by the generation of compounds with electrochemical activities.

CONCLUSION

Optimum conditions for the generation of electrochemically active substances from biacetyl-amino acid mixture in aqueous environment were determined. The compounds with significant reducing abilities were identified by fractionation and separation of reaction mixtures. They are dimethylderivates of dihydroxybenzene formed under conditions in most of the tested amino acids used.

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