Hydroxypyridine Formation in Model System Monosodium Glutamate and 2-Fufural

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Abstract

Monosodium salt of glutamic acid has been approved in Europe as a food additive E 621. It enhances the effect of other taste-active compounds, improving the overall taste of certain foods. There is also an interaction between E 621 and table salt (sodium chloride), and other umami substances such as nucleotides. The aim of the present study was to model a Maillard-type reaction of E 621 with carbohydrates during food processing and to analyze the products of this interaction. Formation of the N-containing heterocyclic compounds as hydroxypyridines, imidazole, pyrazine and pyrazinone is a common feature of the Maillard reaction taking place at temperatures typical for food processing. The formation of 3-hydroxypyridine was observed as reaction product between E 621 and furfural during prolonged but not during short-time heating. The target compound was identified as 3-hydroxypyridine using reverse phase HPLC. The concentration of 3-hydroxypyridine in the reaction mixture was 1 ppm calculated by means of regression analysis. Possible mechanism of this reaction is discussed.

Key words: Maillard reaction, Monosodium salt of glutamic acid, food additive, 3-Hydroxypyridine, HPLC

Introduction

The Maillard reaction was named after the French chemist Louis Maillard (Maillard, L. C. 1912) and comprises an interaction between reducing sugars and amino acids or proteins. The reaction is of great importance for food chemistry and has been thoroughly investigated. During food processing, Maillard-type reactions result in development of colour and flavour components, for example via Strecker degradation of amino acids in the presence of sugar-derived dicarbonyl compounds (Ledl F. and Schleicher E, 1990).

More recently, interactions between glucose and proteins have received significant attention due to their medical implications. There is substantial evidence that these processes contribute to pathophysiological changes associated with diabetes and arteriosclerosis (R. Bucala, H. Vlassara, and A. Cerami, 1992). More over, Maillard-type reactions are considered to be involved in biological aging (H. Vlassara, R. Bucala, and L. Striker, 1994).

The Maillard reaction is typically divided into three main stages. The first initial stage is a nucleophilic attack of an amino group on carbonyl group of a reducing sugar followed by a loss of water to form Schiff base. Further rearrangement leads to formation of N-glycosylamine (a sugar attached to NR₂ group) called Amadori product. The next intermediate stage involves rearrangement and decomposition of the Amadori product to release amino compound and sugar fragmentation. The final stage of the Maillard reaction involves dehydration, fragmentation, polymerization and cyclisation reactions Van den, Ouweland, et. al, 1978; Mouron, J. 1981; Hurrent, R. F. 1982). General scheme of the Maillard reaction is shown in Figure 1 (Van Boekel, M. A. J. S. 2006).

Qualitative and quantitative analysis (Meynier, A. And Mottram, D.S. 1995) indicates that the Maillard reaction products are strongly dependant on pH. Low pH favours dehydration of pentoses and formation of 2-furfural but its concentration decreases as the pH increases. Nitrogen-containing compounds, such as pyridines pyrazines were detected at higher pH, with the lysine model system producing the largest quantities of nitrogen-containing heterocyclic compounds. The pH of the system plays a crucial role in the Maillard reaction (Milic, B.L. and Piletic M.V. 1984; Bernis-Young, G.L.; Haung J.; 1993), because both carbonyl and amine groups tend to attach a proton in acidic medium. On the other hand, the degree of
protonation of these groups is critical because the initial step of non-enzymatic browning is a nucleophilic attack. The higher degree of protonation, the lower nucleophilic power of the amine group (M. Argirova, et. al, 1999).

Furfural is derived from pentoses destruction in the Maillard reaction. Since furfural is a reactive carbonyl compound it may be expected to take an active part in the formation of coloured high molecular weight final products of the Maillard reaction called melanoids. The similarity between the products obtained from D-xylose and glycine (Tan, T.L., et. al, 1950), and furfural and glycine on the other hand, has been shown by elemental analysis, UV, and IR spectroscopy. Based on the similarity between reductones and the products of the Stenhouse reaction, (Nomura, D, 1954) has discussed the interaction between furfural and aniline as a model of nonenzymatic browning. (Haas et. al, 1948) have found that furfural is formed in a concentrate of dried apricots. The removal of furfural by continuous extraction with ethylacetate strongly decreases the rate of browning and vice versa – addition of furfural increases the rate.

Monosodium salt of glutamic acid (monosodium glutamate, MSG) has been approved in Europe as a food additive E621. It enhances the effect of other taste-active compounds, improving the overall taste of certain foods. There is also an interaction between MSG and table salt (sodium chloride), and other umami substances such as nucleotides. With these properties, MSG can be used to reduce salt intake, which predisposes to hypertension, heart diseases and stroke. MSG has been used for more than 100 years to season food. During this period, extensive studies were conducted to elucidate the role, benefits and safety of MSG. At this point, international and national bodies for the safety of food additives consider MSG safe for human consumption as a flavour enhancer (Ronald Walker and John R. Lupien, 2000), although some sides of MSG application are still subjects of public debate (http://www.glutamate.org/).

When MSG is added before thermal processing of particular food, it is plausible to suppose that it can react with carbohydrates or products of carbohydrate degradation in a Maillard-type reaction. A careful literature survey showed a lack of specific research in this direction. Glutamic acid along with other naturally occurring amino acids has been used to obtain a new class of umami test enhancers (Thomas Hofmann, et. al, 2003). Whether such compounds could be formed during thermal processing of foods containing MSG as a food additive so far is not clear. Therefore, the aim of the present study was to model a Maillard-type reaction of MSG with carbohydrates during food processing and to analyze the products of this interaction.
Experimental Materials
Furfural, L-glutamic acid, and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich; NH$_4$HCO$_3$, MeOH (p.a.) and 3-hydroxypyridine were purchased from Merck.

Synthesis
Furfural (25 mmol) and L-glutamic acid (50 mmol) were dissolved in 50% EtOH (40 ml). Concentrated solution of NaOH was added dropwise to the reactant solution until pH 9.4 was achieved. The reaction mixture was refluxed for either 6 or 48 h at 90 °C. In a parallel experiment the same reaction mixture in a closed vial was heated in a thermostat at 90 °C for 48 h as described by Hofmann T. et al, (2003).

Ion-exchange chromatography
Anion exchange separation was carried out on QAE - Sephadex A-50 (40-120 µ) stationary phase packed in a glass column (30x300 mm). Reaction mixture (2 ml) was applied on the top of column and eluted with 100 mM NH$_4$HCO$_3$. Five-ml fractions were collected and the UV-VIS spectrum of each fraction was scanned. Fractions having spectrum similar to that of 3-hydroxypyridine (Figure 2) were further analyzed using HPLC.

HPLC analysis
Reverse phase HPLC (RP-HPLC) was used for qualitative and quantitative analysis of the products of the reaction between MSG and 2-furfural. HPLC instrumental configuration consisted of a quaternary mixer at low pressure Smartline Manager 5000, high-pressure pump Smartline 1000, Photodiode Array Detector PDA 2800 (Knauer, Germany), and a fluorescent detector RF-10A xl (Knauer, Germany). The column used was Purospher 8-star RP-C18 25 cm x 4.6 mm i.d., 5 µm particle size (Merck, Germany).

The composition of the mobile phases was: phase A = 0.1% TFA in water and phase B = MeOH : H$_2$O = 80:20. A gradient elution was used for the separation of components: 0 min 100% A; 2 min 100% A; 10 min 79% A; 15 min. 0% A; 23 min. 0% A.

Results
The reaction between MSG and 2-furfural was accompanied by intensive browning of the reaction mixture after 6-h heating and led to variety of substances as evidenced by the HPLC profile (Figure 3). Some of them possess fluorescence (ex. 290 nm/ em. 390 nm) that is typical for hydroxypyridinium derivatives (Argirov, et. al, 2004)
Figure 3. Typical chromatogram of crude reaction mixture obtained from the reaction between MSG and 2-furfural refluxed for 6 h. Violet line – fluorescent detection (ex. 290 nm/em. 390 nm); blue line – UV detection at 299 nm; green line – UV detection at 254 nm; red line – UV detection at 330 nm.

Figure 4. Typical chromatogram of crude reaction mixture obtained from the reaction between MSG and 2-furfural refluxed for 48 h. Violet line – fluorescent detection (ex. 290 nm/em. 390 nm); blue lines – UV detection at 299 nm; green line – UV detection at 254 nm; red line – UV detection at 330 nm.

In order to identify the compound with retention time 8.07 min an initial purification of the crude product was carried out with anionic ion-exchange chromatography. The target compound was identified as 3-hydroxypyridine since its retention time was identical with that of the authentic 3-hydroxypyridine and co-eluted with it (Figure 5A, B). Both, the standard 3-hydroxypyridine and that obtained from the reaction of 2-furfural and MSG had identical UV spectra.
Peak area of authentic 3-hydroxypyridine standard solutions over the concentration range 1 – 25 ppm was used to quantify 3-hydroxypyridine in the reaction mixture obtained from the reaction of 2-furfural and MSG. The concentration of 3-hydroxypyridine in the reaction mixture was 1 ppm calculated by means of regression analysis.

Discussion
Formation of the N-containing heterocyclic compounds as hydroxypyridines, imidazole, pyrazine and pyrazinone is a common feature of the Maillard reaction taking place at temperatures typical for food processing. Several pyridine derivatives have been isolated and identified. Formation of 3-hydroxypyridinium betaines during the Maillard reaction has been firstly reported by (Klinger et al, 2013). (Koch et al, 1998) have found a formation of pyridinium betaines by reaction of hexoses with primary amines obtained during typical food processing and under physiological conditions. Argirov et. al, (2004, 2003)
have described the formation of lysine-3-hydroxy-β-pyridinium derivatives as post-translational modifications of the lysine side chain of proteins taking place during aging of human lens. Horvath et al. (2007, 2010) have isolated two new Maillard reaction products, containing 3-hydroxy-β-pyridinium and 3-hydroxy-picolinic acid moiety and have discussed the mechanism of their formation.

Therefore, we hypothesized that MSG could form hydroxy-substituted pyridine-like N-heterocycles with carbohydrates during food processing and analysed a model system containing MSG and 2-furfural (dehydration product of pentoses) under moderate heating (90 °C) for different periods of time (6 h and 48 h). Here we report the formation of 3-hydroxypyridine as a product of the reaction between MSG and furfural during prolonged but not during short-time heating. Interestingly, this product contains only the amino acid nitrogen but not the carbon skeleton of MSG.

It is plausible to propose that the reaction cascade starts with the condensation of 2-furfural with free amino group of MSG to form a Schiff base, which then undergoes rearrangement to the Amadori product. Further hydrolysis of the aldimine linkage yields 1-(furan-2-yl)methanamine and 2-oxopentanedioic acid (ketoglutaric acid); most likely the latter participate further in the Maillard reaction already as a carbonyl component. A possible mechanism of 3-hydroxypyridine formation is shown in Scheme 1.

![Scheme 1](image_url)

Figure 6. Proposed mechanism of 3-hydroxypyridine formation

In aerobic conditions 1-(furan-2-yl)methanamine is oxidized with ring opening to (2Z)-5-amino-4-oxopent-2-enal (Yueh-Hsiung Kuo, Kae-Shyang Shih., 1991). Further dehydration and cyclization of this dicarbonyl compound yield 3-hydroxypyridine. The presence of oxidizing step in the overall scheme is supported by the fact that only traces of 3-hydroxypyridine were formed during the reaction with limited access of oxygen (in a closed vial in thermostat for 48 h), while the levels of 3-oxopyridine were much higher under refluxing for the same period in the presence of oxygen (data not shown).

One distinguishing feature of 3-hydroxypyridine and its derivatives is their ability to act as photosensitizers and to produce reactive oxygen species, namely singlet oxygen, being irradiated with UVA. Such chromophores have been detected in cataractous lenses (Argirov et. al, 2004) and human skin cells (Wondrak et. al 2004). Metabolism of 3-hydroxypyridine is very slow (Kaiser J-P, Bollag J-M. 1991) and it could accumulate in some specific tissues. In living organism the formation of reactive oxygen species and organic free radicals is a key mechanism of cellular photooxidative stress.

The presence of pyridine photosensitizers and generation of active oxygen species in foods exposed to direct sunlight could influence food quality. Singlet oxygen reacts with various types of double bonds and initiates oxidation of unsaturated
fatty acids. Oxidation reactions in food are not limited to the lipid components; oxidized proteins, carbohydrates, and amino acids also influence the perception, safety, and nutrition value of food. The deleterious effect of produced reactive oxygen can be diminished by both, antioxidants naturally presented in food, for example, carotenoids, vitamin C, and phenolic compounds, and the high molecular weigh brown coloured products of the Maillard reaction named melanoidins that are known to act as antioxidants in common foods. Their antioxidative properties were first observed in the early 1950s. It is supposed that the mechanism of melanoidin antioxidant action is related to direct oxygen scavenging, trapping of electrophilic reactive species, radical scavenging, reducing properties of some melanoidin structures, metal chelation, or synergies.

On the other hand 3-hydroxyypyridine and its derivatives have been considered analogous to Vitamin B6. Among other important biological functions, Vitamin B6 possesses good chelating properties. Formation of coordination compounds between hydroxyl substituted pyridine-like N-heterocyclic ligands and Pd (Huq et. al, 2007), Cu (Castillo et. al, 2001), and other transition metal ions have been reported. As a ligand 3-hydroxyypyridine could sequester reductox active metal ions and thus, could act as an antioxidant. The balance between pro-oxidant and anti-oxidant properties of 3-hydroxyypyridine in foods most likely depends on the food nature and storage conditions.

References
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