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Research Article

Formation of 5-hydroxymethyl-2-furfural (HMF) and 5-hydroxymethyl-2-furoic acid during roasting of coffee

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The formation of 5-hydroxymethyl-2-furfural (HMF) and 5-hydroxymethyl-2-furoic acid (HMFA) during roasting of coffee was studied. At 240°C the maximum concentration of HMF occurs after 3 min with a quick degradation up to 10 min when most of the HMF has disappeared again. Similar to 5-hydroxymethyl-furfural, HMFA is formed in coffee but not in a model system consisting of sucrose, alanine with or without chlorogenic acid. It was shown that HMFA is produced from different precursors than HMF namely glyceraldehyde and pyruvate. The comparison of the laboratory scale roasting with industrial roasting showed that 5-hydroxymethyl-furfural decreases with a higher degree of roasting whereas HMFA did not change. In the laboratory scale experiments, the highest concentration of 5-hydroxymethyl-furfural in coffee (909 µg/g) was obtained after 3 min and the maximum concentration of HMFA after 4 min (150 µg/g). Industrially roasted coffee contained up to 350 µg/g 5-hydroxymethyl-furfural and 140 µg/g HMFA.

Keywords: 5-Hydroxymethyl-2-furfural / 5-Hydroxymethyl-2-furoic acid / Coffee / Model system

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1 Introduction

During the roasting of coffee, many substances are formed due to reactions at high temperatures. These can contribute to the taste and aroma and isolated substances are well known for their harmful effects on humans. However, to date there are no conclusive results on whether coffee is beneficial or harmful or has even no effect on the health status of humans, except for the effects resulting from the xanthine alkaloids (caffeine, theobromine).

One of the substances formed is 5-hydroxymethyl-2-furfural (HMF) and the concentration in commercially available roasted coffee is in the range of 0.3–1.9 mg/g [1]. HMF is present in practically every food containing free carbohydrates like cookies with up to 0.08 mg/g [2]. For reasons of adulteration, the legal limit on HMF in honey is 0.04 or 0.08 mg/g if the honey originates from a country with a warmer climate. Depending on the temperature and/or duration of production or storage the concentration of HMF can reach 9.5 mg/g in caramel products as was shown by Bachmann *et al*. [3]. Other foods containing high amounts of HMF are dried fruits with up to 3.5 mg/g [1, 3], balsamic vinegar with up to 3.2 mg/mL [4] and sweet sherry wines with up to 0.84 mg/mL [5]. 5-Hydroxymethyl-2-furoic acid (HMFA) is also found in roasted coffee among other nonvolatile acids [6].

The acute toxicity of HMF is relatively low and the LD_{50} was shown to be 3.1 g/kg bw in rats [7]. The genotoxic and carcinogenic potential was reviewed by Janzowski *et al.* [8] showing that HMF is not a highly dangerous compound. However, recent results on the metabolic activation of HMF show a genotoxic potential of this compound [9–11]. HMF is metabolised in the kidneys to HMFA and other compounds (mainly a glycine conjugate and the dicarboxylic acid) which are then excreted with the urine. This was shown for rats and mice [12] as well as humans [13]. The known biological effects of HMF and HMFA were recently summarised by Glatt and Sommer [10].

In foods, HMF can be formed by different pathways mainly *via* dehydratisation of hexoses in the presence (Maillard reaction) [14] or absence (caramelisation) [15, 16] of amines to 3-deoxy-2-hexosuloses that can

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Abbreviations: HMF, 5-hydroxymethyl-2-furfural; **HMFA,** 5-hydroxymethyl-2-furoic acid

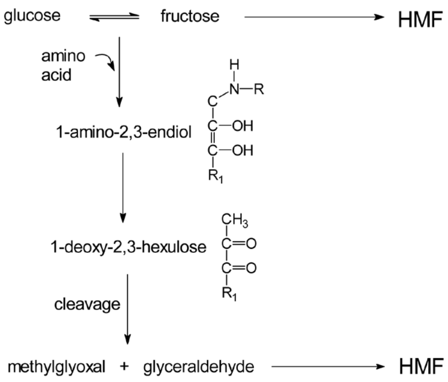

Figure 1. Pathway of the formation of HMF from hexoses (adopted from [17]).

further react to HMF. Another possible pathway goes *via* cleavage of α-dicarbonyl-compounds and recombination of methylglyoxal with glyceraldehyde (Fig. 1) [17].

2 Materials and methods

2.1 Coffee samples

Two different types of green coffee were used for the experiments. One was of the arabica type (Columbia Excelsior Centrals mild – washed) and the other one was an Indian Cherry Robusta. Both samples were purchased from the Berliner Kaffeerösterei (Berlin, Germany). A third type of coffee which was used for comparison of the laboratory scale roasting with a small-scale industrial roasting was an arabica from Brazil which was a gift from Kraft Foods (Bremen, Germany).

2.2 Chemicals

All chemicals were obtained from Fluka (Buchs, Switzerland), except HMFA which was purchased from Matrix Scientific (Columbia, USA). All solvents used were of HPLC grade and purchased from Merck (Darmstadt, Germany). Water was obtained by purification using a Millipore Simplicity 185 system (Molsheim, France).

2.3 Roasting of coffee and model system

The coffee was roasted in a convection home roaster (Heathware Precision Coffee Roaster, Wheeling, IL, USA) with a fixed temperature but different roasting times. The temperature of 200°C was reached after 60 s with a further slow increase to a maximum temperature of 240°C. After roasting the coffee was cooled to room temperature and then stored under nitrogen at –20°C until the analysis.

The model system contained sucrose as the dominant carbohydrate of green coffee and alanine being the dominant amino acid. The two compounds of the model system were dissolved in water and freeze dried to obtain a homogeneous powder which was heated without addition of water. In the case where other compounds were added to the model system (*e.g.* chlorogenic acid) these were given to the solution before freeze drying. Fifty milligrams of this lyophilised powder was heated for 1 min in 1.5 mL glass tubes to 240°C then kept at room temperature for 20 s and cooled on ice. The extraction of the analytes was done with 1 mL of water by vigorous shaking for 5 min and centrifugation for 10 min at 15 000 rpm (Microfuge, Beckmann, Glenrothers, Scotland). The supernatant was then decanted and diluted appropriately with water for the HPLC analysis.

2.4 Analysis of HMF and HMFA

2.4.1 Extraction

Ground coffee (300 mg) was extracted with water in a 2 mL plastic reaction tube by vigorous shaking for 5 min at room temperature. After extraction the mixture was centrifuged for 10 min at 15 000 rpm (Microfuge, Beckmann). The supernatant was then decanted and diluted appropriately with water for the HPLC analysis. The quantitative extraction was tested by repeated extractions using different extraction times and temperatures.

2.4.2 HPLC analysis

The analyses of HMF and HMFA were carried out on a liquid chromatograph Agilent series 1100 (Agilent, Waldbronn, Germany) equipped with a quarternary pump, manual injection valve (Rheodyne, Cotati, CA, USA) and an UV detector. The separation was carried out on a RP column (LiChrosphere 100, RP-18e, 125 × 4 mm^2, 5 μm) with the same material as precolumn (4 × 4 mm^2). HMF and HMFA were eluted with a mixture of water (92%) and methanol (8%) containing 5 mM tetramethyl-ammonium hydrogen sulphate (TMAHS) as ionpairing reagent for the carboxylic acid. The flow was 1 mL/min and the injection volume 15 μL (partial filling of a 50 μL loop). HMF was detected at 280 nm eluting after *ca.* 4.5 min and the HMFA was detected at 255 nm which eluted after *ca.* 5.6 min.

The validation of the analysis was done with software support using VALIDATA (version 3.2, Graz, Austria). For HMF the LOD and LOQ were evaluated as 21 and 73 μg/g, respectively. At a level of 200 μg/g the SD was calculated as 11 μg/g (3.7%). Similar to HMF these parameters were evaluated for HMFA (LOD: 22 μg/g, LOQ: 75 μg/g, SD: 12 μg/g, 3.6%). Both analytes were calibrated in the range of 100–500 μg/g. The recovery was 86% for HMF and 91% for HMFA.

3 Results and discussion

Due to the high concentration of HMF and HMFA in the aqueous extract a clean-up of the samples was not necessary.

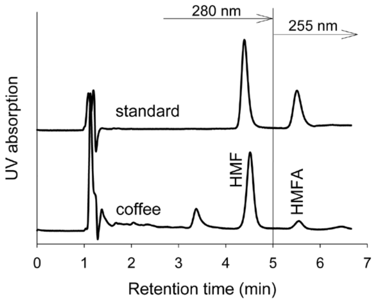

Figure 2. Typical chromatogram of roasted coffee from Brazil showing HMF and HMFA.

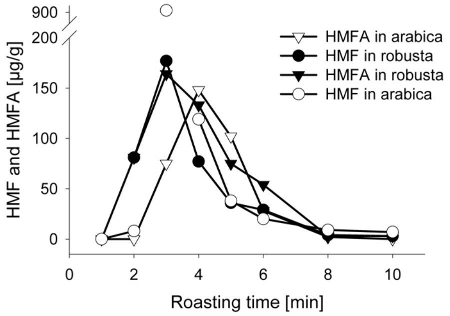

Figure 3. Formation of HMF and HMFA during roasting of coffee in a laboratory scale coffee roaster at 240°C in different types of coffee.

The high dilution with water resulted in a colourless solution of the analytes which could be analysed directly (Fig. 2).

In the laboratory scale coffee roaster a typically roasted coffee is obtained between 4 and 5 min. As can be seen in Fig. 3 the maximum concentration of the two investigated furane derivatives is obtained after 3–4 min. The data shown are mean values of three repeated experiments. The relative variation of the experiments is 5–10%.

In both types of coffee the HMF reached its maximum concentration after 3 min. The extremely high value of HMF (909 µg/g) was repeated in several experiments. However, after 3 min the degradation was rather quick the content of HMF and reached similar levels after 4 min in both types of coffee – arabica and robusta. The levels of HMFA were similar in both types of coffee with a delayed degradation. This would suggest that the acid is formed from the aldehyde by simple oxidation.

Comparison of the roasting process of laboratory scale and industrial scale is difficult since at laboratory scale the roasting process is based on energy input (*i. e.* time–temperature regime) whereas at industrial roasting the process is controlled by colour development. The three degrees of roasting presented here comprise the standard range of coffee quality. When this is compared to the laboratory roasting conditions by measuring the absorption at 420 nm of an ethyl acetate extract this would be equivalent to 4.5–5 min in the small scale roasting device (Fig. 4).

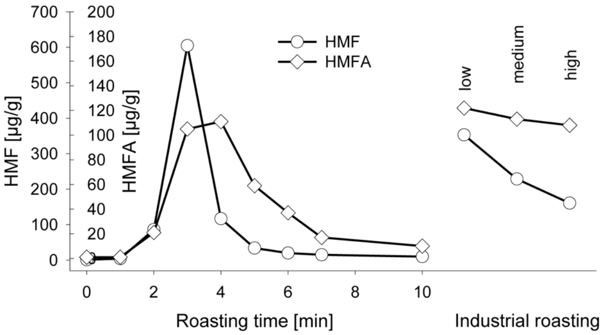

Figure 4. Comparison of HMF and HMFA formation in laboratory scale and industrial scale coffee production (low, medium and high degree of roasting).

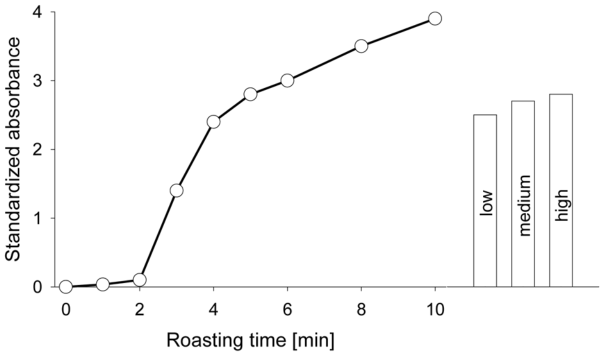

Figure 5. Development of colour during roasting of coffee at laboratory scale compared to industrially roasted coffee (low, medium and high degree of roasting).

The colour development starts after 2 min of roasting. At this time the temperature in the laboratory roasting device has reached 200°C. It is suggested that at this time the water has evaporated from the green coffee and the temperature starts rising in the coffee beans. The water content in the green coffee was 6% (Fig. 5).

To investigate the formation of HMF and HMFA in coffee a model system was established. For simulation of the dry conditions during coffee roasting a lyophilised mixture of sucrose the dominant carbohydrate in green coffee and alanine was heated. The molar ratio of sucrose to alanine is about 0.1 in arabica coffees and 0.05 in robusta coffees. Although HMF was formed in the model system practically no HMFA was found during the reaction (5 mmol/mol based on sucrose) in both, the arabica and robusta model (Fig. 6). HMF was also formed by heating of sucrose but the amount formed was significantly lower. To compare the formation of HMF and HMFA in coffee with the formation in the model system green coffee was ground and heated under the same conditions as the model mixtures (240°C, 1 min).

Since it is well known that the formation of HMF is increased at low pH we added chlorogenic acid (the dominant acid in coffee) and other acids to the model mixture to

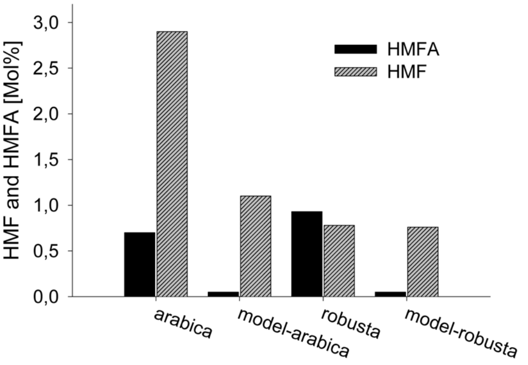

Figure 6. Formation of HMF and HMFA during roasting in the model system and in coffee.

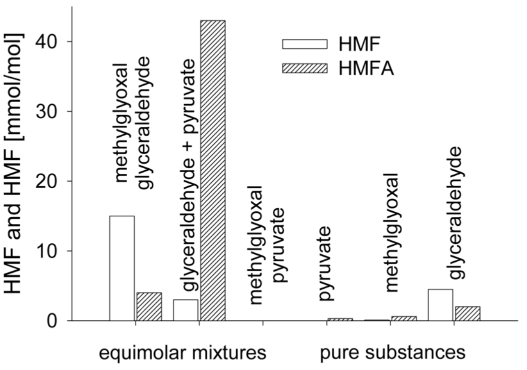

Figure 7. Formation of HMF and HMFA from C_3-precursors by heating to 240°C. The bars show the amount of formed HMF and HMFA based on the amount of precursors used (mmol/mol).

investigate the formation of HMF and HMFA in the presence of these acids (data not shown). These might not only act by reducing the pH but also as potential precursors of HMFA. The addition of chlorogenic acid (290 mmol/mol sucrose) leads to an increase in the HMF formation by a factor of about 10. However, the formation of HMFA did not change. Neither fructose nor the addition of other acidic compounds like gluconic or galactonic acid, glucuronic or galacturonic acid, as well as γ-amino-butyric acid, α-keto-glutarate which are thermal degradation products of amino acids did lead to an increase in the HMFA.

In analogy to the proposed mechanism of HMF formation by Cämmerer *et al.* [17] (Fig. 1) we investigated the formation of HMF and HMFA from C_3-precursors. In ref. [17], it is described that methylglyoxal and glyceraldehyde can be precursors of HMF. The analogous reaction of glyceraldehyde with pyruvate would probably result in a similar compound with the aldehyde changed to a carboxylic acid. To test this hypothetical mechanism the potential precursors were heated under the same conditions as the model system. In the reaction of methylglyoxal with glyceraldehyde 15 mmol/mol (based on glyceraldehyde) of HMF and small amounts of HMFA were formed. On the other hand, when pyruvate was used as a precursor instead of methylglyoxal 43 mmol/mol of HMFA and small amounts of HMF were formed. In the control experiments – heating of a mixture of methyl glyoxal with pyruvate and the pure substances – no substantial amounts of either HMF or HMFA were formed (Fig. 7).

From these results we propose a pathway for the formation of HMFA which is based on a reaction of 2 C_3-units, namely glyceraldehyde and pyruvate *via* aldol addition and elimination of water. In a second step a five-numbered ring is formed by intramolecular ketalisation which eliminates a second molecule of water to give HMFA. Pyruvate is present in green coffee at concentrations of about 600 mg/g [6] and glyceraldehyde is formed during roasting by cleavage of sucrose to monomeric carbohydrates and further to glyceraldehyde and methylglyoxal. Pyruvate and glyceraldehyde can recombine to HMFA by loss of two molecules of water (Fig. 8). To verify the proposed mechanism an equimolar mixture of sucrose with pyruvate was heated to 240°C for 1–5 min. In analogy to the experiments described earlier, HMFA was formed with similar kinetics.

4 Concluding remarks

During the roasting of coffee the Maillard reaction leads to a complex mixture of more or less aroma active and coloured substances. Recent biological experiments showed that HMF – one of these products occurring at higher concentrations – could be activated to highly reactive nucleophiles that can react with the DNA [9–11]. HMF is formed during the roasting process in high amounts at 240°C (up to 900 µg/g coffee). However, the degradation is rather quick and commercially roasted coffee contains less HMF than expected. Additionally, a similar product namely

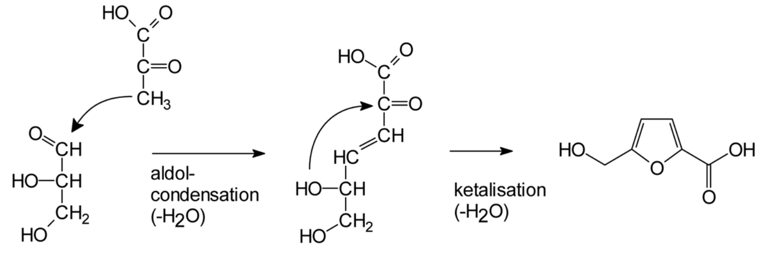

Figure 8. Proposed pathway of the formation of HMFA.

HMFA is formed. Kinetic data suggested that this compound is formed by oxidation of HMF but model reactions revealed that HMFA is a product from different precursors. It is suggested that glyceraldehyde and pyruvate are the precursors of HMFA that combine in an aldol reaction and undergo an intramolecular ketalisation and dehydration to form HMFA. Using these two compounds (glyceraldehyde and pyruvate) it could be shown that HMFA is formed in the model system.

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