Food Research International 61 (2014) 252–256

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

# Mitigation of acrylamide and hydroxymethyl furfural in instant coffee by yeast fermentation

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ARTICLE INFO

*Article history:*
Received 7 April 2013
Received in revised form 23 July 2013
Accepted 26 July 2013
Available online 2 August 2013

*Keywords:*
Coffee
Acrylamide
Hydroxymethyl furfural
Fermentation
Baker's yeast
*Saccharomyces cerevisiae*

ABSTRACT

Acrylamide being known as carcinogenic and hydroxymethyl furfural (HMF) being known as cytotoxic compounds are heat induced process contaminants found in instant coffee. Today's instant coffee production method involves roasting of coffee beans, grinding, flavor and aroma separation, extraction, concentration, and drying steps. During roasting, acrylamide and HMF are formed in varying amounts depending upon the degree of heat treatment as a result of the Maillard reaction. This study was conducted in order to reduce the concentrations of acrylamide and HMF in instant coffee. Instant coffee (20%, w/v) was mixed with sucrose (0–10, w/v) and baker's yeast (*Saccharomyces cerevisiae*, 1–2%, w/v) in a tightly closed glass vessel. The mixture was fermented at 30 °C for 48 h. The kinetics of acrylamide and HMF degradation was investigated. HMF and acrylamide contents were reduced exponentially at varying rates, depending upon fermentation medium and time. After 24 h, HMF concentration was decreased by 61.2%, 75.7%, 93.6% and 99.2% in the fermentation media containing none, 1%, 5%, and 10% of sucrose, respectively. After 48 h, acrylamide concentration was decreased by about 70%. These results revealed that yeast fermentation is promising for the mitigation of HMF and acrylamide in instant coffee.



## 1. Introduction

Coffee bean is an important commodity throughout the world and its trade is getting larger year by year. World coffee exports has been reported to have amounted to 9.42 million bags in December 2012, compared with 9.14 million in December 2011. Exports in the first quarter of coffee year 2012/13 (Oct/12 to Dec/12) have increased by 15% in the same period in the last coffee year (International Coffee Organization, www.ico.org). As a beverage, coffee is widely consumed all over the world. Roasting process is the critical step in the manufacturing of the coffee beverage since the final flavor of the product is heavily dependent on roasting. Roasting is generally performed at temperatures higher than 200 °C. Depending upon the degree of roasting, the product gets different flavors changing from green baggy aroma to freshly roasted and to even burden aroma. Maillard reaction, Strecker degradation and pyrolysis reactions are responsible together for the formation of aroma of the roasted coffee (Bagdonaite, Derler, & Murkovic, 2008).

During coffee roasting, some processing toxicants such as acrylamide and HMF are also formed. Since the first determination of acrylamide in heat-treated food products in 2002 (Tareke, Rydberg, Karlsson, Eriksson, & Tornqvist, 2002), several attempts have been performed in order to investigate the concentration of probable human carcinogen acrylamide in different kinds of foods. Acrylamide is found in varying amounts in most heat-treated foods such as fried potatoes, bakery products and roasted coffee. According to an EFSA report (EFSA, 2012), acrylamide content in foods ranged between 30 μg/kg (soft bread) and 1350 μg/kg (coffee substitutes) in year 2010. A marginal increase was observed in acrylamide contents of 'French fries from fresh potatoes' and for 'instant coffee'. In year 2010, the acrylamide content in instant coffee was 1123 μg/kg (EFSA, 2012). Also in the report of EFSA (2011), the contribution of coffee to exposure to acrylamide has been shown to be up to 40% in high coffee consuming countries.

Besides acrylamide, HMF is formed during roasting of coffee as a result of the Maillard and sugar dehydration reactions. Its content ranges between 100 and 4000 mg/kg (Capuano & Fogliano, 2011). Coffee is known to be the most important source of HMF in daily diet (Arribas-Lorenzo & Morales, 2010). In a study on dietary exposure to HMF from a Norwegian population, coffee was identified as the most important source of HMF (63%), both because of the high levels of HMF in coffee and because of the high consumption (Husøy et al., 2008). HMF is known to be cytotoxic, irritating to the eyes, upper respiratory tract, skin and mucous membranes at high concentrations (Capuano & Fogliano, 2011). It has been recently shown that HMF generated acrylamide from asparagine more efficiently than glucose in model system (Gökmen, Kocadağlı, Göncüoğlu, & Mogol, 2012).

Coffee is mentioned as "a food group where substantial reduction of acrylamide is unlikely without affecting its quality and acceptance or developing additional food safety issues" (Lineback, Coughlin, & Stadler, 2012). It was stated in the acrylamide toolbox of Food Drink Europe (FDE, 2011) that, current mitigation strategies such as asparaginase treatment, use of calcium or magnesium salts, and different roasting

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http://dx.doi.org/10.1016/j.foodres.2013.07.057

technologies are not useful for the reduction of acrylamide in coffee. These strategies are mostly based on the restriction of Maillard reactions which yields loss of aroma/flavor and hence loss of acceptance. For the reduction of HMF concentration in coffee, vacuum treatment was tried, but only 20% of HMF was removed (Quarta & Anese, 2012). This treatment was found to cause the loss of volatile compounds in the product. Previously, fermentation with *Saccharomyces cerevisiae* was performed in order to reduce the content of HMF in roasted malt, and it was shown that it is an effective mitigation method (Akıllıoglu, Mogol, & Gokmen, 2011). Thus, this study aimed to determine the possibility of fermentation operation integrated into instant coffee production in order to reduce the acrylamide and HMF content of the final product. The kinetics of acrylamide and HMF degradation was investigated.

## 2. Materials and methods

### 2.1. Materials

Instant coffee produced by a leading coffee company, and instant yeast (*S. cerevisiae*) was purchased from a local supermarket in Ankara. Methanol, formic acid, potassium hexacyanoferrate, and zinc sulfate were of analytical grade and purchased from Merck (Darmstadt, Germany). Sucrose was purchased from Sigma-Aldrich (St. Louis, MO, USA). HMF and furfuryl alcohol were purchased from Acros Organics (New Jersey, USA) and Sigma-Aldrich (Steinheim, Germany), respectively. Acrylamide (99%) was purchased from Sigma-Aldrich (Deisenhofen, Germany). Syringe filters (nylon, 0.45 μm), Oasis MCX (1 mL, 30 mg) solid phase extraction cartridges, Acquity UPLC HSS T3 column (4.6 × 150 mm, 3 μm), and Atlantis dC18 column (250 × 4.6 mm i.d., 5 μm) were supplied by Waters Corporation (Millford, MA).

### 2.2. Preparation of samples and fermentation

20% (w/v) instant coffee was mixed with varying amounts of sugar (1–10%, w/v) and dissolved in 100 mL of water. Instant baker's yeast (1–2%, w/v) was added. Fermentation was carried out at 30 °C in tightly closed beakers of 250 mL placed in an orbital shaker (100 rpm). Sampling was performed at certain time intervals to monitor the changes in the concentrations of acrylamide and HMF.

### 2.3. Analysis of HMF and its derivatives

HMF and its corresponding alcohol HMF alcohol were determined as previously published elsewhere (Akıllıoglu et al., 2011). The aliquot taken from the fermentation medium (800 μL) was placed in a centrifuge tube and 100 μL Carrez I (15 g of potassium hexacyanoferrate in 100 mL of water) and 100 μL Carrez II (30 g of zinc sulfate in 100 mL of water) solutions were added. The mixture was shaken with a vortex mixer and centrifuged at 10,000 × g for 3 min. Then, 100 μL of supernatant was diluted with 900 μL of distilled water and was passed through a 0.45 μm nylon filter. Then, it was injected onto a Shimadzu UFLC chromatographic system (Shimadzu Corporation, Kyoto, Japan), which is equipped with an LC-20 AD pump, a model DGU-20$A_5$ degasser unit, a SIL-20AHT auto sampler, a CTO-10ASvp thermostatted column compartment, and a model of SPD-M20A diode array detector. The chromatographic separations were performed on an Atlantis dC18 column (4.6 × 250 mm, 5 μm), using the isocratic mixture of 10 mM aqueous formic acid solution and acetonitrile (90:10, v/v) at a flow rate of 1.0 mL/min at 40 °C. Data acquisition was performed, acquiring chromatograms at the detection wavelength of 285 nm for HMF, and 223 nm for HMF alcohol.

To confirm the conversion of HMF to HMF alcohol, aliquots taken from the fermentation medium were analyzed by an Agilent 1200 HPLC system (Waldbronn, Germany), consisting of a binary pump, autosampler and temperature controlled column oven, coupled to an Agilent 6130 MS detector, equipped with an electrospray ionization (ESI) interface. The LC-MS system was operated in positive ionization mode using the following interface parameters: Drying gas ($N_2$) flow rate of 13 mL/min, nebulizer pressure of 40 psig, drying gas temperature of 350 °C, capillary voltage of 4 kV, and fragmentor voltage of 100 eV. The chromatographic separations were performed on an Atlantis dC18 column (4.6 × 250 mm, 5 μm), using the isocratic mixture of 100 mM aqueous formic acid solution and acetonitrile (90:10, v/v) at a flow rate of 1.0 mL/min at 40 °C. Mass spectrum was recorded (m/z range 50–300) to determine furfural derivatives and their potential conversion products. Parent and compound specific ions of 127 and 109 for HMF, and of 129 and 111 for HMF alcohol were also monitored in selected ion monitoring mode.

### 2.4. Analysis of acrylamide

Acrylamide analysis was performed using the method described by Kocadağlı, Göncüoğlu, Hamzalıoğlu, and Gökmen (2012). The aliquot taken from the fermentation medium was centrifuged at 10,000 g for 5 min after Carrez precipitation. Two ml of the supernatant was passed through a preconditioned Oasis MCX cartridge for clean-up. The first 8 drops of the eluent were discarded and the rest were collected into an autosampler vial prior to LC-MS/MS analysis. The sample was injected into an Acquity UPLC HSS T3 column (4.6 × 150 mm, 3 μm) at 40 °C coupled to an Agilent LC-MS/MS system operated in positive ionization mode using the following interface parameters: Drying gas temperature of 300 °C, gas flow rate of 10 L/min, nebulizer pressure of 45 psi, sheath gas temperature of 350 °C, sheath gas flow rate of 11 L/min, capillary voltage of 4000 V, nozzle voltage of 1000 V, and fragmentor voltage of 70 eV. Chromatographic separation was performed by using a mobile phase consisting of 10 mM formic acid in water: 0.1% formic acid in acetonitrile (95:5, v/v) at a flow rate of 0.75 mL/min. Acquisition was performed by monitoring the m/z ratio of 72 for acrylamide, and 55 and 44 for its product ions.

### 2.5. Determination of kinetic parameters

Data were evaluated with MATLAB® v7.0.1. First order degradation rates of HMF and acrylamide, and first order formation rate of HMF alcohol were evaluated using the following equations, respectively;

$$C = C_0 \times [\exp(-k \times t)]$$
$$C = C_{max} \times [1-\exp(-k \times t)]$$

where C: concentration at any time, $C_0$: initial concentration, $C_{max}$: maximum concentration, $t$: time, and $k$: rate constant.

### 2.6. Statistical analysis

Differences between kinetic model parameters of samples and differences between acrylamide reduction percentages were evaluated with ANOVA. Tukey's b test was used to determine the significant differences ($p < 0.05$) by using IBM SPSS Statistics 19.

## 3. Results and discussion

### 3.1. HMF reduction

Instant coffee containing about 1500 mg/kg of HMF was fermented anaerobically with *S. cerevisiae* (baker's yeast). HMF reduction was monitored in 20% (w/v) instant coffee containing media prepared with different concentrations of sugar (0, 1, 5, 10% sucrose) and 1% yeast. As can be seen in Fig. 1a, HMF concentration decreased exponentially during the fermentation process. At the end of the 24 h long fermentation, the concentration of HMF was reduced by 61.2%, 75.7%, 93.6% and 99.2% in the fermentation media containing no sugar, containing 1%, 5%, and 10% sucrose, respectively.