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Research Article

Determination of HMF in Some Instant Foods and Its Biodegradation by Some Lactic Acid Bacteria in Medium and Food

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Abstract

The aims of this study were to determine HMF level in some foods and to reduce their level by biodegradation. Coffee whitener, cappuccino, hot chocolate, instant coffee, instant powdered mix (coffee, coffee whitener and sugar) and aromatized cocoa were subjected to HMF analysis in the scope of this study. The HMF determination of samples was carried out using a high performance liquid chromatography. Color properties of the samples were determined according to standard procedures and their relations with HMF content were also investigated. The biological degradation of HMF with lactic acid bacteria was also studied with broth media and a model food system (reconstituted milk).The average HMF levels were 12.59 mg/kg for coffee whitener, 572.49 mg/kg for cappuccino, 660.29 mg/kg for hot chocolate, 1804.91 mg/kg for instant coffee, 871.56 mg/kg for instant powdered mix and 980.94 mg/kg for aromatized cocoa. The addition of lactic acid cultures reduced the HMF content of the samples. HMF levels of broth media and model food inoculated with lactic acid bacteria (*L. lactis, L.bulgaricus, L. cremoris*) decreased about 25 % as a result of HMF biodegradation.

Abbreviations

HMF: Hydroxymethylfurfural
MR: Maillard Reaction
MRS: De Man-Ragosa-Sharpe
TCA: Trichloroacetic Acid
DAD: Diode Array Detector
HPLC: High Performance Liquid Chromatography
ODS: Octadecylsilane
SAS: Statistical Analysis System
ANOVA: Analysis of variance

Introduction

Heat treatment is widely used in the food industry for many different purposes. Heat processing applications may cause desirable/undesirable reactions between food components such as sugar and amino acid. The determination of these undesirable compounds in terms of food safety and nutrition is very important. Because of these compounds may cause serious health the problems persist [1-6]. Therefore, many researchers have identified the Maillard Reaction (MR) products in the heat treated food such as infant formulas, dairy products, cereal products, fruit juice and their concentrates. Hydroxymethylfurfural (HMF) is known as one of the most common intermediate MR products of over processed foods [7,8]. In many food, HMF level is also used as a marker for determining the intensity of the thermal processing [9]. In recent years, studies over five hundred of food samples showed high levels (1-9.5g/kg) of HMF. In addition, HMF has been determined in tobacco, cigarette smoke and wood fumigation [10,11]. Irritation of high concentration of HMF on upper respiratory tract, eye, mucous membranes and skin has been reported as well as toxic effects. Initiating factor in colon cancer and tumorigenic effects of HMF has been demonstrated by the findings from other epidemiological studies on mice.The studies indicate that in the body, the HMF turns into 5-sulfoxymethylfurfural which is a genotoxic compound. Dietary intake of HMF is considerably more than other food toxicants [12-16].

Cancer cases in the world are increasing every day. The difficulty of their treatments and majority of the budget allocated has been seen. Considering their effect on the formation of cancer

of some compounds in foods, the importance of the issue is better understood. Therefore, it is necessary to determine the levels of HMF in different foods. Also, it is required scientific studies for preventing HMF formation or reducing its amount [17].

In terms of product quality and human health, there is sufficient information in the literature about the importance of HMF. Therefore, we aimed to determine HMF levels of some instant foods (coffee whitener, cappuccino, hot chocolate, instant coffee, instant powdered mix (coffee, coffee whitener and sugar) and aromatized cocoa) in this study. In addition, it is aimed to determine the effect of lactic acid bacteria on HMF level in to medium (MRS-De Man-Ragosa-Sharpebroth) and food (reconstituted milk).

Material and Method

HMF, acetonitrile, oxalic acid, Trichloroacetic Acid (TCA) and MRS was obtained from Sigma and Merck. Lactic acid bacteria (*Lactobacillus reuteri, Lactobacillus helveticus, Lactobacillus plantarum, Lactococcuslactis, Lactobacillus bulgaricus, Lactobacillus acidopylus and Lactobacillus cremoris*) were provided from microbiology laboratories in Erciyes University Food Engineering Department. Instant foods (five different commercial brands of each sample) and reconstitute milk was purchased from the market.

Color Determination

Automatic color measurement device (Lovibond RT Series Reflectance Tintometer, UK) was used in the examples of the color determination. The device was calibrated with standard calibration scale. *L, a, b* values (*L*: Brightness *a*: redness, *b*: yellowness) were recorded.

HMF analysis

Sample preparation flow chart of the HMF analysis performed using chromatographic methods was given in Figure 1. C18 column was used in the method. Chromatograms were determined at 284nm with DAD (Diode Array Detector). Sample to the HPLC system was applied using injection block (Rheodyne, USA). Injection volume was 20 µL. An ODS (octadecylsilane)-modified silica column was used. Analysis was carried out at 25°C using an isocratic mobile phase of acetonitrile–water with acetic acid-1% (5:95, v/v), the flow rate was adjusted to 1 mL/min. In defining conditions, the system pressure is measured as 132 bars. Retention time has been based on the definition of HMF. Quantitative analysis of HMF in the samples was performed using the standard calibration curve.

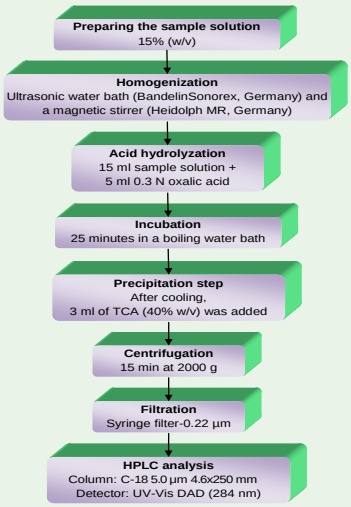

Figure 1: Sample preparation flowchart of the HMF analysis.

Biodegradation of HMF by lactic acid bacteria

Considering the level of HMF of instant food, level was set between 150 to 250 mg/L in broth medium for the biodegradation studies. Therefore, desired level of HMF was provided by the addition of HMF standard compound to medium. After placing the tube broth sterilized in the autoclave (Nuve OT 4060, Turkey). Seven types of lactic acid bacteria (*Lactobacillus reuteri, Lactobacillus helveticus, Lactobacillus plantarum, Lactococcuslactis, Lactobacillus bulgaricus, Lactobacillus acidopylus* and *Lactobacillus cremoris*) were inoculated into the prepared tube and incubated at 37°C for pre testing. At

Table 1: The average value of HMF in instant food samples.

Hydroxymethylfurfural (mg/kg)		
Sample type	$\bar{x} \pm SD$	$\bar{\bar{x}}$
Coffee whitener	14.45±0.31	12.59
	17.83±0.30	
	9.50±0.12	
	8.58±0.13	
	10.48±0.26	
Cappuccino	397.15±8.51	572.49
	396.65±5.98	
	617.15±13.64	
	601.40±6.77	
	850.08±15.51	
Hot chocolate	459.20±8.09	660.29
	725.88±15.54	
	689.73±8.97	
	719.48±36.70	
	707.18±21.64	
Instant coffee	2810.88±84.18	1804.91
	2006.90±23.67	
	1154.65±12.12	
	1591.75±6.15	
	1460.35±8.91	
Instant powdered mix	823.20±18.22	871.56
	880.45±7.20	
	974.58±6.43	
	881.23±13.13	
	798.33±10.75	
Aromatized cocoa	593.60±9.25	980.94
	963.55±17.90	
	877.42±10.00	
	1048.70±9.92	
	1421.45±11.88	

$\bar{x}$: Average, SD: Standard deviation, $\bar{\bar{x}}$: The average of the average

the end of 24 hours HMF level of medium was determined. After these preliminary trials, best results bacteria (*Lactococcuslactis, Lactobacillus bulgaricus and Lactobacilluscremoris*) that were tested again in food model (reconstituted milk). For this, HMF level of reconstituted milk with a dry matter content of 12% was set between 150-250 mg/L. Inoculations was carried out as applied in a broth medium. Each of the three bacteria has been incubated with separate/ combine (1:1:1 ratio). HMF levels were detected at the end of 3, 6, 9 and 24 hours.

Statistical analysis

SAS (Statistical Analysis System, 1988) was carried out for statistical analysis [18]. Analysis of Variance (ANOVA) was used in the general linear modeling procedures. Duncan test was used to determine differences between groups.

Table 2: The color values of instant food samples.

Sample type	*L*	*a*	*b*
Coffee whitener	93.88±0.10	0.32±0.02	10.31±0.04
	92.78±0.39	0.02±0.00	7.23±0.07
	93.86±0.12	0.22±0.01	12.58±0.06
	94.26±0.15	0.68±0.02	10.88±0.07
	93.27±0.19	1.35±0.01	11.74±0.07
Cappuccino	71.82±0.58	2.99±0.05	7.72±0.12
	86.88±0.50	0.79±0.05	5.47±0.09
	80.07±0.16	2.64±0.05	6.98±0.08
	77.35±0.48	3.72±0.08	9.81±0.08
	77.64±0.55	2.82±0.08	8.16±0.14
Hot chocolate	52.99±0.20	8.88±0.06	10.88±0.06
	47.65±0.06	11.06±0.04	14.63±0.05
	44.35±0.20	11.67±0.07	14.18±0.09
	48.25±0.13	8.45±0.01	11.17±0.01
	47.96±0.08	8.55±0.01	11.56±0.03
Instant coffee	34.12±0.45	13.66±0.14	25.41±0.19
	18.76±0.48	11.95±0.30	15.18±0.72
	26.73±0.17	13.77±0.07	21.29±0.22
	30.03±0.42	11.39±0.13	20.09±0.09
	18.13±0.29	10.88±0.11	12.41±0.25
Instant powdered mix	75.61±0.20	3.08±0.05	6.63±0.05
	61.79±0.06	5.14±0.05	11.05±0.04
	60.77±0.39	4.49±0.05	9.85±0.04
	57.35±0.44	6.30±0.08	14.95±0.11
	57.67±0.91	5.12±0.11	12.10±0.38
Aromatized cocoa	43.70±0.53	11.37±0.28	14.86±0.03
	44.14±0.48	12.15±0.08	17.97±0.07
	52.77±0.14	11.05±0.02	17.18±0.03
	45.87±0.05	10.67±0.02	15.87±0.03
	86.15±0.11	5.74±0.02	0.61±0.02

L: Brightness *a*: redness, *b*: yellowness

Results

HMF average values of the samples and their overall average were given in the Table 1. Chromatograms and calibration graph of the HMF standard compound are given in Figure 2 and 3. The HMF levels as a result of research; it was determined as 8.58-17.83 mg/kg in coffee whitener group, 396.65-850.08 mg/kg in cappuccino group, 459.20-725.88 mg/kg in hot chocolate group, 1154.65-2810.88 mg/kg in instant coffee group, 798.33-974.58 mg/kg in instant powdered mix and 593.60-1421.45 mg/kg in aromatized cocoa. Color values (*L, a, b*) of instant foods were shown in Table 2. *L* values of the coffee whitener group were determined as the highest unlike instant coffee group. A negative correlation between the levels of HMF and *L* values was determined (r= - 0.7137). *a* values of the coffee whitener group were determined as the lowest unlike instant coffee group. A positive correlation between the levels of HMF and *a* values was determined (r= 0.6951). This situation can be explained by the formation of the brown compound as a result of the Maillard reaction (non-enzymatic

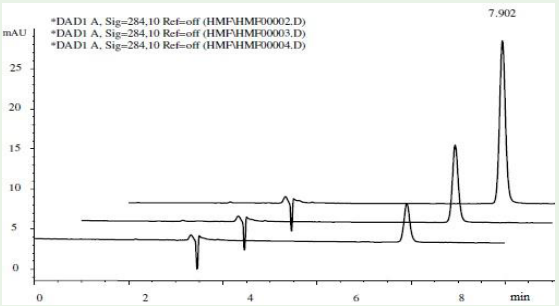

Figure 2: Chromatograms of HMF standard compound.

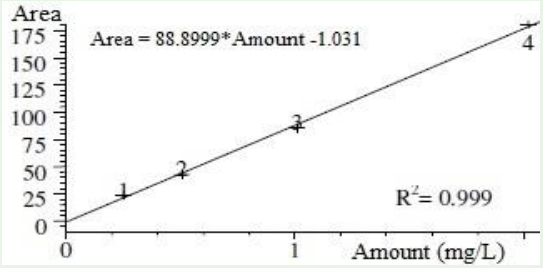

Figure 3: Calibration curve of HMF standard compound.

browning). A research carried out has revealed that the daily intake of HMF was 30-150 mg in humans [12]. The same researchers have suggested that this value could be increased further by the intake of acrylamide and furan compounds in the body. In another study conducted on rats, it has been reported to increase skin tumors and create skin lesions with application of 10-25mmol HMF. Body weight basis (200 mg/kg), HMF was developed liver tumor [19]. Our research findings have highlighted that is necessary to be more careful about consumption of the certain instant foods.

After HMF addition into the medium (MRS broth) and sterilization, HMF level of medium was measured as 217.45 mg/L. HMF levels of the medium at the end of 24 hours incubation were quantified as 219.90±3.46 mg/L in control group, 181.75±3.73 mg/Lcontaining *Lactococcuslactis*, 164.70±2.59 mg/Lcontaining

Lactobacillus bulgaricus and 165.60±1.88 mg/L containing *Lactobacillus cremoris.*

The biodegradability data of HMF in the reconstituted milk were demonstrated in Figure 4. After HMF addition in to the reconstituted milk and sterilization, HMF level was measured as 189.65±0.34 mg/L. HMF levels of the milk at the end of 24 hours incubation were quantified as 144.55±1.21 mg/L containing *Lactococcuslactis*, 145.05±1.00mg/L containing *Lactobacillus bulgaricus*, 142.53±0.32 mg/L containing *Lactobacillus cremoris* and 143.58±1.79 mg/L containing their combination.

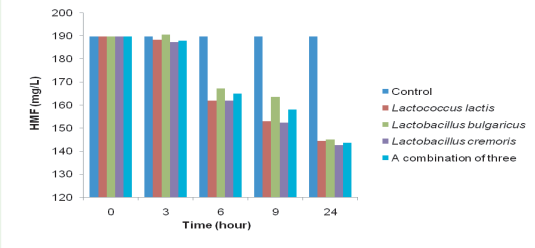

Figure 4: Changing of HMF in the reconstituted milk during the incubation.

Studies on the decreasing of HMF formation in food were very limited [20-21]. Some enzymes are reported to be effective in reducing furfural in the presence of NADPH. Effective on the furan compounds of the hydrolytic enzyme group that was considered.In a research done, degradability of HMF with the help of microorganisms was thought and *Saccharomyces cerevisiae* was used for the research. This yeast in reducing the amount of certain furfural compounds were determined to be effective butany effect on the HMF [20]. Lactic acid bacteria were tested in order to reduce the amount of HMF in our study. Lactic acid bacteria have reduced the HMF level (the rate of 16.42-24.26% in MRS broth; 23.32-24.66% in reconstituted milk) after 24 hours. Reducing effects of HMF of *Lactococcuslactis* and *Lactobacillus cremoris* have been shown to be higher than the *Lactobacillus bulgaricus* at the end of 6th and 9th hours. At the end of 24 hours, the rate has been approximately the same for all statistically ($p<0.05$).

As a result, the HMF content of some instant foods was determined by this study. In addition, biodegradability of HMF has been demonstrated in the media and the food. In light of our research findings, we can suggest that the standardization requirements about HMF in instant coffee and related products group. The people who consume risky foods in terms of the HMF content, it is believed that the benefits of consuming the food that is rich in lactic acid bacteria such as kefir, yogurt and probiotics.

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