# Hydroxymethylfurfural (HMF) Formation in Milk and Dairy Products

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**Abstract**

Milk and dairy products are encountered non-enzymatic browning reactions due to the heat process and storage temperatures. These reactions occur in foodstuffs containing sugar and amino acids. As a result of the reaction, undesirable compounds are formed in foods, so that the natural structure of the food is deteriorated. It is of great importance to control and observe the 5-Hydroxymethyl-2-furaldehyde (HMF) compound in milk and dairy products during the production process and storage period as a result of the browning reaction due to the heat treatment. Formation of (HMF), which is heat treatment indicator used as a chemical parameter to determine whether most food products with sugar concentration, such as fruit juices, honey, molasses, jams, milk and dairy products, are stored under right conditions and whether the appropriate heat treatment is carried out during the production process. In this study, heat treatment was applied to milk and dairy products during production and hydroxymethylfurfural (HMF) formation as a result of non-enzymatic chemical reactions have been investigated and evaluated.

*Keywords: Milk, HMF, Hydroxymethylfurfural, Heat Treatment, Maillard Reaction*

## Introduction

The Turkish Food Codex Legislation Statement Regarding Raw Milk and Heat-Treated Drinking Milk (Official Gazette, issue 14.02.2000/23964) defines raw milk as the secretions of a mammary gland of one or more cows, sheep, goats or buffalos; which is not heated above 40 $^0$C or exposed to an equivalent treatment. A heat-treated drinking milk is defined as being processed through a heat treatment such as pasteurization, UHT, or sterilization (but not boiling). This type of milk subsequently yields a negative result in the alkaline phosphatase test (Anon., 2000).

The nutritional content of the milk can vary according to the female mammal it has been sourced from. However, it contains nutritional elements which help the newborns be nourished and be immunized (Anon., 2018). Milk is an indispensable food for the growth and development of newborn mammals. Milk contains other important ingredients that contribute to the health of the mammal; such as proteins and peptides (enzymes, enzyme inhibitors, immunoglobulins, growth hormone and other hormones, growth factors, anti-bacterial agents), fatty acids, vitamins, and minerals. This is why dairy products are one of the most important food items for the nutrition of mammals (Maijala, 2000; Miller et al., 2000; Fox & McWeeney, 2003).

Raw milk is consumed after heat treatments in terms of food safety. The main reason behind this procedure is to destroy and prevent the proliferation of the microorganisms in milk, a fertile medium. Initially, the milk of healthy mammals does not contain harmful bacteria. However, the milk may be contaminated with noxas living in the mammary ducts and the nipples while it is passing through these ducts. Contamination can be caused by several other factors such as milking in non-hygienic conditions and storage in improper temperatures. This can lead to the contamination of milk with pathogenic microorganisms and occurrence of the pathogenesis among the consumers (Anon., 2016).

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The milk may start to spoil after being milked in case it is contaminated by spoilage bacteria. Heat-treatment of milk is the most common way used for preventing spoilage, killing the pathogenic bacteria while extending the shelf-life and providing stability (for storage and sales). The main heat treatment methods and norms are presented below (Unal and Besler, 2008).

- UHT (Ultra High Temperature) Sterilization, at 135-150 $^0$C, 2 -20 sec.

- LTLT (Low-Temperature Long Time) Pasteurisation, at 62-65 $^0$C, 30 –32 min.

- HTST (High-Temperature Short Time) Pasteurisation, at 72-75 $^0$C, 15 – 30 sec.

- HP (High Pressure) Pasteurisation, at 85-127 $^0$C, 2 -4 sec.

Heat treatment is commonly used in the food industry for several different purposes. The heat treatment may lead to (desirable/undesirable) reactions between components such as sugars and amino acids. The type of these reactions is very important for food safety and nutrition issues. As the result of various research studies, the complex compounds formed may cause serious health problems (Chavez-Servin et al., 2005; Oral et al., 2014). Several researchers have defined the Maillard Reaction (MR) products in heat-treated food items such as baby foods, dairy products, cereal products, juices and their concentrates.

Hydroxymethylfurfural (HMF) is one of the most common MR by-products in the over-processed foods. In several food products, the HMF level is used as an indicator of the absence of thermal processing. In a research study, researchers have examined over five hundred food items and they have found high levels of HMF (1-9.5 g / kg) (Oral et al., 2012; Oral et al., 2014). HFM is the most commonly known by-product of the MR. A temperature of 120 °C or above is required for the formation of these chemical changes (non-enzymic browning reactions) to occur in dairy products.

The reaction starts with the interaction between the ϵ-amino group of lycine and the carbonyl group of lactose (Morales et al., 1992).

As is known, the number of cancer incidents is gradually increasing in the populations every day. It is known that cancer is hard and costly illness to be treated. Some of the chemicals found in food items can lead to cancer development. Thus, the sources and the formation of these chemicals should be better investigated. This requires the determination of the HMF levels in different food items. In addition, further research is required for the prevention of HMF or to decrease its abundance (Oral et al., 2015).

**The Maillard Reaction**

The MR was first defined by the French chemist Louis Maillard. However, the first scientist that was able to provide a coherent analysis of the process was Hodge (Figure 1). The Maillard Reactions start with the condensation between the carbonyl groups of the sugars (reducing agents) and the amino groups of the amino acids. This reaction is responsible for the brown color and the mature flavor of heat treated items (Martin et al., 2000).
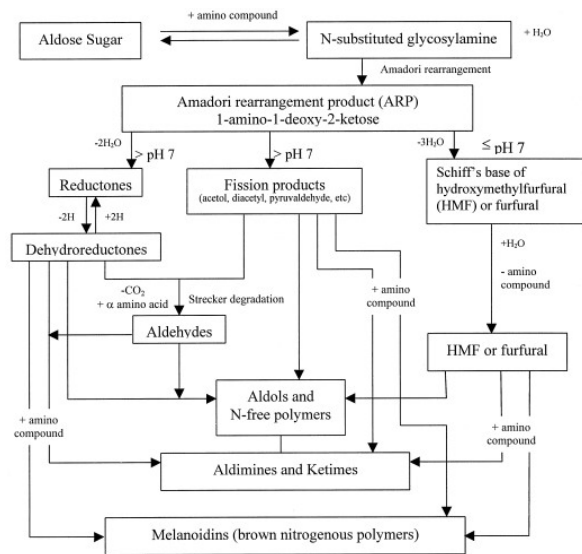


**Figure 1:** The schema of the Maillard reaction, adapted by Hodge (Martin et al., 2000)

The MR simply occurs in three steps. The first step is the condensation reaction between the reducing sugar and the amino acid. The reaction yields in water and a Schiff base (Figure 2). Next, the Schiff base turns into aldoseamine. In this step, the aldoses turn into ketoseamine (1-amino-1-deoxyketose) through the Amadori rearrangement, and the ketoses turn into 2- amino-2-deoxyaldose through the Heyns rearrangement (Figure 3) (Celebi, 2006).

The second step is where the color change begins. This step might be followed by three different possible reactions. The first possible reaction forms the most important byproducts of the MR:

1-amino-1- deoxyketose reacts with a different aldose molecule to form the diketoseamine molecule (a more stable compound). This new compound can be separated into smaller molecules, such as monofructoseamine or 3-deoxyosulose (Celebi, 2006; Anet, 1964; Wedzicha & McWeeny, 1974).

The second possible reaction occurs through the enolization of the Amadori products. This reaction is pH-mediated. If the pH value is below 7, the pentoses turn into furfural and the hexoses turn into HMF. If the pH is above 7, the products are much more unstable (Yıldız et al., 2010; Celebi, 2006).
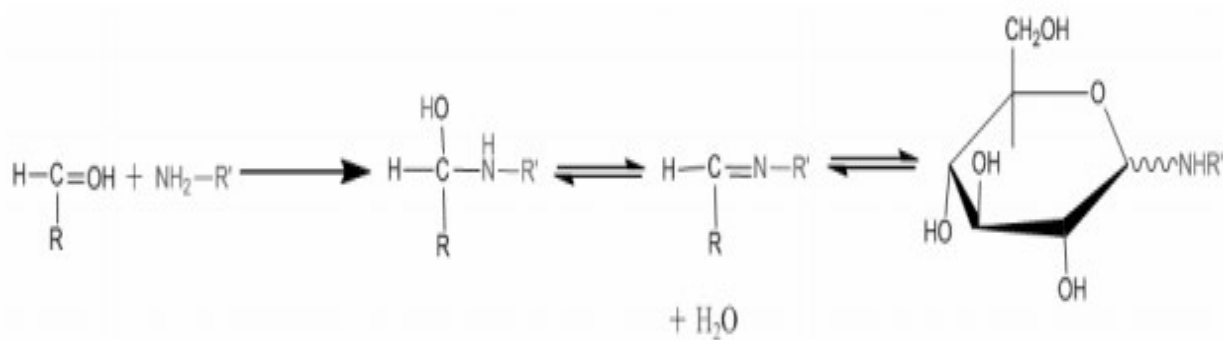


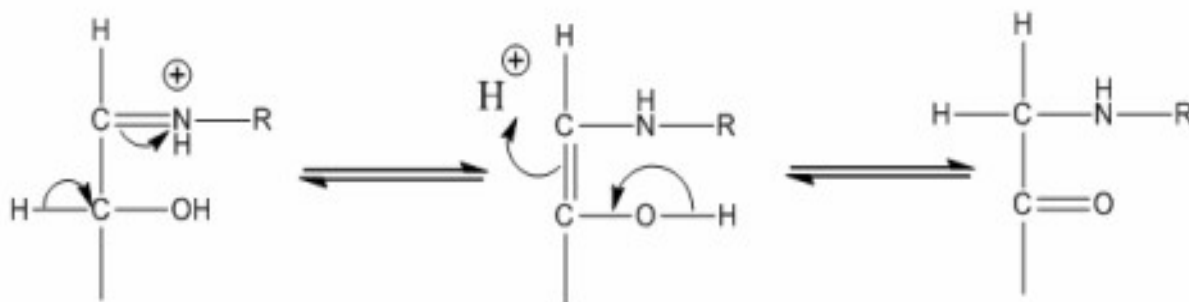**Figure 2:** Condensation reaction



**Figure 3:** The Amadori rearrangement

The third and final possible reaction is named as the Strecker degradation. The carbonyl groups (C=O) and the amino groups (N-H) condense and lead to $CO_2$ formation - which is the distinctive character of this reaction. This step is accepted as the beginning of aroma development (Celebi, 2006; Coca et al., 2004).

In the third step; the compounds that have formed in the second step combine with the amine groups ($-NH_2$), the aldol groups condense and the aldehydes and amines polymerize; and melanoidin is formed. Melanoidin compound (Figure 4) is the end-product of the non-enzymatic browning reactions forms, which is in heterocyclic structure and consists of dark-colored molecules with a high molecular weight. The aromatic molecules also form as a result of series of reactions. These series of reactions are extremely complex and similar to chemical compounds such as HMF, dihydrofurans, furan, pyruvaldehyde, and dimethylpirazines (Edward, 2000).
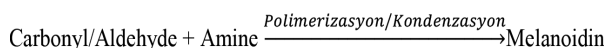
$$\text{Carbonyl/Aldehyde + Amine} \xrightarrow{Polimerizasyon/Kondenzasyon} \text{Melanoidin}$$

**Figure 4:** The third step of the Maillard reactions

## Formation of Hmf in Milk and Dairy Products

### HMF Formation in Milk

Milk is one of the most important foods for mammals and it is rich in nutritional content. Milk is a basic food that contains almost all the nutritional items that are good for overall health and are required for the formation of organisms (Yetismeyen, 1997). The heat treatment of raw milk causes some chemical and biochemical reactions and leads to changes in the carbohydrate, protein and vitamin composition of milk. Also, heat treatment can alter the nutritional quality and the sensorial properties of milk (Ferrer et al., 2000; Arena et al., 2017).

One of the major reactions that occur in milk due to heat treatment is the MR. This reaction occurs between the free amino groups of proteins and the aldehyde groups of the reducing sugars (Molares & Jimenez-Perez, 1999; Pellegrino et al., 1995; Van

Boekel, 1998). The MR has a complex mechanism. However, it is generally summarized in three steps that are the initial, further and final steps (Yildiz et al., 2010).

The last step of the reaction is the separation of the Amadori product (lactulosylycine) and the formation of furfural or HMF. This reaction is mediated by the pH level. The final step of the reaction consists of the formation of dark-colored nitrogen polymers and melanoidins (Martins et al., 2001). As a result of the MR, the amount of lycine decreases and there is protein polymerization, yielding aroma compounds, mutagenic and antioxidative compounds. The level of the Maillard reaction is determined by heat-treatment indicators. The heat-treatment indicators of this reaction include furosine, carboxymethyllycine, lactulose, furfural, and HMF (Martins et al., 2001; Ames, 1998).

HMF is a byproduct of milk heat treatment and it can be used as a parameter of the intensity and the quality of the heat treatment process (Berg & Van Boekel, 1994). The amount of HMF depends on the nutritional content of the milk, the intensity of the heat treatment and the storage conditions. Morales et al. (2000) have found that HMF levels increase with increasing the processing intensity.

Maillard Reaction also affects the carbohydrate composition of the milk. Ledesma-Osuna et al. (2008) and Leiva et al. (2017) have determined that the monosaccharides more readily react compared to the reducing disaccharides (Ledesma-Osuna et al., 2008; Leiva et al., 2017). Jansson et al. (2014) have found that the lactose-free milk is a better medium for the MR. This study has found that the hydrolysis of lactose (a disaccharide, milk sugar naturally found in milk and dairy products) to glucose and galactose provide better reactivity values (with $-NH_2$) for the MR (Jansson et al., 2014). This was determined through the use of lactulose and furosine as heat-treatment indicators (Jansson et al., 2014; Evangelisti et al., 1999; Tossavainen & Kallioinen, 2007).

## HMF Formation in Powdered Milk

The whole fat powdered milk, that is produced for the confectionary industry, can develop a brown color when it is produced through the cylinder (roller) method. This is due to the MR that occurs between the carbon and protein complexes (Metin, 1996).

Marquez et al. (1992) have studied the effects of heat treatment on powdered milk. They have found that the application of the microwave method directly increased the HMF levels in the final product. The HMF levels were determined to be 12.5167 mg/kg for the microwave drying method and 0.3-0.9 mg/kg for the air-drying method (Marquez et al.,1992).

Baldwin and Ackland (1991) have investigated the effects of storage period on the HMF levels of milk. They have found that after twelve months of storage, the HMF levels increased from 5.6 mg/kg to 21.35 mg/kg. The HMF concentration of skimmed milk powder also increases with increasing storage duration (Baldwin & Ackland, 1991).

## HMF Levels in Milk and Dairy Products

Chen et al. (2009) have measured the HMF levels of milk samples (two regular and seven powdered farms) that were obtained from the consumer's market. Among these, two powdered milk samples (#3 and #9) had HMF concentrations that were lower than 0.50 μg / g, and thus, could not be measured. The rest of the samples contained HMF concentrations ranging between 0.54 μg / mL and 2.25 μg. Baby formulas were heat treated to extend the shelf-life; however, this process increased the HMF concentrations between 0.6 μg / g and 2.25 μg / g. The findings of the study are shown in Table 1 (Zhijuun Chen & Xiaomei Yan, 2009).

**Table 1:** The HMF levels in milk and dairy product samples

| No | Examples | HMF |
|----|----------|-----|
| 1 | Fruit-Flavored Milk | 0.59 μg/mL |
| 2 | Same brand as Sample 1, different batch | 0.54 μg/mL |
| 3 | Powdered milk | N/A* |
| 4 | Same brand as Sample 3, different batch | 1.59 μg/mL |
| 5 | Baby Formula | 2.25 μg/g |
| 6 | Same brand as Sample 5, different batch | 1.27 μg/g |
| 7 | Imported baby formula (New Zealand) | 2.10 μg/g |
| 8 | Imported baby formula (Deutschland) | 0.60 μg/g |
| 9 | Baby Formula | N/A* |

*N/A: Not available

Morales et al. (1996) have measured the HMF values in milks subjected to different heat treatments: 9 pasteurized, 36 UHT-treated, and 6 sterilized samples. The samples were obtained from some large Spanish milk producers. The researchers tried to choose products with similar expiry dates. The HMF levels were measured through the HPLC system and TBA methods. It was determined that the HMF levels of the UHT products were between 3.6-6.0 μm for 53% of the samples (HPLC method) and 6.0-9.6 μm for 61% of the samples (TBA method). The findings suggest over-processing as the HMF levels of 25% of the UHT products matched the HMF levels of sterilized milk (9.6-12 μm). It was concluded that the producers overlook the role of the heating and cooling phases and purposefully over-process the milk (Morales et al., 1996).

Urgu et al. (2017) have conducted a similar study in Izmir, Turkey. They have analyzed 6 pasteurized, 29 UHT and 2 lactose-free milk samples. They have chosen products with similar expiry dates. The findings are presented in Table 2 (Urgu et al., 2017).

**Table 2:** The total HMF values of the milk samples

Total HMF Values (µmol/L)

| Brands | Pasteurized Whole Milk | Pasteurized Low Fat | UHT Whole Milk | UHT Half-Fat Milk | UHT Fat Free | UHT Lactose Free |
|---|---|---|---|---|---|---|
| 1 | - | - | 4.90±0.27[hA] | 3.44±0.20[fB] | 3.52±0.18[bB] | 35.73±2.35[a] |
| 2 | - | 1.48±0.13[b] | 6.99±0.70[f] | - | - | - |
| 3 | - | 1.15±0.04[b] | 8.10±0.30[deA] | 7.81±0.03[bAB] | 7.49±0.11[aAB] | - |
| 4 | 4.03±0.16 | - | 5.72±0.10[ghA] | 3.80±0.19[fB] | - | - |
| 5 | - | - | 5.92±0.15[gA] | 4.89±0.21[eB] | - | - |
| 6 | - | 4.30±0.92[a] | 8.77±0.45[cdA] | 5.29±0.14[deB] | - | - |
| 7 | - | - | 12.74±0.63[aA] | 5.72±0.44[dB] | - | - |
| 8 | - | - | 9.55±0.20[bcA] | 5.91±0.46[cdB] | - | - |
| 9 | - | - | 6.01±0.28[gA] | 3.71±0.06[fB] | - | - |
| 10 | - | - | 9.04±0.23[bcA] | - | 6.39±0.61[aB] | - |
| 11 | - | 1.34±0.11[b] | 4.91±0.14[hA] | 2.50±0.30[gB] | 2.16±0.37[cB] | - |
| 12 | - | - | 7.46 ±0.13[ef] | - | - | - |
| 13 | - | - | 9.71±0.19[bA] | 8.89±0.18[aA] | - | - |
| 14 | - | - | 7.09 ±0.06[f] | - | - | - |
| 15 | - | - | - | 6.52±0.16[c] | - | - |
| 16 | - | 4.78±0.42[a] | - | - | - | - |
| N | n=2 | n=10 | n=28 | n=22 | n=8 | n=4 |
| Average±SD | 4.03±0.16[C] | 2.61±1.71[C] | 7.64±2.19[B] | 5.32±1.94[BC] | 4.89±2.47[BC] | 31.54±5.93[A] |

n= Number of samples analyzed, SD: Standard deviation, Lowercase letters indicate the difference in each column (P<0.05). Capital letters indicate the difference between UHT milk (whole, half-fat, fat free UHT milk) on the same line.

The findings indicate that the HMF levels are positively correlated with the treatment temperature. The lowest HMF levels were determined in the pasteurized milk products. The lactose-free milk samples were found to have higher HMF values, due to the high reactivity of glucose and galactose. However, this study did not find a correlation between color change and the level of HMF (Urgu et al., 2017).

Hurtado et al. (1997) have analyzed powdered milk and liquid baby formula that have been kept at 37°C for six months including 12 samples of Spanish baby formula (six diluted and six liquid) and 10 samples of commercial powdered milk. All of the commercial products were analyzed before the expiration date, and it was found that they only contained HMF and furfural. It should be noted that the HMF levels were higher than the furfural levels in all samples. The HMF and furfural levels of the liquid and diluted baby formulas were between 38.3 - 189.1 and 11.5 - 24.5 µg / 100 mL, respectively (Albalá-Hurtado et al., 1997).

Surprisingly, the powdered baby formulas had a higher HMF content compared to commercial powdered milk samples. Similarly, free furfural content is found to be lower in commercial powdered milk samples. However, HMF and furfural were found at a lower level in liquid milk formulas for babies compared to powdered baby milk formulas. (Albalá-Hurtado et al., 1997

Sunds et al. (2018) have stored two batches of skimmed and whole-fat milk at different controlled temperatures (10, 20, 30, 40 and 50°C) and three different cycles that simulated real-life condition. The cycles were as follows: cycle 1, 10 to 30°C; cycle 2, 20 to 40°C; cycle 3, 30 to 50°C. The samples that were stored at 10 to 40°C and the cycles 1 and 2 were analyzed in 24-week periods. The samples that were stored at 50°C and the cycle 3 were analyzed in an 8-week period. The furosine concentration was chosen as an indicator of MR. It was determined that the furosine concentration significantly increased with temperature and storage time. The furosine concentration was 1.1 times higher in cycle 2 compared to cycle 1, and 3 times higher in cycle 3 compared to cycle 2. These findings show the importance of storing dairy products at 20°C. However, it may lead to difficulties for the countries with warm climates. In addition, it was observed that the samples that were stored at 30 and 40°C had visible color changes after 24 and 6 weeks of storage respectively. This indicated the importance of storage below 30°C (Sunds et al., 2018).

Dmytrów et al. (2010) have analyzed sterilized goat milk sourced from two different suppliers in Poland. The milk samples were kept at room temperature (21 ± 2°C) for 6 months in their original Tetra Pak packages. When the HMF content of the milk samples was analyzed, it was found that samples of one of the producers always had lower values compared to the other (Graph 1). These findings support the notion that Maillard Reactions resume after being packaged, as the HMF content significantly increased during the storage (Dymtrow et al., 2010).
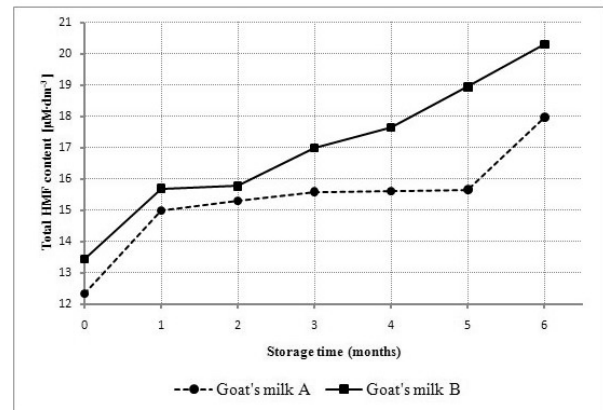


**Graph 1:** The changes in total HMF values of samples A and B at room temperature.

**Prevention of HMF Formation**

The MR has favorable outcomes as it is responsible for the flavor of many food items; however, it can also produce undesirable carcinogenic and toxic effects (Yildiz et al., 2010). Thus, researchers have been trying to develop methods for the prevention of HMF formation in different food products.

Burdurlu & Karadeniz (2003) indicate that it is hard to prevent the MR when milk and milk products are heated, due to their sugar and amino acid contents. Several suggested methods are as follows: keeping a stable temperature during processing and storage, storing in low humidity, inhibition of the amino/carbonyl group reactions through inhibitors, breaking down the glucose through an enzymatic reaction (Burdurlu and Karadeniz, 2003).

It has been reported that the rate of non-enzymatic browning reactions can decrease through the decomposition of D-glucose (by the D-glucose oxidase that is found in foods) and the subsequent methylation of the amine groups (Bolin & Steele, 1987; Daniel & Whistler, 1985).

Richardson (2001) reported that the following precautions can be taken to block the non-enzymatic browning reactions: keeping the pH below the isoelectric points of amino acids, proteins and peptides, keeping the temperature at minimum degrees during processing and storage, dilution of

the food in order to increase the distance between molecules and, choosing non-reducing sugars if possible (Richardson, 2001).

Friedman & Molnar-Perl (1990) have found that N-acetyl-L-cysteine and L-cysteine compounds' sulfur-containing groups had antioxidative and antitoxic properties. These groups react with the mutagens, carcinogens and other toxic compounds and inhibit HMF production (Friedman and Molnar-Perl, 1990). Most thiol compounds (R-SH) also have antioxidant properties: they can replace sulfide groups and prevent the enzymatic/non-enzymatic browning reactions and the subsequent production of compounds such as HMF, furfural, and methylfurfural (Naim et al., 1993).

Haleva-Toledo et al. (1999) have prepared buffer solutions, with and without arginine, that contained L-cysteine, N-acetyl-L-cysteine and had a pH of 3 and temperature of 50 $^0$C (Table 3). The prepared solutions were kept in 20 mL light-proof bottles at 70 $^0$C for two days. It was concluded that HMF formation increases in the presence of acids. At pH=2, the presence of L-cysteine and N-acetyl-L-cysteine decreases HMF levels and at pH=5 (between 5-10 mM) it completely inhibits the formation of HMF (Haleva-Toledo et al., 1999). Friedman & Molnar-Perl (1990) indicate that the sugar may react with the thiols to inhibit HMF formation (Friedman and Molnar-Perl, 1990).

Antal et al. (1990) have found that; the decomposition of levulinic acid, and the subsequent polymerization of its products to humic acids lead to the decreased formation of HMF (Antal et al., 1990). Kroh (1994) indicates that in the presence of several chemicals (N-butanol, dioxin, polyethylene, glycol, etc.), HMF decomposes into levulinic acid to decrease the HMF concentration. Following the reactions lasted for 10 hours, they have found that different saccharides and monosaccharides react with phenylalanine at 98°C, and that the presence of these reactions leads to decreased HMF production at decreased levels (Kroh, 1994).

**CONCLUSION**

The rapid increase in world population, increases the need for food resources. These resources need to be efficiently used and be more available; thus, the improvement of the shelf-life and stability of these products have been subjected to various intensive research studies. Heat-treatment is an especially popular method for foods. Heat treatment is one of the oldest and most effective food preservation methods. However, the intensity of the treatment can lead to some chemical changes in the structure of the food. Thus, the procedure must be handled with care. HMF is one of the metabolites that result from the intensity of the heat treatment. It was determined that it is mostly found in intensely heat-treated baby formulas. It was concluded that the intensity of the heat treatment varies according

**Table 3:** The effect of the thiols on HMF formation in buffer solutions with or without arginine
(Haleva-Toledo et al., 1999)

| pH | | w/o Thiol | Cys | | | AcCys | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1mM | 5mM | 10mM | 1mM | 5mM | 10mM |
| **HMF** | | | | | | | | |
| 3 | Glc | 5 ± 1 | 1.4±0.1 | 0.7±0.2 | 0.2±0.1 | 2.7±0.4 | 2±0.5 | 0.6±0.2 |
| | Glc + Arg | 92 ± 4 | 90±5 | 25 ± 6 | 6 ± 1 | 118 ± 5 | 77 ± 7 | 13 ± 1 |
| 5 | Glc | 0.2 ± 0.1 | 0.1 ± 0.1 | N/A | N/A | 0.1 ± 0.4 | N/A | N/A |
| | Glc + Arg | 6 ± 1 | 7 ± 1 | 3.4 ± 0.6 | N/A | 6 ± 1 | 3 ± 1 | 2.5 ± 0.3 |

(Arg: Arginine, Glc: Glucose, Cys: L-cysteine, AcCys: N-acetyl-L-cysteine, N/A: Not Available)

to the target consumers, where it is sometimes overdone on purpose. This is exemplified by the HMF levels of pasteurized milk, which are similar to those of UHT milk, where it should be lower. The HMF level can be reduced by scientifically reviewing the heat treatment procedures and correctly applying the determined principles.

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