



**ISTANBUL AYDIN UNIVERSITY**  
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# IJFER

**INTERNATIONAL JOURNAL OF  
FOOD ENGINEERING RESEARCH**

**Year 4 Number 1 - 2018 ISSN: 2149-5777**

- **Contamination of Dioxins in Foods**  
*Banu EŞDER, Güner ARKUN*
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## **Aims and Scope**

International Journal of Food Engineering Research (IJFER) is an international , peer-reviewed journal devoted to the publication of high quality original studies and reviews concerning a broad and comprehensive view of fundamental and applied research in food science&technology and their related subjects as nutrition, agriculture, food safety, food originated diseases and economic aspects.

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## ***From The Editor***

*Istanbul Aydın University Faculty of Engineering has started to publish an international journal on Food Engineering, denoted as “International Journal of Food Engineering Research (IJFER)”. We have especially selected the scientific areas which will cover future prospective food engineering titles such as Food Processing, Food Preservation, Novel Technologies, Food Safety, Food Quality etc. and their related subjects as nutrition, food and health, agriculture, economic aspects and sustainability in food production.*

*We have selected only a few of the manuscripts to be published after a peer review process on many submitted studies. Editorial members aim to establish an international journal IJFER, which will be welcomed by Engineering Index (EI) and Science Citation Index (SCI) in short period of time.*

***Editor in Chief***  
***Prof. Dr. Güner ARKUN***

# International Journal of Food Engineering Research (IJFER)

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# Contamination of Dioxins in Foods

Banu Eşder<sup>1</sup>, Güner Arkun<sup>1,2</sup>

## Abstract

Dioxin and dioxin-like compounds are complex chemical materials occurring as intermediates and by-products during the production process of chlorination in many chemical industries. Dioxins can conserve their stable chemical structure in natural environment due to their hydrophilic physical character and low solubility in water. They accumulate in both food and living body because they have lipophilic physical character that makes them be able to dissolve only in fatty tissue, their catabolism can occur only at higher temperatures. Since the mechanism of disintegration in the living body is so difficult, dioxins lead to many adverse effects on human health and cause diseases such as cancers, chloroacne, wasting syndrome, growing abnormalities, congenital anomaly etc. It is very important to determine the presence and levels of the dioxin complex compounds in both ecosystem and industrial processes according to the statutory legislation for protecting the health of living beings and preventing environmental pollution.

**Keywords:** *Dioxin, dioxin-like compounds, human health, toxicity, Dioxin analyzing techniques*

## Introduction

In parallel with the rapid development of the industry, it is observed that industrial production worldwide has also increased on a global basis. As a result of these developments, the number and diversity of the product portfolio that we use in our daily lives have also increased. Apart from the products used, many unwanted byproducts are produced as a result of the industrial production processes and also as a result of user wastes. [11] If these byproducts are not eliminated by recycling, they return into our living sources; water, soil, air and food sources and also they adversely affect the general health of people.

Dioxins are colorless, odorless, and crystalline solids containing C, H, O, and Cl, which are formed, unintendedly, in consequence of the exposure of organic compounds to high temperatures in the presence of chlorine during industrial operations, and are also water-soluble and volatile chemical compounds. These components were produced exclusively until 1970 and were used in many branches of the industry. Despite being banned, after many years, they can still be found in systems and in many environments.

In this article, general information about the chemical and physical properties of dioxins; their sources, toxicity, how they affect our lives and their negative effects on living life is given, and also their importance for our country, the communicate and regulations and how the components of these chemicals are analyzed in the food will be reviewed.

## Chemical and Physical Properties of Dioxins

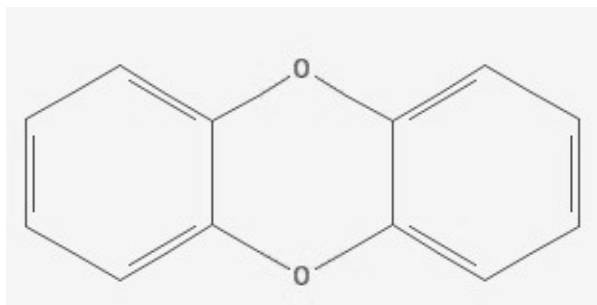
The term “dioxin” is a common name given to two large chemical groups with similar structures but containing a chlorine molecule in different proportions; there are a variety of different dioxins because chlorine is found in different positions in these complex chemical molecules. The simple structure formula of dioxins is dibenzo-para-dioxins (DD) molecule. Dioxins and similar compounds are formed by the para-carbon atoms of the two benzene rings combining with 2 oxygen atoms in 3 different ways and have a 3-ring structure. [14] These compounds are also present in the environment as dioxins in tetra, penta, hexa and octatypes. There are 75 types of complex chemical groups of dioxin classes: 2 monochloro dibenzo-p-dioxins (MCDDs), 10 dichloro dibenzo-

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p-dioxins (DCDDs), 14 trichloro dibenzo-p-dioxins (TrCDDs), 22 tetrachloro dibenzo-p-dioxins (TCDDs), 14 pentachloro dibenzo-p-dioxins (PeCDDs), 10 hexachloro dibenzo-p-dioxins (HxCDDs), 2 heptachloro dibenzo-p-dioxins (HpCDDs) and 1 octachloro dibenzo-p-dioxins (OCDD). The numbers in the naming of complex structures vary depending on where the chlorine atom is located in the structure of the compound. [15]



**Picture 2.1.1:** Chemical structure of dibenzo-p-dioxin(DD)molecule(URL 1)

There is a strong bond between the number of chlorine in the dioxins and the position of the molecule in the molecule to the stable structure; it is generally known that those containing 4 or more chlorine atoms form a more stable structure than other types of molecule structures, which makes their catabolism very difficult. Another characteristic of the chlorine atoms found in dioxins is that they play a role in determining the toxicity of the structure. When the number of chlorine molecules is high, the toxicity is high because the catabolism in the liver becomes significantly difficult. In other words, chlorine atoms make up the structure of the molecule that has a higher electron affinity.

As a result, these complex molecules bind to Ah receptors in the body and cause the formation of carcinogenic effects to start. When dibenzo-p-dioxin (DD) molecule is examined, the structure can be easily catabolized as a result of the absence of chlorine in the atomic structure, and in the liver turns into mercapturic acid removed from the body by way of excreting the toxic property

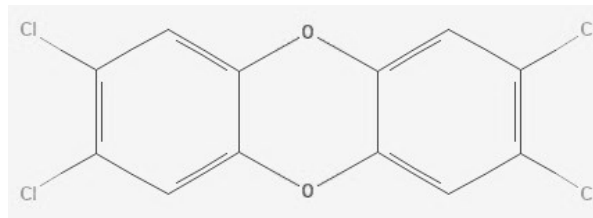
that is less than others. Other dioxins containing chlorine atom cannot be catabolized in the liver and excreted from the body, therefore they become accumulated in the body; this type of pollution is called “persistent organic pollution (POP)”.

The chemical and physical properties of the whole family of dioxin chemical complex compounds were not investigated, and it was determined that dioxins have hydrophobic properties that are not soluble in water and are not dissolved in water. For this reason, the catabolism in the living structures are very difficult; and these compounds, containing lipophilic properties, can be stored in the fat tissues of living organisms and are also called “environmental contaminants” which are stable in nature. [1]

In order to name a compound as dioxin, it must contain the following properties:

1. The molecule must combine with 2 oxygen atoms of carbon atoms of the para position of two benzene rings in 3 different ways to represent 3-ring structural properties,
2. The molecule must combine to Ah receptors,
3. It should be able to accumulate the compound by showing lipophilic properties, and thus by passing through the food chain without collapsing between living things,

Dioxins are considered to be the most toxic chlorinated organic compounds, and the most toxic ones are tetra-, penta- and hexachloro chemical complex compounds in the groups (2,3,7,8-tetra-p-dioxins (TCDD), 2,3,7,8-penta-p-dioxins (PeCDD)... as.). [2]



**Picture 2.1.2:** Chemical structure of 2,3,7,8-tetra-p-dioxin (TCDD) molecule (URL 2)

The physical properties of dioxins are used for describing how they can be found in the environment. Because dioxins and their derivatives have hydrophobic properties, they do not dissolve much in water and therefore have the low vapor pressure. Due to these properties, they are able to dissolve very quickly to the gas phase in the atmosphere more than they can in water.

They are resistant to environmental conditions because of the high temperatures and stable compounds of dioxins, thus they can increase their concentration in the living body and cause toxic effects to be observed over time.

The chemical and physical properties of dioxin complex molecular species are examined in detail and given in Table 2.2.1;

**Table 2.2.1:** Properties of Dioxin Compounds

Chemical & Physical Properties	TrCDD	TCDD	PeCDD	OCDD
Molecular Weight	287.5	322	356.4	459.8
Color	Colorless White	Colorless	Colorless	N/D
Physical State	Solid	Crystal/Solid	Solid	N/D
Melting Point	163 °C	306 °C	206 °C	332 °C
Boiling Point	374 °C	446.5 °C	N/D	510 °C
Density (at 25°)	N/D	1.827 g/mL	N/D	N/D
Solubility Water (at 25°)	4.75x10 <sup>-3</sup> mg/L 20°C	3.2x10 <sup>-8</sup> mg/L 20°C	1.18x10 <sup>-8</sup> mg/L 20°C	7.4x10 <sup>-8</sup> mg/L 20°C
Vapor Pressure (at 25°)	6.46x10 <sup>-8</sup> mm Hg	7.4x10 <sup>-10</sup> mm Hg	6.6x10 <sup>-10</sup> mm Hg	8.25x10 <sup>-13</sup> mm Hg
Henry's Law Constant (at 25°)	37.9x10 <sup>-6</sup> atm.m <sup>3</sup> /mol	101.7x10 <sup>-6</sup> atm.m <sup>3</sup> /mol	2.6x10 <sup>-6</sup> atm.m <sup>3</sup> /mol	6.74x10 <sup>-6</sup> atm.m <sup>3</sup> /mol
Degradation (Atmospheric Lifetime)	0.9 day	2 days	2.4 days	9.6 days
Conversion factors in air at (25°/760 mm Hg)	1 mg/m <sup>3</sup> = 0.0850 ppm 1 ppm = 11.76 mg/m <sup>3</sup>	1 mg/m <sup>3</sup> = 0.0759 ppm 1 ppm = 13.17 mg/m <sup>3</sup>	1 mg/m <sup>3</sup> = 0.0686 ppm 1 ppm = 14.58 mg/m <sup>3</sup>	1 mg/m <sup>3</sup> = 0.0532 ppm 1 ppm = 18.81 mg/m <sup>3</sup>

### **Sources of Dioxins**

The paper production industry is shown as one of the most important sources that cause dioxin pollution in the environment. The paper containing chlorophenol chemicals used in the packaging process for the preservation of raw materials contaminates the environment.

Three types of chemical manufacturing processes—bleaching of wood pulp in paper manufacturing, chlorine, and chlorine-derivative manufacturing, and halogenated organic chemical manufacturing—lead to the production of DLCs; DLCs, primarily the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Due to the formation of high-rate carcinogenic byproducts in result of these processes, restrictions have been introduced in many countries and in the new century many research studies related to this subject has been carried out. The industrial processes especially applied in Scandinavia, the USA, Canada and Sweden are changed to ECF bleaching method, in other words, using chlorine dioxide chemical production and/or TCF bleaching method, that is, chlorinated processes are preferred.

The amount of chlorine atom charge increases depending on the amount of salt increased in the logs carried overseas. It is also known that hydrocarbon structure, such as lignin in wood, has an important effect on dioxin formation and, during overseas transportation, it creates chlorinated dioxin molecules that are more dangerous in a chemical process with chlorine atoms. In addition, it pollutes the environment with liquid wastes and/or atmospheric conditions and affects the food chain. [16]

During the production of various chlorophylls used as fungal, insecticide and bactericide, dioxin is released as a by-product especially in the presence of copper. [22] As a result of many pesticides and industrial-chemical processes, chlorophenols and chlorophenolic herbicides (2,4,5-T) are released as byproducts. The determination of these products was based on the years 1986, and these and similar by-product formation processes have been banned in today's manufacturing processes.

There are TCDD isomers at 2000-5000 ppm levels in commonly used pharmaceutical materials (Medicine, Dentistry, and cosmetic products).

Recently, polymer-based products are used for making our lives easier. The products that may contain dioxin are plastic plates and glasses, PET bottles, foam materials, paper tissues, milk and juice cartons, children's diapers and napkins. [4] These materials i.e. plastic bottles containing liquids, being exposed to high temperatures –kept under the sun, hot liquid contact, over keeping in the package-may result in contamination of dioxin.

Natural disasters such as the eruption of volcanoes and forest fires are also result in dioxin release to the atmosphere. In addition, as a result of the combustion reactions of hospital (medical waste), home, sewage, and city residues, many dioxins are exposed to the air and, as stated previously, they can be dispersed to distant areas by atmospheric formations such as air currents, winds, rain, etc. from the environment where their destructions/ degradation are not very easy [22]. The largest source of dioxin contamination in the atmosphere is the OCDD and its components (Germany 47% and Netherlands 82%).

Ignition processes are one of the primary sources of industrial dioxins. [3] As a result of many burning reactions, organic and inorganic chlorinated dioxin chemical sources are obtained as byproducts. Organic materials that are not fully burnt provide a ready environment for the presence of carbon in ash and the formation of dioxin-form chemical compounds. More organic matter and less burnt matter contribute to the formation of a high-form dioxin chemical complex. The tempering of industrial products especially metals at high temperatures (higher than 350 °C), production and casting processes as well as recycling processes for scrap metals may also produce dioxine as a result of chemical processes caused by high temperatures [12]. However, at temperatures above 800 °C, dioxins can easily be degraded.

Other potential products of dioxin can be listed as engine and mineral oils, fluids and hydraulic fluids, paint and wood preservatives etc. In addition, dioxin is widely used in the cosmetic and drug industry as well as detergents, cleaners, shampoos, shower gels and liquid soaps due to its foaming/effervescence properties. The packaging of dioxin-containing products is especially suitable for peg, oksinol, nonoxynol, polysorbate60, polysorbate80, polyethylene glycol, polyethylene, polyoxyethylene. Furthermore, the carcinogenic chloroform chemical and dioxin complex component is formed by the reaction of the chlorine ions in water due to the use of the triclosan chemical in the packaging in antibacterial soaps, deodorants, toothpaste and shower gels.

## Dioxins Toxicity and Toxicokinetics

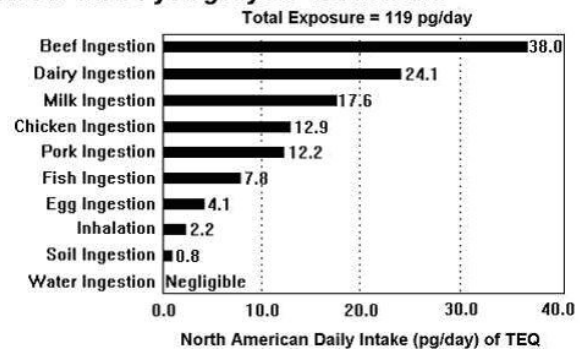
### Dioxins Toxicity

All dioxins are not equally toxic. Only 7 out of 75 dioxins have a high level of toxicity. The most important factor for these chemicals to cause a toxic effect in human body is the compatibility of the molecule with the “AhR receptor”. The chemical attached to the receptor is more toxic than the loose bound. [14] In determining the toxicity rates of the dioxins, the basis of the species known to be the most toxic and its derivatives, and toxicity equivalence factor (TEF) are considered. For this reason, the TEF value of the most toxic derivative which is TCDD is 1; whereas the least toxic PCDD type of dioxin is 0,1. In addition, toxicity values are indicated by TEQ i.e. Total Dioxin Equivalence Factor. According to the World Health Organization (WHO), the TCDD dose values, which can be administered daily as a result of research, has been reported to be 1.4 pg/kg TEQ for humans.

When samples taken from the atmosphere after various burning processes were examined, maximum values of 926 g (1990) and 3870 g (1989) were observed in west Germany and England, respectively. These chemicals are then stored in the air and/or soil by emission and then accumulate in the bodies of living organisms, including the food chain, exposing them to toxic effects. [1] An example to explain the effects and

damages of dioxins is the possibility of health problems caused by the cows grazing near burning ovens in Germany. When an accumulation of fatty tissues was examined, it was determined that dioxin is not excreted from the bodies of cows and instead stored in the milk, due to the lipophilic properties of the molecule, during the metabolism activities of the animal while grazing.

### This is where you get your dioxin from:



**Picture 2.1.5:** Daily dioxin consumption TEQ amounts in North America [18] (Picture 2.1.5).

This graph shows the amounts in picograms of nutrients and TEQ that enter the human body by dioxin complex molecule on a daily basis.

Fish products is thought to be contaminated with dioxins through waste water. It was determined that dioxins exist in rain, erosion and industrial wastes were combined with suspended substances in the water, and in the sediment of water in the bottom of the water resources such as lakes, oceans, and rivers. Fish living in these kind of environments may be contaminated with dioxins. In this way, dioxins enter the food chain. As a result of a research conducted on 25 fish farms using commercial feed, it is stated that fish contains more dioxins than salmon that live in other natural environments.

Dioxins, on the other hand, were found to be not present when fish were washed with water since it accumulates in the leaf-wrapping wax layer.

It has been determined that there may be contamination in plastic packaging and that it may pass to people through milk products. According

to the studies, 8 PVC food packaging materials were examined for the presence of dioxin and it was determined that the PVC had dioxin 2.6-6.9 ng TEQ values per kilogram. The researchers reported that the maximum amount of dioxins in food is estimated to be 0.07 ng TEQ, due to the risk of carrying. [13]

### **The Effects of Dioxins on Human Health**

Dioxin hazards can also be carried from one living organism to another through contamination. The transfer of dioxin to feed, animal and animal food products by air and/or soil in the environment through the human consumption continues.

One of the most dangerous properties of dioxin compounds is that their destruction is difficult, in other words, they are resistant to biological, photolytic and chemical degradation. In this way, they can reach the highest points of the food chain at high concentration without any loss of quantity, and can also be carried away from the environment by means of atmospheric transport mechanisms.

Synthesis and/or production of dioxin complex molecules in any industrial or laboratory environment is prohibited except for the diffusion of the environment through transport. Dioxins can only be analyzed in a laboratory environment, although it is not legal. Dioxins in nature are formed as a result of the exposure of chemical substances to high temperatures during chemical processes. Most of the compounds formed by air are found at high levels in the soil, especially by accumulation/storage method in fat tissues. These complex molecules, which are then transferred to plant sources through the intake of nutrients from the soil, can rise to the upper levels in the food chain through the consumption of contaminated plants by animals. Due to the lipophilic properties, the molecules that accumulate in the fat tissues of animals, which are stable structures, are exposed to dioxins by taking both animal and plant-based foods without being destroyed. It has been determined that there is more accumulation of dioxins than those with high-fat content. Biological half processes were for about a few years for TCDD, recently it is 7-8 years and for PCDF 13 years. [18] Dioxins

are metabolized primarily in the liver, converted to water-soluble complex compounds, and excreted by 30% out of the body through urine.

It can be transferred to humans through packaging, drinking water as well as respiratory and skin contact. This is because of the fact that 90% of dioxin poisoning in humans being due to food chain. [11]

Abnormalities seen in humans due to dioxins are observed if the amount of toxins accumulated in the body is higher than the specified dose (14ng/kg). If the concentration of dioxin in the body is less than this value, it is converted to water-soluble complex compounds in the liver and excreted through urine; however if it is high and passes through the bloodstream by any means, they may cause DNA mutations. These cancer cells do not show up in the standard tests until they have multiplied to a few billion. Dioxins have been shown to cause major digestive, liver and breast cancers, and have caused abnormalities such as defective kidney formation. In addition, cardiovascular toxicity due to dioxins, nausea, difficulty in breathing, high blood pressure, asthma and acne-style lesions may occur in the skin. [1,9]

Women who are exposed to dioxins pass this chemical to their babies through breastfeeding. Thus, babies are under the possible risk of dioxin exposure [19]. During the lactation process, babies take the dioxins accumulated in the fatty tissues of mother's milk into their bodies [20] and according to a study conducted in Japan, it causes mental retardation and cognitive retardation in 8-year-old children in the sense declining their mental ability [21].

The most prominent example of dioxin poisoning is the case of Victor Yushchenko, the strongest candidate in the elections of 2004 in Ukraine. In a few weeks, the lesions developed in the form of acne on the face and his skin became grayish. Yushchenko's body contained 50.000 times more dioxins than the level that human body can tolerate. (Picture 2.1.6)



**Picture 2.1.6:** Vicky Yushchenko, a candidate in Ukraine's presidential election, was poisoned by dioxin (www.basinbulteni.com, 14 November, 2016)

TCDD type dioxin was found in his body, which was also used in many civilians during the Vietnam War. In addition, after the industrial accident in 1976 (Seveso/Italy) during the examination in the region chlorine-linked acne was found to occur on many people and children's faces. 2,3,7,8-TCDD dioxin derivatives spread to the area of 15 square meters affect about 37,000 people and cause poisoning cases; 3300 animals were found dead and 447 people have skin lesions caused by serious diseases. [10]

The mean daily TEQ intake was determined as 6.3 pg/kg body weight in males and 6.1 pg/kg body weight in females between the ages of 1 and 11. This study shows that the TEQ intake decreases as the age progresses. It was determined that 3.5 and 2.7 TEQ pg/kg body weight levels were found to be in the range of 12-19 ages of men and women; while 2.4 and 2.2 pg/kg body weight concentrations were found among men and women at 20-79 ages, respectively. Men who were 80 years old and above found to contain 1.8 pg/kg body weight, in women 2 pg TEQ intake/kg body weight was determined. These results show that more TEQ intake is possible in new born infants and children at growing ages with low body weight [26]. Toxic equivalence of dioxins are given in (Picture 2.1.7.).

2,3,7,8-TCDD	1	2,3,7,8-TCDF	0,1
1,2,3,7,8-PeCDD	1	2,3,4,7,8-PeCDF	0,3
		1,2,3,7,8-PeCDF	0,03
1,2,3,4,7,8-HxCDD	0,1		
1,2,3,6,7,8-HxCDD	0,1	1,2,3,4,7,8-HxCDF	0,1
1,2,3,7,8,9-HxCDD	0,1	1,2,3,7,8,9-HxCDF	0,1
		1,2,3,6,7,8-HxCDF	0,1
1,2,3,4,6,7,8-HpCDD	0,01	2,3,4,6,7,8-HxCDF	0,1
OCDD	0,0003	1,2,3,4,6,7,8-HpCDF	0,01
		1,2,3,4,7,8,9-HpCDF	0,01
		OCDF	0,0003

(T = tetra, P = penta, Hx = hexa, Hp = hepta, O = octa)

**Picture 2.1.7:** Toxic Equivalence of Dioxins (World Health Organization)

#### Analysis Methods of Dioxins

Chromatographic techniques are generally used as the analysis methods of dioxins.

Mainly two methods are commonly used in dioxin analysis;

- HR-GCMS; to determine the source,
- Biotests; contamination frequency, location determination, and size

However, since it has complex structured molecules, specific sample preparation procedures are applied only after a series of different sieving process. The analytical stages are given below.

**1. Dioxin extraction processes;**

The weighing of the sample (the weighing of the sample depends on the fat oil ratio of the analysis sample.)



Adding cyclohexane, propanol (to enable the oil and dioxins in the oil phase to be transferred to cyclohexane phase with solvents)



Evaporation in order to obtain oil



**Picture 2.1.8:** Econo Prep Equipment

Separation of the obtained oil into dioxin and PCB fractions with FMS device

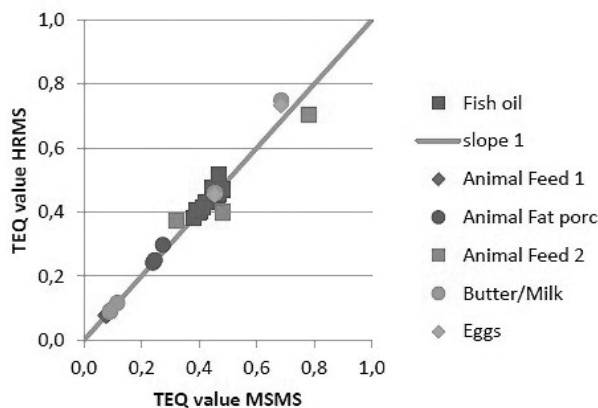


Obtaining data and evaluation process



**Picture 2.1.9:** APGC/LC-MS-MS Equipment (Waters)

The classical method used in dioxin analysis is GC (Gas Chromatography) System and GC-MS (Gas Chromatography combined with Mass Chromatography) System for scanning purposes. GC-MS-MS gas spectroscopy-tandem mass spectroscopy is an approved method of analysis according to EU regulations No. 589/2014 and No. 709/2014, June 2014. The accuracy of the sensitivity measurements and the accuracy of the results of the analyses performed in mass spectroscopy depends on the number of ions measured. For the samples with relatively low detection limits needed for dioxin analysis, analysts have caused the need for the technologies that do the detection at the trace level. Therefore, GC-MS-MS devices developed to achieve higher quality results with developing technology, have been produced with high-efficiency electronic ionization sources which produce more ions. Figures 2.1.8, 2.1.9)



**Picture 2.1.10:** TEQ values for GC-HRMS and GC-MS results (ng/kg) [25]

Available methods for the determination of dioxin and dioxin-like substances are specified in the relevant American and European regulations [23, 24]. The methods used in the analysis are carried out using the EPA 1668B and EPA 1613 methods determined by the United States Environmental Protection Agency (EPA).

Two analysis methods are used within the scope of dioxin analysis

- Dioxin analysis in fixed-source emissions (TS EN 1948/2-3)
- Dioxin analysis in food samples (EPA 1668B/EPA 1613)

In dioxin analysis of fixed-source emissions, it is possible to scan and detect toxic dioxins in the area where they exist. This analysis provides work safety in enterprises. Air, water and soil cleaning of areas from production units is determined through these analyses. Various chemical, physical and microbiological tests are applied to samples taken from these areas and their suitability is determined.

As with all analytical methods, the sample preparation phase is extremely important in dioxin analysis. It is known that consumption material costs and solvent consumption rates in the automatic systems used to prepare dioxin samples are higher than the manual (classical) methods. For this reason, many large and/or small laboratories are using dioxin analysis methods and they complete the sample preparation phase by using classical methods. However, as a result of some analytical studies carried out with the help of the latest technology, classical sample preparation methods are changed towards less expensive automated methods. Also, since this automatic sample preparation method is applied to all matrices, users can easily apply it to their different samples.

### **Legal Regulation of Dioxins on Food**

Dioxins are very stable complex compounds including hydrogen, carbon, oxygen and chlorine atoms in their structures. As a result of the research study carried out, it was determined that they were harmful and triggered by the carcinogenic effect mechanism. These effects, especially as a result of plastics to high temperatures are revealed and many restaurant companies have abandoned the use of plastic and paper products. However, there are some legal gaps in Turkey concerning these matters.

While there are legal sanctions in developed countries on this issue, there is not enough dedication and resources for the implementation of deterrent official procedures in Turkey. In European

countries, there have been restrictions on the use of plastic materials in contact with food, adhered to certain rules. However, there is limited control in Turkey. In our country, these issues need to be dealt with more care and the necessary legal regulations and control systems should be established.

Analysis of dioxin complex chemicals is carried out by the official laboratories of the Ministry of Food, Agriculture and Livestock in Turkey on the foods to be imported and exported.

The levels of dioxins obtained as a result of analyses are evaluated according to the values defined in the regulations on foods i.e regulation on the contaminants of the foods of Turkish Food Codex and the regulation on undesirable substances in Feeds. During exportation of foods, when the product parties belonging to the samples analysed for dioxins and found to be contaminated at exceeding levels are rejected in importation and export and recalled as the result of official controls. When the high levels of dioxins are determined in the parties, the parties are collected and destroyed [6].

EFSA and WHO, the leading work institutions in Europe, continue their research on dioxins and its derivatives worldwide by increasing their research on dioxins and their effects on human health, such as chemical structure, toxicity and the effects on human health. The European Union organizes meetings where certain groups are involved in this issue and conduct studies to determine the maximum daily dioxin level that a person can be exposed to and non-toxic on a mass basis, including the age/gender discrimination arising from the research.

Dioxin and its derivatives are included in the carcinogenic substances group by (EPA) and the (WHO). The American (EPA) states that as a result of experimental studies conducted in guinea pigs, the higher levels of dioxin molecule that can cause harm to humans, the higher the risk of cancer in the body. This value was determined as the lowest dioxin load of 14 ng/kg.



The European Parliament has increased the pressure to create legal legislation to limit the use of this toxic substance, and urged some institutions to take action and emphasized that it should be implemented by using different methods as a result of the technological developments and elaborately examining, developing, and even taking the necessary measures for further analysis of the methods of analysis.

In addition, the world market, especially in Poland and the Czech Republic some exported products through the transmission of meat and animal products a result of analysis dioxin chemical substances containing processed meat and animal products were collected from the market and destroyed.

As a result of such developments, Japan, which is one of the countries concerned about the quality of the products imported to their countries and the health attributes, has reported that it has increased the control of food imports from Germany. Some products imported from Germany as a result of previous research and analysis are pork and pork processed products as a result of the discovery of dioxin chemicals, and thus dioxin was ordered to be analyzed in the imported products.

In the guar gum, which is also known as guarana, the members of the European Union have determined that there is dioxin at the highest values due to the analysis of the guar bean legume, which is also known as guarana, obtained from India. For this reason, following the inspection of suppliers, they increased the frequency of analysis of imports and included some criminal procedures in order to take more deterrent measures for firms. Guaran, guaran beans; dairy products (especially yogurt), and mayonnaise are used quite often, and as a result accumulation of toxic effects have been determined in the body human.

While the other countries in the world, especially European countries are taking more positive steps and critical measures on this issue, Turkey's attitude about this issue is relatively slow and not constructive with respect to taking precautions.

Thus, concerning the dioxins, necessary controls and precautions must be regulated and performed in terms of food safety.

## **CONCLUSION**

Today, toxic chemicals continue to threaten our health and environment all over the world. In other words, it is almost impossible to be not exposed to chemicals in our daily life. It is known that continuous industrial processes and their products pollute our environment and our lives. The chemical complex components of dioxin, which has been in our lives for almost thirty years, is among the most harmful chemicals.

Dioxins are not found naturally in the environment; they are formed as a result of various industrial processes. Their harmful effects on the environment and human are well-known. Dioxins are released as main and/or by-product in result of a process in which they are involved combustion reaction in the presence of chlorine during the production of a substance containing chlorine and bromine. The process of burning and burning in industrial processes, processing and forming of metal and similar products, disposal of solid wastes, bleaching and cooking of paper pulp are the results of the conversion of chlorinated compounds into dioxin complex components.

When the dioxins are formed, atmospheric weather conditions and/or water can be released to areas far away from the environment and thus can be accumulated in soil and plants in solid or gas phase. Dioxins being hydrophilic, the substances they have a dense accumulation in animal tissues and soil. As a result of this accumulation, they easily enter in the food production chain through consumption of contaminated products. This is why the entry of dioxin into the human body is taken by means of animal foods such as meat, chicken and fish, and animal products such as milk and eggs.

In order to provide a healthier society and a clean environment for future generations, everyone needs to be more sensitive about it. The most important task falls to the food authority and

senior officials of the countries. In particular, waste that causes pollution through pre-treatment process and disposals. According to the types of industrial processes, such as burning and control of burning processes and control of foods for dioxin contamination levels. In addition, preparation of regulations, official control of dioxins in foods; developing and using reliable methods of analyses are the main activities in order to prevent and control of dioxins in terms of the food safety.

## REFERENCES

- [1] Arıkan, D., Yetim, H., Sağdıç, O., Kesmen, Z. 2009. Dioxin contamination in food and its effects on human health. *Journal Of Food Technologies*, 12, 9-15.
- [2] M. Van den Berg, L.S. Birnbaum, M. Denison, M. De Vito, W. Farland, M. Feeley, H. Fiedler, H. Hakansson, A. Hanberg, L. Haws, M. Rose, S. Safe, D. Schrenk, C. Tohyama, A. Tritscher, J. Tuomisto, M. Tysklind, N. Walker, R.E. Peterson, 2005 re-evaluation of human and mammalian genetic equivalence factors for dioxin and dioxin-like compounds of the World Health Organization, *Toxicology. Science*. 93 (2006) 223–241.
- [3] RN. Hoover. Dioxin dilemmas, *J Natl Cancer Inst.*, 91:745-746, 1999.
- [4] G. Bröker, P. Bruckmann, H. Gliwa, Study of dioxin sources in North Rhine-Westphalia, *Chemosphere*, 38 (8): 1913-24, 1999.
- [5] Carcinogenic substances in chicken feed, (no date)  
<http://www.ntvmsnbc.com/id/25167591/received> from the address.
- [6] The additives used in products covered within the scope of this communiqué must be in compliance with Annex 1 of this communiqué and 2nd section of the “Turkish food Codex regulation”, 29.12.2011 date ve 28157 Number Official Gazette (no date)  
[http://www.gkgm.gov.tr/mevzuat/kodeks/kodeks\\_yonetmelik/bulasanlar\\_yonetmelik.html](http://www.gkgm.gov.tr/mevzuat/kodeks/kodeks_yonetmelik/bulasanlar_yonetmelik.html) received from the address.
- [7] J. Tuomisto, T. Vartiainen and J.T. Tuomisto, Synopsis on Dioxins and PCBs, National Institute for Health and Welfare, (no date)  
<http://www.thl.fi/thl-client/pdfs/81322e2c-e9b6-4003-bb13-995dcd1b68cb> received from the address.
- [8] P. Boffetta, K.A. Mundt, H.O. Adami, P. Cole, J.S. Mandel, TCDD and cancer: a critical review of epidemiologic studies, *Crit. Rev.Toxicol.*, 41(7): 622-36, 2011.
- [9] F. Şahbaz, J. Acar. Dioxins and the Dioxin contamination possibility on Foods, *Food*, 18 (4) 243-245, 1993.
- [10] Dioksin Danger; Are the dioxins the most dangerous chemical in our environment? (no date)  
[http://en.opasnet.org/w/Are\\_the\\_dioxins\\_the\\_most\\_dangerous\\_chemicals\\_in\\_our\\_environment%3F](http://en.opasnet.org/w/Are_the_dioxins_the_most_dangerous_chemicals_in_our_environment%3F) received from the address.
- [11] Yalçın, H., 2015. Dioxin and polychlorinated biphenyls. *Clinics In Turkey, J Food Hyg Technol – Special Topics.*, 1, 38-47.
- [12] O. Çiftçi, Investigation of dioxin and similar compound levels in the butter consumed in elazığ and its environments, *Firat UniversityJournal of Health Care*, 22(5):289-292, 2008.
- [13] Lau, O. W. and Wong, S. K. 2000. Contamination in food from packaging material. *J. of Chromatography A*, 882 (1-2), 255-270.
- [14] Hişmioğulları, Ş.E, Hişmioğulları, A.A., Aşkar, Kentaş, T. 2012. Toxic effects of dioxin and similar chemicals. *Balıkesir Journal of Health Care*, 1, 23-27.
- [15] Baytok, E., Bingöl, N.T. 2013. The toxins that enter our food table and our lives: dioxin. *Journal of YYU Vet.*, 24, 45-49.
- [16] Vural H., 1995. Dioxin and furan isomers in terms of food pollution. *Ecology Environment Derg.*, 15, 45-49.

[17] Güneş G. 2007. Dioxin and furan formation mechanisms and removal technologies. Master's Thesis. Yildiz Technical University, Graduate School Of Natural And Applied Sciences, Department Of Environmental Engineering. İstanbul.

[18] Güler, Ü.A, Kundakçı, Ö. 2014. The effects of dioxin and similar compounds on human and environmental health, *Karaelmas Journal of Science and Engineering*, 4, 71-75.

[19] Gürsoy, O. 2001. Dibenzodioxin (PCDDs) and furan ( PCDFs) compounds and their importance in milk and products. *Journal of Engineering*, 2, 234-241.

[20] Ulaszewska, M.M., Zuccato, E., Davoli, E. 2011. PCDD /Fs and Dioxin-Like PCBs in human milk and estimation of infants' daily intake: A review. *Chemosphere*, 83, 774-782.

[21] Sun, S.J., Zhao, J.H., Liu, H.J., Liu, D.W., Ma, Y. X., Li, L., Horiguchi, H., Uno, H., Iida, T., Koga, M., Kiyonari, Y., Nakamura, M., Sasaki, S., Fukatu, H., Clark, G.C., Kayama, F. 2006. Dioxin concentration in human milk in Hebei Province in China and Tokyo, Japan: Potential dietary risk factors and determination of possible sources. *Chemosphere*, 62, 1879-1888.

[22] Şahbaz, F., Acar, J. 1993. Dioxin and dioxins are likely to infect food. *Hacettepe University Faculty of Engineering Department Of Food Engineering*, 18, 243-245.

[23] Uçar, Y. 2015. The effect of yogurt and kefir fermentation on dioxins, furans, polychlorinated biphenyls and indicator polychlorinated biphenyls, *Ankara University Institute of Science and technology*, Ph.D. thesis, 97 s.

[24] Uçar, Y., Traag, W., Immerzeel, J., Kraats, C., Van der Lee, M., Hoogenboom, R., Van der Weg, G., Celik Cakirogullari, G., Oymael, B., Kilic, D. 2011. Levels of PCDD/Fs, PCBs and PBDEs in butter from Turkey and estimated dietary intake from dairy products. *Food Additives and Contaminants, Part B Vol. 4, No. 2*, 141–151.

[25] Shimadzu GCMS ApplicationNote GCMS-TQ8030 (SCA\_280\_079) Quantitative analysis of Dibenzo-p-dioxins (PCDD) and polychlorinated-p-Dibenzofurans (PCDF) in food and feed with the tandem mass spectrometer (03/05/2018)

[https://www.chromnews.com/tr\\_tr/optimize-edilmis-eser-seviyede-dioksin-analiziyle-gida-zincirini-koruma-ve-mevzuata-uyumluluk-saglama/received-from-the-address](https://www.chromnews.com/tr_tr/optimize-edilmis-eser-seviyede-dioksin-analiziyle-gida-zincirini-koruma-ve-mevzuata-uyumluluk-saglama/received-from-the-address).

[26] Anonymous, 2000 (03/05/2018)

[http://ec.europa.eu/dgs/health\\_consumer/library/pub/pub08\\_en.pdf](http://ec.europa.eu/dgs/health_consumer/library/pub/pub08_en.pdf) received from the address.

## INTERNET SOURCES

**URL 1**><https://pubchem.ncbi.nlm.nih.gov/compound/68512548#section=2D-Structure>

**URL 2**><https://pubchem.ncbi.nlm.nih.gov/compound/15625#section=2D-Structure>

# 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 °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 (Majjala, 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 °C, 2 -20 sec.
- LTLT (Low-Temperature Long Time) Pasteurisation, at 62-65 °C, 30 –32 min.
- HTST (High-Temperature Short Time) Pasteurisation, at 72-75 °C, 15 – 30 sec.
- HP (High Pressure) Pasteurisation, at 85-127 °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.

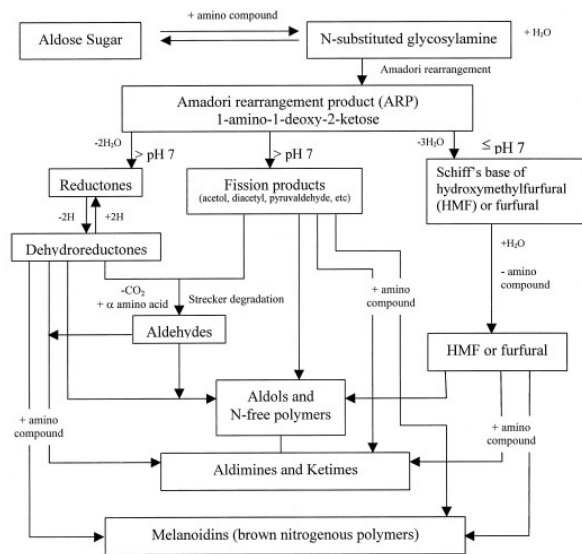
Hydroxymethylfurfural (HMF) is one of the most common MR by-products in the over-processed foods. In several food products, the HMF level is used as an indicator of the absence of thermal processing. In a research study, researchers have examined over five hundred food items and they have found high 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 lysine 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).



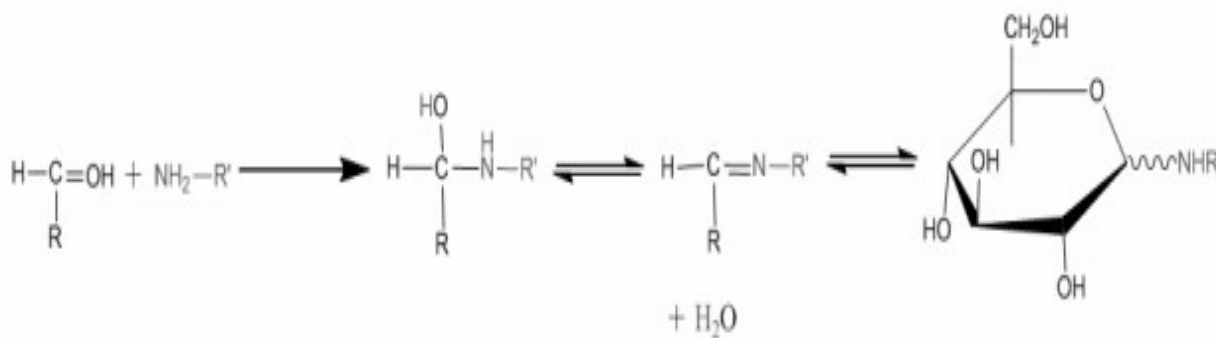
**Figure 1:** The schema of the Maillard reaction, adapted by Hodge (Martin et al., 2000)

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).

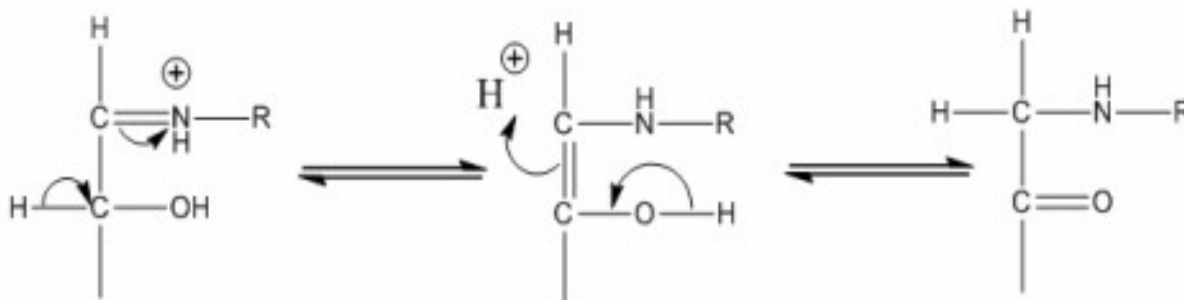
The second step is where the color change begins. This step might be followed by three different possible reactions. The first possible reaction forms the most important byproducts of the MR:

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).



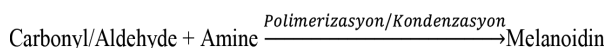
**Figure 2:** Condensation reaction



**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<sub>2</sub> 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<sub>2</sub>), 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).



**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 (lactulosylglycine) 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 lysine 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, carboxymethyllysine, 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<sub>2</sub>) 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

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

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).

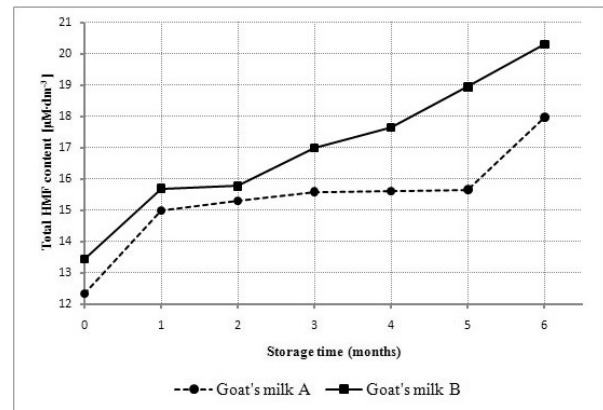
Hurtado et al. (1997) have analyzed powdered milk and liquid baby formula that have been kept at 37°C for six months including 12 samples of Spanish baby formula (six diluted and six liquid) and 10 samples of commercial powdered milk. All of the commercial products were analyzed before

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  $\mu\text{g} / 100 \text{ mL}$ , 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 ( $21 \pm 2^\circ\text{C}$ ) 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 °C (Table 3). The prepared solutions were kept in 20 mL light-proof bottles at 70 °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
<b>HMF</b>								
<b>3</b>	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
<b>5</b>	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.

## REFERENCES

- [1] Albalá-Hurtado, S., Veciana-Nogués, M. T., Izquierdo-Pulido, M., & Vidal-Carou, M. C. (1997). Determination of free and total furfural compounds in infant milk formulas by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 45(6), 2128-2133.
- [2] Ames, J.M., (1998). Applications of the Maillard reaction in the food industry. *Food Chemistry* 62(4): 431-439.
- [3] Antal, M. J., Mok, W.S.L., Richards, G.N., (1990). Mechanism of formation of 5-hydroxymethyl-2-furaldehyde from D-fructose and sucrose. *Carbohydr. Res.* 199, 91–109.
- [4] Anonim, (2000). Türk Gıda Kodeksi Çiğ Süt ve Isıl İşlem Görmüş İçme Sütleri Tebliği. T.C. Resmi Gazete, Sayı: 23964. Tarım ve Köyişleri Bakanlığı, Ankara.
- [5] Anonim, (2016). *Çiğ Süt Neden Isıl İşlemden Geçmeli*, Süt Hakkında Aklımızda bir şey kalmasın, <http://www.hurriyet.com.tr/paylas/sut-hakkinda-aklinizde-bir-soru-kalmasin-2361>.
- [6] Anet, E.F.L.J., (1964). 3-Deoxyglycosuloses (3-deoxyglucosones) and the degradation of carbohydrates, *Advances Carbonhydrate Research* 19; 181-218.
- [7] Anon., 2018 (<https://www.foodelphi.com/tag/sutun-tanimi/>, Accessed May 2, 2018).
- [8] Arena, S., Renzone, G., D'Ambrosio, C., Salzano, A.M., Scaloni, A., (2017). Dairy products and the Maillard reaction: A promising future for extensive food characterization by integrated proteomics studies. *Food Chemistry* 219: 477–489.
- [9] Baldwin, A.J., Ackland, J.D., (1991). Effect of preheat treatment and storage on the properties of whole milk powder. Changes in physical and chemical properties. *Netherland Milk Dairy Journal*, 45, 169-181.
- [10] Berg, H.E., Van Boekel, M.A.J.S., (1994). Degradation of lactose during heating of milk. 1. reaction pathways. *Netherlands Milk and Dairy Journal* 48: 157-175.
- [11] Burdurlu, H. S. and Karadeniz, F. (2003). Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry*, 80, 91-97.
- [12] Bolin, H. R. and Steele, R. J. (1987). Nonenzymatic browning in dried apples during storage. *Journal of Food Science*, 52(6), 1654-1657.
- [13] Chavez-Servin, JL, Castellote, AI, Lopez-Sabater, (2005). C. Analysis of potential and free furfural compounds in milk-based formula by high-performance liquid chromatography evolution during storage. *Journal of Chromatography A*, 1076: 133-140.
- [14] Coca, M., Garcia, M.T., Gonzalez, G., Pena, M., and Garcia, J.A., (2004). Study of colored compounds formed in sugar beet processing, *Food Chemistry* 86(3); 421-433.
- [15] Çelebi, I., (2006). Color formation in wheat starch based glucose syrups and use of activated carbons for sugar decolorization, a thesis of Master, Natural and Applied Sciences of Middle East Technical University, p: 27-33.
- [16] Daniel, J. R. and Whistler, R. L., (1985). Carbonhydrates. In 'Food Chemistry', O. R. Fennema (Ed.), second edition, Marcel Dekker, p. 70-137, New York.
- [17] Dmytrów, I., Mituniewicz-Małek, A., & Balejko, J., (2010). Assessment of selected physicochemical parameters of UHT sterilized goat's milk. *Electr. J. Pol. Agric. Univ. Food Sci. Technol.*, 13(2), 09.

- [18] Edward, W.P., (2000). The Maillard Reactions. In: *The Science of Sugar Confectionery*, Edited by W.P. Edwards, Royal Society of Chemistry Publication, Cambridge, pp. 9-13.
- [19] Evangelisti, F., Calcagno, C., Nardi, S., Zunin, P., (1999). Deterioration of protein fraction by Maillard reaction in dietetic milks. *Journal of Dairy Research* 66: 237-243.
- [20] Ferrer, E., Alegría, A., Courtois, G., Farré, R., (2000). High-performance liquid chromatographic determination of Maillard compounds in store-brand and name-brand ultra-high-temperature treated cows' milk. *Journal of Chromatography A* 881(1-2): 599–606.
- [21] Fox PF, McWeeney PLH., (2003). *Advanced Dairy Chemistry. Volume 1. In Chapter 1: Milk Proteins: General and Historical Aspects. Third Edition, Part A* New York, Springer Verlag Publish.
- [22] Friedman, M.; Molnar-Perl, (1990), I. Inhibition of browning by sulfur amino acids. I. Heated amino acid-glucose systems. *J. Agric. Food Chem.* 38, 1642-1647.
- [23] Haleva-Toledo, E., Naim, M., Zehavi, U., Rouseff, R.L., (1999). Effects of L-Cysteine and N-Acetyl-Lcysteine on 4-Hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol), 5-(Hydroxymethyl) furfural, and 5methylfurfural Formation and Browning in Buffer Solutions Containing either Rhamnose or Glucose and Arginine *J. Agric. Food Chem.* 47, 4140-4145.
- [24] Jansson, T., Clausen, M.R., Sundekilde, U.K., Eggert, N., Nyegaard, S., Larsen, L.B., Ray, C., Sundgren, A., Andersen, H.J., Bertram, H.C., (2014). Lactose-hydrolyzed milk is more prone to chemical changes during storage than conventional ultrahigh-temperature (UHT) milk. *Journal of Agricultural and Food Chemistry* 62(31): 7886–7896.
- [25] Kroh, L.W., (1994). Caramelisation in food and beverages. *Food Chem.*, 51, 373-379.
- [26] Ledesma-Osuna, A.I., Ramos-Clamont, G., Vázquez-Moreno, L., (2008). Characterization of bovine serum albumin glycosylated with glucose, galactose and lactose. *Acta Biochimica Polonica* 55(3): 491-497.
- [27] Leiva, G.E., Naranjo, G.B., Malec, L.S., (2017). A study of different indicators of Maillard reaction with whey proteins and different carbohydrates under adverse storage conditions. *Food Chemistry* 215: 410-416.
- [28] Maijala K., (2000). Cow milk and human development and wellbeing. *Livestock Production Science.* 65: 1-18.
- [29] Martins, S. I., Jongen, W. M., & Van Boekel, M. A. (2000). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology*, 11(9-10), 364-373.
- [30] Martins, S.I.F.S., Jongen, W.M.F., Van Boekel, M.A.J.S., (2001). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology* 11: 364–373.
- [31] Marquez, F. M., Gomez, M., Hernandez, E.G., Villanova, B.G., (1992). New spectrophotometric methods for measuring hydroxymethylfurfural in powdered milk. *Journal of Dairy Research*, 59, 225-228.
- [32] Metin M., (1996). 5. Sütün Karbonhidratları, 13. Süte Uygulanan Isıl İşlemler Süt teknolojisi 129-130, 526-527.
- [33] Miller GD, Jarvis KJ, McBean LD. (2000). *Handbook of Dairy Foods and Nutrition.* (Ed: Jensen, Kroger). The Importance of Milk and Milk Products in the Diet. New York. CRC Press. 4-24.
- [34] Morales, F. J., Romero, C., & Jimenez-Pérez, S. (1992). An enhanced liquid chromatographic method for 5-hydroxymethylfurfural determination in UHT milk. *Chromatographia*, 33(1-2), 45-48.
- [35] Morales, J. F., Romero, C., Perez-Jimenez, S., (1996). Evaluation of heat-induced changes in Spanish commercial milk: hydroxymethylfurfural and available lysine content, *International Journal of food science & technology*, v.31 no.5, pp 411-418

- [36] Morales, F.J., Jiménez-Pérez, S., (1999). HMF formation during heat-treatment of milk-type products as related to milkfat content. *Journal of Food Science* 64(5): 855-859.
- [37] Naim, M., Wainish, S., Zehavi, U., Peleg, H., Rouseff, R. L., Nagy, S., (1993). Inhibition by thiol compounds of off-flavor formation in stored orange juice. I. Effect of L-cysteine and N-acetyl-L-cysteine on 2,5-dimethyl-4-hydroxy-3(2H)-furanone formation. *J. Agric. Food Chem.*, 41, 1355-1358.
- [38] Oral R.A, Dogan M, Sarioglu K, Toker OS., (2012). 5-hydroxymethyl furfural formation and reaction kinetics of different pekmez samples: effect of temperature and storage, *International Journal of Food Engineering*. 2012; 8: 1556-3758.
- [39] Oral R.A, Dogan M, Sarioglu K., (2014). Effects of certain polyphenols and extracts on furans and acrylamide formation in model system, and total furans during storage. *Food Chemistry*. 2014; 142: 423-429.
- [40] Oral R.A, Mortaş M, Dogan M, Sarioglu K, Yazici F., (2014). New Approaches to Determination of HMF. *Food Chemistry*. 2014; 143: 367-370.
- [41] Oral, R.A., Dogan, M., Sarioglu, K., Kayacıer, A., & Sagdic, O. (2015). Determination of HMF in Some Instant Foods and Its Biodegradation by Some Lactic Acid Bacteria in Medium and Food. *Ann Chromatographia, Sep Tech*, 1(1), 1004.
- [42] Pellegrino, L., Resmini, P., Luf, W., (1995). Assessment (indices) of heat treatment of milk. In: *Heat-induced changes in milk*, Edited by P.F. Fox, Brussels, International Dairy Federation, 9501: 409–453 p.
- [43] Richardson, P., (2001). *Thermal technologies in food processing*. Woodhead Publishing, 294, England.
- [44] Sarımeahmetoğlu B., Güvenli Süt Tüketimi [online], *Besin Hijyeni ve Teknolojisi*, Ankara Üniversitesi, <https://www.foodelphi.com/tag/sutun-tanimi/>.
- [45] Sunds, A. V., Rauh, V. M., Sørensen, J., & Larsen, L. B. (2018). Maillard reaction progress in UHT milk during storage at different temperature levels and cycles. *International Dairy Journal*, 77, 56-64.
- [46] Tossavainen, O., Kallioinen, H., (2007). Effect of lactose hydrolysis on furosine formation in skim milk during pasteurization. *Milchwissenschaft* 62(2): 188-191.
- [47] Urgan, M., Saatli, T. E., Türk, A., & Koca, N. (2017). Isıl İşlem Görmüş İçme Sütlerinde (Pastörize, UHT ve Laktozsuz UHT Süt) Hidroksimetilfurfural İçeriğinin Belirlenmesi. *Akademik Gıda*, 15(3), 249-255.
- [48] Ünal R. N., & Besler H. T., (2008). Beslenmede sütün önemi. *Sağlık Bakanlığı Yayın*, (727).
- [49] Wedzicha, B.L., and McWeeny, D.J., (1974). Non-enzymic browning reactions of ascorbic acid and their inhibition, The production of 3-deoxy-4-sulphopentosulose in mixtures of ascorbic acid glycine and bisulphite ion, *Journal of the Science of Food and Agriculture* 25; 577-587.
- [50] Van Boekel, M.A.J.S., (1998). Effect of heating on Maillard reactions in milk. *Food Chemistry* 62(4): 403-414.
- [51] Yetişmeyen A., (1995). Süt teknolojisi. *Ankara Üniversitesi Ziraat Fakültesi Yayınları*, (1420/420), 1997.
- [52] Yıldız, O., Şahin, H., Kara, M., Aliyazıcıoğlu, R., Tarhan, Ö., & Kolaylı, S., (2010). Maillard reaksiyonları ve reaksiyon ürünlerinin gıdalardaki önemi. *Akademik Gıda*, 8(6), 44-51.
- [53] Zhijun Chen and Xiaomei Yan, (2009). Simultaneous Determination of Melamine and 5-Hydroxymethylfurfural in Milk by Capillary Electrophoresis with Diode Array Detection, *J.Agric Food Chem.*, 57(19), pp 8742-8747.



# Investigation of Compliance on Good Manufacturing Practices (Gmp) and Hygiene Conditions in Enterprises That Supply Mass Catering Services

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## Abstract

Nutrition which is our basic need to be healthy and to survive has changed since the old days of human history. Mass food production and consumption has increased due to industrial revolution, rapid urbanization, rapid increase in the number of people and women's employment because of economic requirements. Due to these reasons, the number of companies that engage in mass food production has increased rapidly at the present time. In this research, mass food production in the province of İstanbul was inspected, in compliance with the ligibility for Good Manufacturing Practices (GMP) system and hygiene regulations, according to the two-stage audit forms. After the result of audits,67% of companies were rated successful by getting a passing grade compliance with GMP and hygiene regulations. The remaining 33% of companies could not receive a passing grade in audits.

**Keywords:** *Hygiene, Mass Food Production, Good Manufacturing, Practices, Gmp*

## Introduction

Nutrition is a process that has to be performed with awareness in order to acquire adequate and balanced amounts of nutrients that we need to maintain and improve our health and enhance the quality of life. The organizations which provide food for large amount of people outside of their homes are called catering companies and this type of nutrition can be called catering or collective nutrition [1].

Catering companies provide food at gatherings such as weddings, engagement ceremonies, birthday parties as well as some schools and work places. Hence, in order to provide food in such communities, catering firms are more practical and economical. From this point, catering companies may provide more practical and economic solutions [2].

Today, more than half of the population in industrialised countries and 30% of the population in Turkey eat at least one meal out of their home in their daily life. According to the data collected

from 9 EU Member States, the number of the meals eaten out is approximately 35.6 billion in a year. 44,7% of this was provided by catering industry and 55,3% were consumed in restaurants. Similer to EU, mass nutrition systems are developing rapidly in Turkey in parallel with the world [2]. The increasing level of expectations and food safety systems along the food production chain from farm- to –table have led to the continuous development of existing food safety systems. The catering companies should implement integrated food safety and quality management systems in order to ensure Good Manufacturing Practices (GMP) and to become a reputable organization by gaining consumer confidence [3].

The purpose of this study is to examine the suitability of kitchen planning, the amount of used tools, capacity, material, cleaning agents, maintenance frequency, warehouses and production areas with respect to Good Manufacturing Practices (GMP) and hygienic rules at several companies engaged in the production of mass food in İstanbul [3].

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### **Catering Sector in Turkey**

Based on the data about Turkey, in 2017, 4800 catering companies were affiliated to the Ministry of Food and Agriculture and many of these firms are known to provide services in the metropolitan areas. Public catering firms provide employment for approximately 400.000 people. According to the result of a study conducted by the Federation of Turkish Food Industrialists Association (YESIDEF), the half of the food produced is consumed by the ready-made food industry and that the sector turnover is approximately 66 billion TL [4].

### **Food Safety**

The process to supply healthy and reliable food which is produced by suitable and environment friendly methods, checked at all the stages of production, traced starting from the field to the last step at our dining table, is defined as the Food Safety System [5].

The definition that is made by the Food Safety Expert committee is “the whole measures taken to eliminate the chemical, physical, biological and any other damages that may harm food and human health” [5].

### **Hygiene in Mass Food Production Companies**

It's very important for the mass food production companies to strictly follow the hygiene rules and the reliable production methods as well as providing a balanced and adequate nutrition to the consumers. The production is to be stopped if the materials are found to be inadequate to consume or harmful to health [6].

To ensure food safety;

- 1) The production, processing, storage, distribution and sales phases are to be in compliance with normal conditions,
- 2) The information given on the label should contain about the ingredients and the possible harmful effects such as allergic effects [5].

Food hygiene defines the conditions and measures necessary to ensure the safety of food for the human consumption from production to consumption taking of the intended use into account [6].

Hygienic conditions provided in the kitchen prevent contamination of foods with microorganisms during obtaining and storing of the raw materials, and food preparation and serving stages [7].

In catering companies three factors are taken into consideration to ensure hygiene for producing safe food. The physical factors to provide the hygiene in the kitchen and the equipment used for preparing the food. Factors belonging to the production stage are: assuring hygiene of the food and the personnel, and the personnel factors are related to the training personnel about personnel hygiene [8].

### **Food Safety Management Systems and Infrastructure**

The obligations to ensure the sustainable food quality and safety at the catering companies are determined by various legislations, standards and guidelines [9].

The hygiene rules in mass consumption areas are important together with the food safety management system in order to determine and affectively monitor the critical check points, set pre-requirement programs and applications with respect to the success of the industry ensuring the quality and safety in food production and compliance with the EU Norms [10].

(GMP) , which constitute the infrastructure of food production systems, are preventive measures related to the internal and external conditions of the company in order to prevent or reduce the possibility of product contamination from internal and external sources. The actions and activities required to prevent, eliminate or minimize hazards to an acceptable level are defined as control measures (preventive actions). The schematic representation of the relationship between the order and the steps or processes applied in the production of a particular product is important for the system. Therefore, flowcharts are prepared, and critical control points (CCP) are required for each flowchart [10].

### **Critical Control Point (CCP)**

The critical control point (CCP) in the food chain is a place, procedure, process step or link where control can be applied and required to prevent, eliminate or reduce food risks and hazards [11].

It is defined for monitoring the sequence of control parameters, observations or measurements to be applied, to determine if a critical control point (CCP) in the business is under control and whether it complies with the Good Manufacturing Practice (GMP) procedure [11].

In order to control the relevant hazards within the scope of the GMP management system, the control plan must be prepared in accordance with the GMP principles. CCPs must be created for each hazard set in this plan. Critical limits are also determined in order to monitor CCPs and a monitoring system must be prepared [12].

### **Good Manufacturing Practices (GMP)**

The increase in safe food expectations by consumers and producers has brought many applications in food preparation and processing areas. The oldest of these applications is the GMP systems. It is a series of techniques that are essential in the production and distribution of food products for providing and maintaining quality in products, processing, raw materials, product development, packaging, production, warehousing, and continuous implementation of all phases of distribution. GMP is a flexible system in which errors, deficiencies, remediation and additions are made as a result of audits [13].

The fulfillment of the general requirements of GMP and the establishment of systematic network is possible with the designing of the following 11 basic principles [10].

1. Quality management
2. Building, infrastructure, equipment and materials
3. Documentation
4. Personnel and organization
5. Raw product input, product processing, storage and distribution

6. Quality control and proficiency tests
7. Approval and authorisation of all transactions
8. Investigation of errors, clinical follow-up after use of manufactured products
9. Complaints and recalls
10. Storing samples, destroying of problematic and returned products
11. Internal and external auditing.

In line with these principles, GMP aims to establish a comprehensive tracking system for all production lines and organizations [13].

The ISO 22000 Food Safety Management System, one of the main management systems of a food business, is directly related to GMP system. If we want to do a benchmark on behalf of the two; GMP is a pre-requirement program for the ISO 22000 standard. In other words, for a food business that will use the ISO 22000 standard, the right way to start would be fulfilling the GMP requirements [13].

### **General Rules of Good Manufacturing Applications System**

- Deciding on the details of the products to be produced (product types, controls, approvals)
- Write, save and copy everything to be done (standard application methods, laboratories, equipment, etc.)
- Apply everything what you wrote and noted (training, qualification, Process Control)
- Prove what you did with documents (records, audits)
- Identify nonconformities resulting from audits, fix and increase quality (track transactions, query and subtract results) [13]

### **Benefits of GMP Management System**

Main benefits of the GMP system are summarised below:

- Provides compliance with international trade.
- It is a system that controls the processes necessary for the prevention of errors and contamination of various infections, risks, complications that may

occur during production and the organization established to manage the production.

- It is ensured that the product is continuously monitored thus it reaches the consumer with the best quality and healthiest standards.
- Increases the awareness and understanding of food safety within the company.
- Enhances the image of reliability in public and for the consumer.
- Provides competitive advantage in the sector market.
- Ensure that customers are satisfied with existing or future requests.
- Comply with legal requirements.
- Reduces legal penalties in criminal situations that can be supernatural.
- Helps employees to be proud and motivated with the company in which they work.
- Information from third-party auditors provides added value to the company.

As a result; The good manufacturing applications system is a quality approach for food production and provides reliable and effective production by ensuring the professional work of food industry workers [13].

### **Kitchen Planning in Mass Food Production Companies**

Kitchen planning should cover the basic purpose of the kitchen. The main purpose of the kitchen is to produce high quality and hygienic food at a low-cost as much as possible. Kitchen planning contributes to the realization of this objective by enabling the arrangement of the workflow and the methods of providing the working staff with a comfortable working environment. All planning, work and movements must be seated on specific international professional standards based on policies and procedures [14].

Some of the issues to be considered in the planning of kitchen and cafeteria in the companies of mass food production are as follows: [14]

- The amount of food to be produced and the number of people to be served,

- The shape of the food presentation to be prepared (school, dormitory, barracks, hospital etc.),
- The average age, number of the group to be served,
- Presentation time, number of meals and form of presentation,
- The type of menu to be presented (optional, tabldot, etc.),
- The way in which food is purchased, frequency and storage conditions,
- The types and capacities of devices, machines, tools and equipment to be used for preparation, cooking and service of food,
- Number of personnel working in the kitchens of the companies (special needs of personnel, shower, toilet, dressing cabinets),
- Size of the area allocated to the kitchen in companies,
- Budget allocated for the application of kitchen plan in companies.

If appropriate, conditions are provided at the entrance of food enterprises; hygiene turnstiles must be present. If this condition cannot be achieved at the entrance of the business, there should definitely be a hygiene tourniquet area in the kitchen entrances [15].

The order of these tourniquet systems should be as follows:

- Boot washing system,
- Washbasin for hand washing and disinfecting,
- Hand Wash – disinfecting part,
- Input and output turnstiles,
- The disinfectants must be located in the mop section [15].

### **MATERIAL and METHODS**

#### **Material**

In this research 6 catering companies in Istanbul district have been subjected to investigation. These companies have been grouped two by two as small, medium and large size companies. The production capacities of these companies vary between 500 and 1000 serving/day.

Two factors were effective for the selection of Istanbul as the research site: there are several mass food production companies and a similar research has not been performed before in Ctering companies located in Istanbul.

### Methods

In this study, the general planning of the kitchen, storage facilities and the tools used for the production have been audited with respect to the suitability and adequacy with the standards. Audits are the results of long-term observations in firms by the researcher himself, and the relevant documentation (inventory list and maintenance planning and forms), including the responsible cook, warehouse supervisor and food engineer or technician, who are personally seen in the place of the equipment. Item list and maintenance repair form were reviewed.

The standards used in the research were based on an average of 1000 servings/day production capacity and 50 m<sup>2</sup> production area [16].

The suitability of the kitchen capacities of the companies that were investigated has been examined and evaluated according to the standards of GMP and the Food Hygiene Regulation published in the Official Gazette numbered 28145 [14].

In this study, units and physical properties of the units that should be present in the mass food production facilities have been examined and evaluated according to the standards of good manufacturing practices and the Regulation of Supervision and Control of Food Safety and Quality published in the Official Gazette numbered 27009 [14].

In line with the standards, the existence of the facilities which should be present at all mass food production companies (personnel recreation room, toilet, shower, cold and dry air depots, chemicals and cleaning agents storage, vegetable products washing and preparation, meat products preparation, preparation of pastries, baking and dishwasher), and the physical conditions of the

kitchen (wall, ceiling, floor, doors and windows, electricity and water installations, ventilation, lighting, drain, mosquito nets) have been evaluated [16].

According to the capacity of the companies involved in the research, the coefficients were found based on the standard of the tools and equipment required to be present in each section of the company's kitchens. The number of tools to be found is determined by multiplying these coefficients [13].

These coefficients are as follows:

- 1. Company: Standard x 10 = (10000 servings/day)
- 2. Company: Standard x 10 = (10000 servings/day)
- 3. Company: Standard x 5 = (5000 servings/day)
- 4. Company Standard x 4 = (4000 servings/day)
- 5. Company Standard x 1 = (1000 servings/day)
- 6. Company Standard x 1 = (1000 servings/day)

The statistical evaluation of the findings was not possible due to insufficient number of instruments present in the companies. The results were evaluated as numbers and percentages [16].

**Control Form Method** A control form has been prepared to evaluate the conformity to the standards of GMP and the hygiene rules of the 6 food companies at different sizes, which were scaled according to the number of daily serving meals.

### Data Collection and Evaluation

Data Collection the studies were carried out in 3 stages.

**Stage 1:** 12 control forms were used at this stage. Each audit form was evaluated over 100 points and the minimum 60 points were considered as the limit for qualification [13].

*Investigation of Compliance on Good Manufacturing Practices (GMP) and Hygiene Conditions in Enterprises That Supply Mass Catering Services*

The audit forms consist of the following headings; general hygiene, general cleaning and sanitation, personnel hygiene, warehouses, goods acceptance and handling, water supply and water, ice, steam quality, food waste and waste management, dishwasher, in-house, toilets and other areas, pest control, food production, cooking and preparation, quality management system documents, registrations and training.

After these 12 forms are filled in separately and evaluated over 100 points, all results are collected and divided into 12. If the resulting average score is 60 and above, this indicates that the business has received a passing grade in the first stages.

**Stage 2:** At this stage, the company was inspected for a second time. This time the rectification status of the findings of the first stage had been checked by using the form entitled “Kitchen and Operation Plan Control List”.

The form includes a total of 100 questions under various headings. If businesses receive a score of 60 and above, they are deemed to have received enough points from the audit.

**Stage 3:** At this stage, the resulting points from the first and the second stages are collected and divided into two. If the result is a score of 60 and above, the company is considered to have passed good production practices and hygiene competence.

**Evaluation by Production Capacities**

In the research, the mass food production companies are grouped into large 33.3% (N:2), medium 33.3 (N:2) and small 33.3 (N:2) size enterprises according to their daily production (servings/day) capacities. The following chart (table 1) shows the production capacities, production areas and personnel numbers of the companies participating in the study [9].

**Table 1:** Daily production capacity, production area and the number of personnel of the enterprises

Company Size	Daily Production Capacity (Servings/Day)	Production Area (m <sup>2</sup> )	Number of Personnel
<b>Big</b>			
1.Firm	10000	450	150
2.Firm	10000	400	120
<b>Medium</b>			
3.Firm	5000	250	70
4.Firm	4000	200	60
<b>Small</b>			
5.Firm	1000	100	20
6.Firm	1000	50	15

According to Table 1, the number of personnel employed in the enterprises increased the size of the operation together with the number of employees.

**Kitchen Areas**

In Table 2, the qualification statuses of the food companies participating in the audit are given according to the daily serving capacity of the kitchen areas [5].

**Table 2:** Production information of the enterprises studied in.

Company Size	Daily Production Capacity (Servings/Day)	Production Area size (m <sup>2</sup> )	Required Area (m <sup>2</sup> )	Field evaluation (%)
<b>Big</b>				
1.Firm	10000	450	500	10% insufficient
2.Firm	10000	400	500	20% insufficient
<b>Medium</b>				
3.Firm	5000	250	250	Adequate
4.Firm	4000	200	200	Adequate
<b>Small</b>				
5.Firm	1000	100	50	Adequate
6.Firm	1000	50	50	Adequate

According to GMP standards, 50 m<sup>2</sup> area is enough for a business with a production capacity of 1000 servings/day [13].

According to the results of this study (Table 2), the production capacity of large enterprises has been confirmed to be insufficient. Production areas are enough for the daily production capacities of all medium and small enterprises. The production area of the 5th Firm has a 50% excess size compared to the amount given in the standard. This indicates the existing excess of unnecessary area. The wider production area than necessary can lead to increased dead area, unnecessary workforce, electricity and other expenses.

### Physical Requirements of the Kitchen

The physical conditions of the kitchens; the floor (tiles waste dirty water drains), walls (2 meters or more tiles at height), doors and windows (corrosion resistant, easy to clean), ventilation (natural and mechanical ventilation sufficiency), lighting (suitable illumination level for the purpose), water (continuous hot water system), as well as the physical design of Good Manufacturing Applications according to the standards and compliance status of hygiene regulation have been investigated [10].

Among the companies representing large-scale named as, firm 1. and firm 2. demonstrate full compliance with physical standards. Medium scale establishment, firm 3 is complying in terms

of floor design, illumination adequacy and water supply. The floor is designed with light colored, nonslip, easy to clean and quick-wear-resistant tiles as written in standards and regulations. In addition, the waste water drains on the floor are at adequate sizes and located where they should be. The partially or completely inadequate points in the firm 3 are as follows; wall, door and window designs. The wall design is partly inadequate because of the absence of protective metal coatings at the level of hand carts on the walls.

Door and window designs are completely inadequate and inappropriate according to standards and regulations. The doors in this firm are not self-closed. Another reason for non-conformance is that they are produced from a rapidly worn substance. The windows also did not have opaque glass where necessary, thus direct sunlight hit the production area [5].

It has been observed that insufficient fields are more than used areas in the firm 4 which is grouped as medium scale enterprise. In this case, it shows that the facility is inadequate both in terms of GMP standards and the regulations. It was also found that dark colored tiles were used in most areas of the floor. This situation causes difficulty for the detection of contaminated areas. Doors and

windows are not manufactured from corrosion resistant material. There is no opaque glass used on the windows. The flynet is not available in all opening windows. There is no protective equipment in case of refraction of illumination fixtures. For this reason, although illumination is adequate, it is found to be inappropriate. Since a refraction of illumination fixtures may lead to contamination of the food that is being produced [7].

The firm 5, grouped as a small size company, can be shown as a good example for its category. The most prominent error in design is that the floor is designed from a dark tile. In addition, the floor of the cold and dry storage areas is covered with parquet [8]. In this case, storage areas are not acceptable. There are no other unacceptable points except the floor design.

The firm 6, grouped as a small business, is partially inadequate because although the wall is tiled in dark colors, it does not contain metal preservatives at the level of carts. Doors and windows are not made of corrosion-resistant material and the doors are not self-closed. The use of opaque glass in windows generally shows compliance with the standards. However, there is no fly-net at any opening window [9].

Considering the physical design of the companies involved in the audit, most firms generally comply with GMP standards and regulations. The fourth business, grouped in medium scale, has remained far behind of other companies in terms of physical design competence. In addition, the physical design of the fourth business does not generally conform to GMP standards and regulations. It is inevitable that the company should have a move in its physical redesign and modifications which will have a positive effect on the company in the future [11].

### **Results of the First Stage Audits**

6 companies that are participating in the audit were subjected to 12 inspection forms in the first stage. These 12 inspection forms are prepared according

to the rules of GMP standard and regulations. In the first phase, the deficiencies of the enterprises have been reported with results from the 12 audit forms. For the elimination of these deficiencies, firms are given a week of time. How successful businesses are in the first audit phase is given in Table 3.

**Table 3:** Average results of the first stage audit forms of enterprises

Company Size	Qualification Score*	Points received**
<b>Big</b>		
1. Firm	60-100	74
2. Firm	60-100	76
<b>Middle</b>		
3. Firm	60-100	61
4. Firm	60-100	53
<b>Small</b>		
5. Firm	60-100	68
6. Firm		
7. Firm	60-100	48

\* 60-70 score enough, 70-80 score successful, 80-90 score very good, 90-100 score is excellent

\*\* According to the GMP standard inspection form of good manufacturing applications, 60 points have been regarded as the limit in terms of qualification [16].

### **Results of the Second Stage Audit Forms**

At the end of the first audit, the report is prepared to resolve the deficiencies for the businesses participating in the audit. After enough time (approx. 1-2 weeks), the companies were re-inspected in order. The results of the second stage controls are given in Table 4.

**Table 4:** Results of the second phase control forms of the enterprises

Company Size	Qualification Score*	Points received**
<b>Big</b>		
1. Firm	60-100	70
2. Firm	60-100	74
<b>Middle</b>		
3. Firm	60-100	65
4. Firm	60-100	53
<b>Small</b>		
5. Firm	60-100	65
6. Firm	60-100	50

\* 60-70 score is enough, 70-80 score is successful, 80-90 is very good and 90-100 score is defined as excellent

\*\* According to the GMP standard inspection form of good manufacturing applications, 60 points have been regarded as the limit in terms of qualification [16]

### Results of the Final (Third) Stage Audits

In the third stage i.e. the final stage, the results were obtained by averaging the two phases of the first and second audits. In the following table (Table 5), the average results are given for the scores that firms have received from the final inspection .

**Table 5:** The results of the third (Final) audit forms of the enterprises

Company Size	Qualification Score	Points received
<b>Big</b>		
1. Firm	60-100	72
2. Firm	60-100	75
<b>Middle</b>		
3. Firm	60-100	63
4. Firm	60-100	52
<b>Small</b>		
5. Firm	60-100	66
6. Firm	60-100	49

According to the result of two-stage auditing, 2 firms' audits have passed and 2 firms were found to be sufficient. The remaining two firms did not receive a pass in the first and second controls. Among the companies studied in, the most successful one was the second firm with the result of 75 points. In general, 67% of the participating firms in the audit have scored a pass through the inspection.

### Conclusion and Discussion

In parallel with the increasing of mass food production and consumption, more companies are involved in this business every other day. It is very important that the companies and the employees should follow the personal and the environmental hygiene and cleanliness prerequisites as well as the suitability of the food produced by these companies for human health [10].

As consumers, most of us can define the meaning of quality. However, we may face with difficulties in determining the quality and quality assessment as an individual. In literature, the word "quality" is defined as the 'excellence level'. Since everyone has a different level of excellence, it is not possible to be in line with everyone's personal standards. For this reason, certain standards are created for the food production and consumption areas by people who are accepted as experts in the food industry. The main purpose of these standards can be defined as producing food products in the highest hygiene conditions with the lowest cost of consumer appreciation. However, the use of the word 'control' in combination with the term quality makes it a better meaning. The use of these two words together emphasizes the hygiene and sanitation control during the creation of food quality [13].

For this reason, "Food quality control" services play an important role in the control of harmful substances and diseases that are transmitted to human through food. The control is for ensuring whether the food is produced under hygienic conditions using techniques adequate for human



consumption together with the accepted conditions of production, process, storage, marketing places and the hygiene of the people working at these stages [16]. If food hygiene, cleanliness and sanitation rules are not provided adequately starting from production to the presentation of the product to the consumers, toxic substances occurring in the foods and the proliferation of microorganisms cause food contamination which puts human health at risk [10].

Infectious diseases that are becoming a preventable health problem in the world and Turkey. The problem also found at significant degrees in the food sector, which pose an important risk. Since various problems in the production, storage and distribution stages of food supply chain still exist in our country, the level of hygiene and sanitation conditions are important research subjects to be investigated. It has been shown by many research studies that are conducted in recent years that a significant portion of food poisonings may arise due to the lack of personal and environmental hygiene habits of staff working food manufacturing, consuming and selling facilities where adequate importance is not given to the environmental hygiene [13].

In this study, it should be emphasized that the mass food production companies commonly disregard the GMP standards and regulations. It should be also expressed the facts of our country with respect to food poisoning which poses a big threat to human life [5].

In addition to these objectives, the study was strengthened scientifically by investigating the degree to which mass food production companies are in line with the relevant regulations and good manufacturing standards in Turkey. Besides, to which degree the GMP and the regulations follow the facts of Turkey and how much work is done to comply with the hygiene rules were also investigated.

6 firms in Istanbul which has allowed auditing in their establishments and production areas has participated in this research. Comparing the production capacity of each business, the qualification status of the kitchen and production area design, tools, equipment and units has been inspected. In addition, quantity capacity assessment has been made for the units required to be present in the business. The degree of functional use of the materials such as cleaning materials, chemical disinfectants, the operator's maintenance and repair frequency, general appearance, usage instructions and physical sufficiency, and their conformity to GMP standards and regulations were assessed [10].

The production area which was available in 4 of the 6 companies participating in the audit, had enough size according to the production capacity and the number of employed personnel. The second of the production areas of the two remaining firms were found to be insufficient by 20% of the first 10%. 66,6% of the companies had enough capacity in terms of production areas. It has been found that the physical conditions that must be in accordance with the Good Manufacturing Standard were in line with the standards in 67% of the firms. Areas where physical conditions were found appropriate in all enterprises are 33% of the floor design and 33% of the wall design were found to be at 17% of illumination in 50% of doors, windows and mosquito nets. Hot water and ventilation installations were capable of adequate capacity in all firms [16].

In all the companies participating in the research, the units with the most deficiencies with respect to the capacity of vehicles and equipment, respectively, are 80% in the vegetable preparation unit, 75% in dishwashing units, 70% in meat and pastry preparation units, purchasing and the control unit was determined to be 65%, 60% in the depot units and 40% in the cooking unit. When the vehicle and equipment capacities of enterprises were generally assessed in terms of quantity, it was determined that 66% of all businesses were inadequate [6].

The results of the inspection forms used in the first stage were successful except for 2 companies. The success rate at the first stage was 67%. In this case, 67% of enterprises have succeeded in obtaining general hygiene, cleanliness and sanitation, personnel hygiene, warehouses, goods acceptance and transport, water supply, and used water, ice and steam, food waste and waste management, dishwashing, in-house toilets and other areas, pest control and combatants, food production, cooking and preparation, quality management system documents, records and training [13].

During the second inspection, the deficiencies were not corrected. In addition, the results of the combined result of companies taking note of the positive and negative changes from the first inspection to the second audit were observed in the next control i.e. in the final inspection. According to the results of last inspection, 4 firms have received a pass score while 2 firms have failed. The companies that fail are generally lacking in terms of general physical requirements, the necessary tools and equipment, general hygiene rules, food warehouse order and rules, personnel hygiene and personal cleanliness. After the second inspection, some companies were observed to have an increase in these points and some of them have not fulfilled their requirements [13].

As a result of the research, most of the food production companies that participated in the audit were successful in their GMP standards and the qualifications they had to carry on the basis of the regulations in a well-demonstrated and applied manner. The 6 companies engaged in the production of collective food were determined to be lower in small and medium sized enterprises in compliance with standards and hygienic aspects. The main reasons for this situation are as follows; manufacturers and employees do not comply with the personal cleaning, hygiene and sanitation rules at adequate levels, the raw materials used in

production not comply in terms of quality GMP and hygiene standards, the general production is not in accordance with the rules specified in the standards, employees do not know the rules of hygiene and sanitation at adequate levels.

It was also deduced as a result of our research that the following measures should be taken in order to increase the hygienic qualities of the establishments engaged in mass food production. The risk and critical points must be concentrated in all phases, from production to consumption. Hygiene rules and controls and microbiological studies do not always provide the reliability of food. For this reason, hazard analyses should be developed at critical control points (CCP) in enterprises and using control systems to produce, prepare, cook, refrigerate, reheat, prepare and prepare for the service. Failures and nonconformities in the basic stages such as holding, storing, etc. In addition, the cold chain, deep freezing process and the equipment cleaning should be taken care of. Personnel hygiene and cleanliness of the operation should be emphasized, cleaning and disinfection of surfaces in contact with food should be effectively done, cross contamination sources and their causes should be avoided. In addition, employees should be educated and be conscious in terms of hygiene, personal cleaning, operation cleanliness and hygiene rules. Health checks and porter analyses of personnel in contact with food and food equipment are required periodically. Effective and periodic food control must also be carried out by the official organizations.

Finally, GMP system is a system that provides, protects and improves food safety. Mass food production companies must apply this system and fully implement it for eliminating or minimizing the problems that may occur in their businesses. In summary, GMP system is an effective tool in production and preparation of safe and quality foods .

## REFERENCES

- [1] Aksu, H. (2012). *Gıda Hijyeni ve Sanitasyon*. İstanbul Aydın Üniversitesi, Anadolu Bil Meslek Yüksek Okulu, Gıda Teknolojisi Programı Ders Notları, İstanbul.
- [2] Sezgin A., Özkaya F. (2013). *Toplu Beslenme Sistemlerine Genel Bakış*. Akademik Gıda 12(1): 124-128.
- [3] Pellegrini, D. C. P., Paim, D. S., Lima, G. J. M. M., Kich, J. D., Coldebella, A., Cardoso, M. R. I. (2013). *Application of a good manufacturing practices checklist and enumeration of total coliform in swine feed mills* Semina: Ciências Agrárias, 34: 3767–3776.
- [4] Anonim, (2017). *Hazır Yemek Sektörü Sektörel Bilgiler*. (Erişim tarihi:01.04.2017).
- [5] Santana, N. G., Almeida, R. C. C., Ferreira, J. S., Almeida, P. F. (2009). *Microbiological quality and safety of meals served to children and adoption of good manufacturing practices in public school catering in Brazil*. Food Control, 20: 255–261.
- [6] Bilici, S. (2008). *Toplu Beslenme Sistemleri Çalışanları İçin Hijyen El Kitabı*. Ankara: T.C. Sağlık Bakanlığı Temel Sağlık Hizmetleri Genel Müdürlüğü.
- [7] Bilgin B, Erkan ÜC. (2013). *Bir Hazır Yemek İşletmesinde HACCP Sisteminin Kurulması*. Tekirdağ Ziraat Fakültesi Dergisi, 5 (3): 267-281.
- [8] Cevizci, S. ve Önal, A. E. (2009). Halk Sağlığı Açısından Hijyen ve İyi Üretim Uygulamaları. Türk Hijyen ve Deneysel Biyoloji Dergisi. 66, 73-82.
- [9] Çopur, U., Yonak S. ve Şenkoyuncu, A. (2012). Gıda Güvenliği ve Denetim Sistemi, Erişim Tarihi: 22.05.2012, [www.zmo.org.tr/resimler/ekler/6bf16f1f0372a63\\_ek.pdf](http://www.zmo.org.tr/resimler/ekler/6bf16f1f0372a63_ek.pdf)
- [10] EC-Asean Economic Cooperation (2015). Guidelines on HACCP, GMP, Comprehensive GHP for ASEAN food SMEs. [http://ec.europa.eu/food/training/haccp\\_en.pdf](http://ec.europa.eu/food/training/haccp_en.pdf)
- [11] Erkan, N., Üçok Alakavuk, D. ve Tosun, Y.Ş. (2010). *Gıda Sanayinde Kullanılan Kalite Güvence Sistemleri*. Journal of Fisheries Sciences, 2, 88-99.
- [12] Koç A., Bölük, G. ve Aşçı S. (2008). *Gıda Güvenlik Standartlarının Gıda İmalat sanayiinde Yoğunlaşmaya Etkisi*. Akdeniz Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi, 16, 83-115.
- [13] Merican, Z. (2017). *GMP and HACCP: are you confused? Paper Presented at Symposium on Current and Innovative Approaches to Microbiological Food Safety Management organized by ICMSF, ILSI-SEAR, AVA*. Singapore, October 3-4, 2017
- [14] Mahmutoğlu, T. (2009). *Gıda Endüstrisinde “Güvenli Gıda” Üretmek*. Ankara: ÖDTÜ Yayıncılık.
- [15] Merdol, T.K., Beyhan, Y., Çiğirim, N., Sağlam, F., Tayfur, M., Baş, M. ve Dağ, A. (2009). *Toplu Beslenme Yapılan Kurumlarda Çalışan Personel İçin Sanitasyon-Hijyen Eğitimi Rehberi*, Ankara.
- [16] Oliveira, C. A. F. de, Cruz, A. G. da, Tavolaro, P., Corassin, C. H. (2016). *Food Safety: Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), Hazard Analysis and Critical Control Point (HACCP)*. Chapter 10. In: Antimicrobial Food Packaging, 129–139.
- [17] Özbek, Ş. F. ve Fidan, H. (2010) *Türkiye ve Avrupa Birliğinde Gıda Standartları, Selçuk Tarım ve Gıda Bilimleri Dergisi*, 1 (24), 92-99.

# An Investigation on the Deterioration of Packaged Chicken Doner

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## Abstract

The aim of this study was to investigate the deterioration in packaged chicken doner related to microbiological and chemical analyses when stored under normal, vacuum and modified atmospheric (MAP) conditions at 0, 10 and 20°C temperatures. For each storage temperature 16 samples of freshly cooked doner were taken each one packaged with 100 g Polypropylene (PP) packages. The results were evaluated according to *Salmonella* spp; *Listeria monocytogenes*, total volatile nitrogen (TVN) and pH for each sample and temperature were analyzed in two days intervals in duplicate according to Turkish food regulation. The deterioration value related with microbial and chemical analysis were reached within 20, 14 and 7 days at 0°C under modified atmospheric (MAP), vacuum and normal storage conditions respectively but it was deteriorated in one day when stored at 20°C at any storage conditions

**Keywords:** Deterioration, packaged chicken doner, *Salmonella* spp, TVP, MAP.

## Introduction

Factors such as increase in living standards, the orientation of more women to business life, practical solutions for daily food needs, the variety of ready-made meals and the increase in well-made advertisements also increase the consumption of fast foods. Fast foods that have the highest consumption rate are hamburger, pizza, and döner (Öksüztepe and Beyazgül, 2014).

To begin with, meat doner kebab, a traditional Turkish meal, was first serviced in 1820 by Sinegin Hafız (a nickname) in Kastamonu. Chicken doner, on the other hand, first appeared in Saudi Arabia. It was prepared from chickens brought from Denmark and it is said that turkey meat was also added for obtaining a different flavor (Kuscu, 2007). There are doners made from various materials such as fish, pekings duck and vegetables in Turkey (Kuşçu, 2007). Doner's raw material is made from lamb, veal, poultry meat which is also mixed with onion, water, milk, yogurt, fat, tomato paste, lemon juice, vinegar and spices in order to get high flavor and nutrition value during cooking (Kayaardı et al., 2013; Jöckel and Stengel, 1984; Acar 1996). The same manner is also used for the preparation of the

poultry meat doner (TGK Meat and Meat Products Communiqué, 2012). In the production of poultry meat, non-animal origin proteins, starch and starchy materials and soy and soy products cannot be used (Cebirbay and Aktaş, 2007). After the meats are arranged in the DONER platform, the excess on the edges can be shaped by cutting with a knife into an oval, cut conical (TSE, 1995; TSE 2003). Modified Atmosphere Packaging (MAP) is a study with the aim of reducing the microbiological reactions and biochemical events that occur in the product by changing the gas rates in the atmosphere of the environment where the product is located (Tülin and Sülfer, 2017). The MAP has started to be widely used in a variety of products such as fruits and vegetables, meat and meat products and also in dairy products in accordance with the demand for fresh vegetables (Doğu, 2009). The first report on MAP studies to increase the shelf life of the fish was observed in the 1930 (Özoğul et al., 2006). The French company Scope used the MAP for the packaging of the food products in 1974. This was to inhibit the development of aerobic microorganisms in white meat products (Kılıç and Çaklı, 2004). Chicken products are preferred more due to their high nutritional value, low price, and

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low-fat content, but their shelf life is very short even in the refrigerator. Therefore, the use of MAP is quite common for preserving chicken products (Mood et al., 2016).

The purposes of this research was to investigate the deterioration of the packaged chicken doner related to microbiological and chemical effect on the quality of doner when stored at different temperatures such as 0, 10 and 20°C and under normal, vacuum, (MAP) conditions.

## **Material and Methods**

### **Sample Preparation**

All samples of cooked chicken doner were obtained from a doner processing company (Gursoy Gıda Ltd.Co. Istanbul,Turkey), all products were collected randomly from the same batch and were immediately brought to laboratory in portable refrigerated containers. Then each sample was weighed in 100 g pieces on the clean bench of the lab. All 72 samples were put in sealed polypropylene bags under normal vacuum and MAP conditions and were kept at 0, 10 and 20°C until further analysis. For modified atmosphere packaging Food 35 gas was used and set at 20% gas mixture (35% CO<sub>2</sub>, 65% N<sub>2</sub>) that was provided by Habaş company in Turkey. Following the 24 days for each temperature, the samples were taken out of the refrigerator every three days for experiment.

### **pH measurement**

The pH values of the samples were measured at three days intervals by Mettler Toledo, Seven Compact S210-K and results were given in figures 1, 2 and 3.

### **Estimation of Volatile Nitrogen**

10 g of comminuted sample was taken with 50 ml of fresh tap water into homogenizer (Ultraturrax, IKA, Yellowline D125), after 10 seconds of homogenization the sample was washed in the 250 ml distillation flask of macro apparatus with 250 ml fresh tap water and 1-2 g of added MgO (Sigma-Aldrich, 13138). The solution placed in the distillation apparatus was connected to receiving flask added 25 ml of 2% boric acid solution. The

flask containing the sample in the heater was set to boil exactly for 10 minutes and distilled for 25 minutes after it starts boiling. After distillation, a few drops of methyl red (Merck, 1.06076.0100) were added dropwise and titrated with 0,1 N sulfuric acid. The amount of 0,1 N sulphuric acid consumed for the color change was determined and calculated TVN values are given in Figures 4, 5 and 6 (Wiley, 1973).

### **Microbiological Analysis**

For determining *Salmonella spp* predence and *Listeria monocytogenes* microorganisms were estimated according to TS EN ISO 6579 and TS EN ISO 11290-1 standards respectively and the total number of microorganisms were counted (TS EN ISO 4833-1) as given in figures 7, 8 and 9.

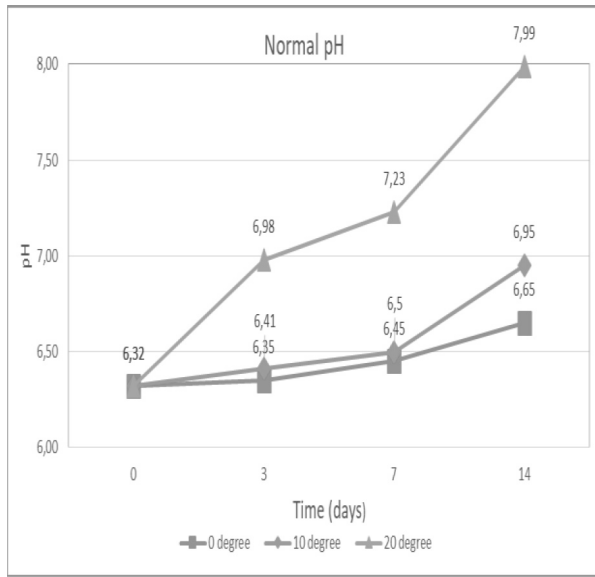
For each storage condition, 72 specimens were used, 100 g each, in parallel. All samples were stored at 0, 10 and 20 degrees for 24 days (FRITERM refrigerant, FEM 30 32 type).

$$\frac{(\Sigma C)}{((1. \text{ number of dilution Petri dishes} * 1) + (\text{number of dilution Petri dishes} * 0,1))} * (\text{1st dilution coefficient})$$

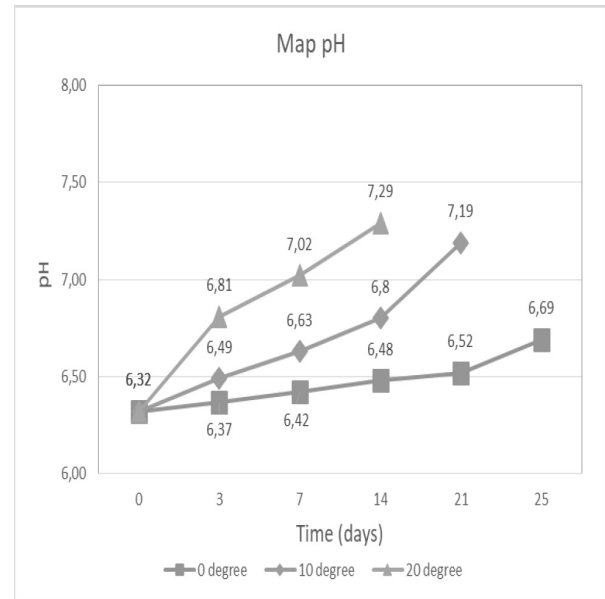
### **Results and Discussions**

PH value of cooked chicken doner samples, that analyzed at 0, 10 and 20°C, were changed from 6.37 to 6.61, 6.68 and 7.98 under MAP, vacuum and normal conditions, respectively during the 24 days of storage. The cooked chicken doner was not given any pH standards. The other research indicated that pH value of the cooked red meat doner was estimated between 5.4 to 6.3, for mixed doner in range of 5.8-6.79; as mentioned by Cebirbay and Aktaş (2007) and also given in TSE (2003), that differences between two doners may be due to the composition of meats.

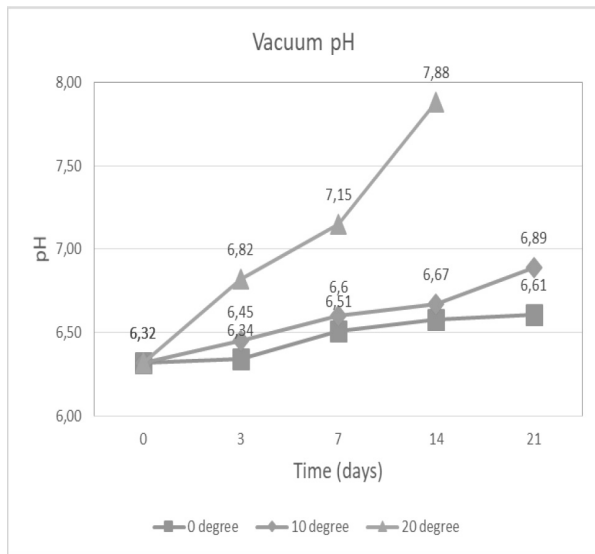
The amount of pH changes in chicken doner samples, in accordance with other studies, shows that it is significantly affected by storage temperature (Çiçek et al., 2013).



**Figure 1:** pH values of cooked chicken doner under normal packaging



**Figure 3:** pH values of cooked chicken doner under MAP

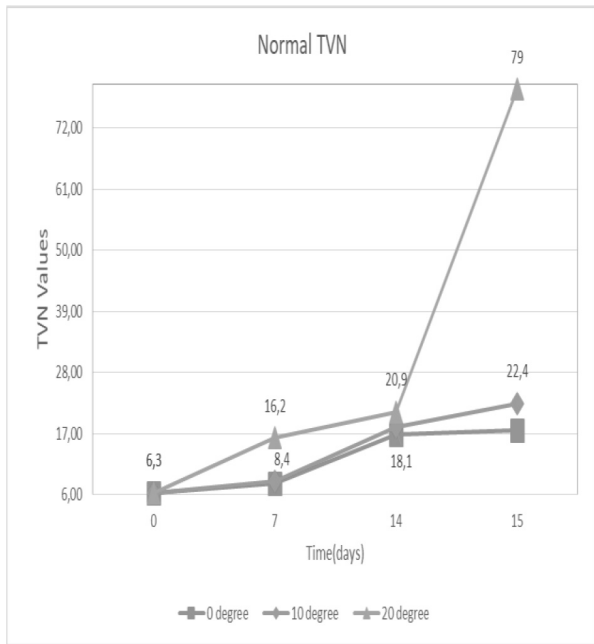


**Figure 2:** pH values of cooked chicken doner under vacuum packaging

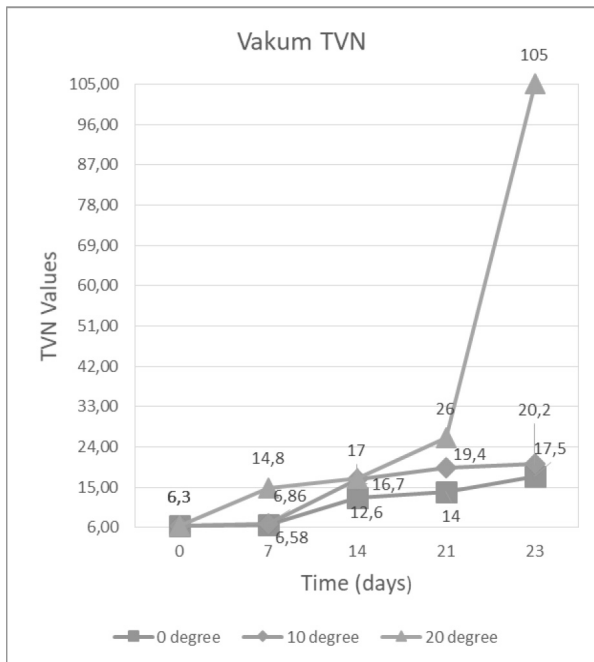
**TVN value of cooked chicken doner**

The amounts of TVN for the cooked doner at different storage conditions at different time and temperatures were shown in Figures 4, 5 and 6. Acceptable limit values of TVN for cooked chicken doner were reported by Economou et al., to be between 4-10 mgN/100g 2009)..

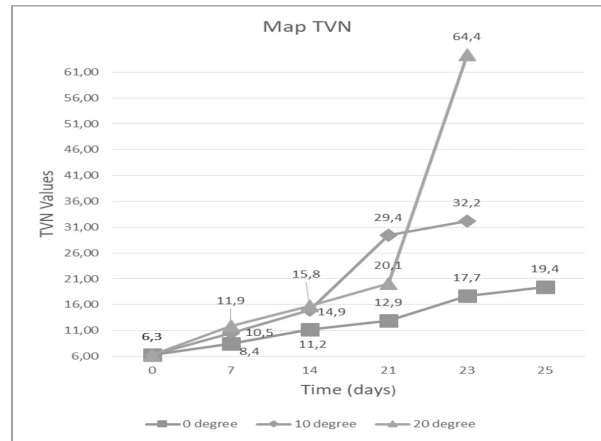
The TVN content under normal atmospheric packaged sample initially was estimated to be 6.3 mgN/100g, after being stored at 0, 10 and 20°C for 25 days in time intervals, TVN was increased to the upper limit of 10 mgN/100g and after 7, 5 and 1 days respectively as shown in Fig.4. According to these values, the amount of TVN was significantly increased during storage at 0°C and 10°C but high accumulation TVN occurred when the sample was stored at 20°C.



**Figure 4:** TVN mg N/ 100g cooked chicken doner under normal packaging



**Figure 5:** TVN mg N/ 100g cooked chicken doner under vacuum packaging



**Figure 6.** TVN mg N/ 100g

Cooked chicken doner under MAP packaging

Initially 6.3 mg N/100g content of TVN in vacuum packaged cooked chicken doner, after storage at 0, 10 and 20°C for 25 days in time intervals, increased above limit value of 10 mgN/100g after 9,8 and 1 days, respectively (Fig 5). Comparing this result with normal atmospheric conditioned sample, the amount of the TVN was slightly different during storage at the same time and temperatures, which may be due to the absence of oxygen. For storage under MAP conditions at 0, 10 and 20°C, cooked chicken doner initially contains 6.3 mg N/100g, after storage for 25 days in time intervals was increased overhead of 10 mgN/100g (Fig 6) It was indicated that for MAP packaged samples TVN was increased when the temperature increased. All the cooked chicken doner spoilage where in agreement a recommended by Economou et al., 2009. All the samples were spoiled at 20°C after being stored one day at any condition; this data is also supported by others (Fallah et al., 2016).

*Salmonella spp* and *Listeria monocytogenes* assays were performed but they were determined as negative when the total bacterial counts were taken into account. In the sample that was packed under normal air, vacuum and MAP conditions initial total bacteria content were determined as 3, 3. After being stored at 0°C in time intervals of 10, 8, and 8 days respectively; total bacteria were increased above the limit value of 10<sup>6</sup>. After the storage of packed

chicken doner under MAP, vacuum and normal air conditions at 10 °C, the sample which initially contains 3,3 total bacteria was increased overhead of 10<sup>6</sup> after storage in time interval and reached the highest value after 6 and 4 days, respectively. All the samples were spoiled after being stored one day at 20°C under any storage condition, this also is in good agreement with the information given by other researchers (Al-Shadefat and Bassam, 2011; Eker et al., 2011).

Temperature dependence of the Maillard reaction was modelled with the Arrhenius equation as follows:

$k = k_0 \cdot e^{-E_a/RT}$  by using Arrhenius equations, activation energy for MAP, vacuum and normal air storage conditions were estimated as 41572, 26190 and 25774 j/kmol respectively. The larger the activation energy was given, the slower the degradation rate.

### Conclusions

The storage temperature had strong effect on the denaturation of cooked chicken doner at 10 and 20°C related with TVN, pH and growth of the microorganisms. This investigation indicates that the deterioration of packaged chicken doner happens as fast as one day at 20°C, however it can be stored about 10 days at 0°C under MAP conditions.

### REFERENCES

[1] Acar, MS., (1996), “Kasaplık Hayvan Etleri ve Tavuk Etinden Yapılan Döner Kebapların Mikrobiyolojik kalitesinin Karşılaştırmalı Araştırılması”, İstanbul Üniversitesi, Sağlık Bilimleri Enstitüsü, Besin Hijyeni ve Teknolojisi Anabilim Dalı, Doktora Tezi, İstanbul.

[2] Al-Shadefat, Bassam, (2011), “Tüketim sürecinde döner kebablarda *Salmonella* Spp. varlığının araştırılması”, Besin Hijyeni ve Teknolojisi (Vet) Anabilim Dalı, Doktora Tezi, Konya

[3] Cebirbay M.A., Aktaş N., (2007) “Türk Mutfağı'nın Geleneksel Yiyeceği: Döner Kebab”, Selçuk Üniversitesi Mesleki Eğitim Fakültesi, Çocuk Gelişimi ve Ev Yönetimi Bölümü, Beslenme Eğitimi Bilim Dalı, 42070 Kampüs/KONYA.

[4] Çiçek E., Karabıyık Ş., Kurbanduriyer H., (2013), “Dana Etinin Bazı Fizikokimyasal ve Mikrobiyolojik Özellikleri Üzerine Farklı Ambalajlama Yöntemleri ve Depolama Süresinin Etkisi”, Gazi Osman Paşa Üniversitesi Ziraat Fakültesi Dergisi Tokat, 30(2),62-70.

[5] Doğu E., (2009), “Marine Edilmiş Pişmeye Hazır Tavuk Etlerinin Modifiye Atmosfer Paketleme İle Muhafazası”, Yüksek Lisans Tezi, İstanbul Teknik Üniversitesi Fen Bilimleri Enstitüsü.

[6] Economou T., Pournis N., ve diğ, (2009), “Nisin –EDTA Treatments and Modified Atmosphere Packing to Increase Fresh Chicken Meat Shelf-Life”, Food Chemistry 114 1470-1476, Greece.

[7] Eker Yılmaz F., Muratoğlu K., Aydın A., Bostan K., (2011), “Pişmiş döner kebablarda mikrobiyolojik kalite ve mikrobiyel gelişim üzerine bir araştırma”, Kafkas Üniversitesi Veteriner Fak. Derg.

[8] Fallah F., Emrahimnezhad Y., Sis N.M., Sadabadi M.G., (2016), “The Effect Of Different Levels of Diet Total Volatile Nitrogen of Performance, Carcass Characteristics and Meat Total Volatile Nitrogen İn Broiler Chickens, Department of Animal Science”, Faculty of Animal Science and Veterinary Medicine, Shabestar Branch, İslamic Azad University, Shabestar, İran.

[9] Jöckel, S., Stengel. G., (1984), “Döner Kebab Untersuchung und Beurteilung Einer Türkischen Spezialitat”, Fleischwirtschaft, 64 (5), 527-538.

[10] Kayaardı S., Obuz E., Demircioğlu Karaca S., Textural, (2013), “Chemical And Sensory Properties Of Doners Produced From Beef, Chicken And Ostrich Meat”, Celal Bayar Üniversitesi, Kafkas Üni. Vet. Fak. Derg., 19(6):917-924



- [11] Kılıç B., Çaklı Ş., (2004), “Su Ürünlerinin Modifiye Atmosferde Paketlenmesi”, Ege Üniversitesi Su Ürünleri Dergisi, 349-353.
- [12] Kuşçu E.A., (2007), Popüler Kültür Beslenme Biçimleri Örnek Olay: Döner Kebap, Ankara Üniversitesi, doi:10.1501/0001501
- [13] Mood F.G., Mohsenzadeh M. ve Diğ., (2016), “Microbial and Chemical Spoilage Of Chicken Meat During Storage At İsothermal and Fluctutation Temperature Under Aerobic Conditions”, İnan.
- [14] Öksüztepe G., Beyazgül P., (2014), “Elazığ’da Satılan Pişmiş Et ve Tavuk Dönerlerin Mikrobiyolojik Kalitesi”, F.Ü. Sağ. Bil. Vet. Derg; 28 (2): 65 - 71 <http://www.fusabil.org>
- [15] Özoğul Y., Özoğul F., Külay E., (2006), “Modifiye Atmosfer Paketlenen Balık ve Balık Ürünlerine Etkisi”, Ege Üniversitesi Su Ürünleri Dergisi, 23,(1-2):193-200
- [16] TGK, (2009), Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği Tebliği, TEBLİĞ NO: 2009/6, Sayı: 27133.
- [17] TGK, (2012), Türk Gıda Kodeksi Et ve Et Ürünleri Tebliği, TEBLİĞ NO: 2012/74, Sayı: 28488.
- [18] *TS EN ISO 11290-1, 1997*
- [19] *TS EN ISO 4833-1, 2013*
- [20] *TS EN ISO 6579-1, 2017.*
- [21] TSE, (1995), TS 11658, “Döner yapım Kura zlları-Pişmemiş”, Türk Standartları Enstitüsü, 1-9.
- [22] TSE, (2003), TS 11859, “Döner Eti-Pişmemiş”, Türk Standartları Enstitüsü, 1-7.
- [23] Tülin A., Sülfer Ö., (2017), “Doğal Bileşiklerin Modifiye Atmosfer İle Kombinasyonunun Meyve ve Sebzelerde Ürün Kalitesi ve Muhafaza Ömrü Üzerine Etkileri”, Türkos Tüm Ürün, Kap ve Ambalaj Standartları Sempozyumu.
- [24] Wiley J., (1975), “Laboratory Techniques in Food Analysis D. Person (National College Of Food Technology, Univ. Of Reading, Weybridge, Surrey, U.K.)”, New York and Toronto, 1973, 35 pp.

## ***Bacillus* spp. Responsible for Spoilage of Dairy Products**

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### **Abstract**

This review's subject is *Bacillus* genus involved in the spoilage of dairy foods. Despite the improvement of production processes and hygiene applications, spore-forming bacteria are still an emerging problem in milk and dairy products. Spore-formers have adaptable spores, and thus they are prevalent in nature and food processing lines. Spore formation allows these bacteria to survive under heat treatments. Among the spore-forming bacterias, the genus *Bacillus* is of particular importance in dairy industry.

**Keywords:** *Milk, Dairy Products, Bacillus spp., Spoilage in Dairy Products*

### **Introduction**

In 1950s, introduction of cooling and cold storage techniques to raw milk was an obligatory technological step, and thus bacteriological quality of milk and dairy products has been significantly improved. After this, the acidification of raw milk caused by the growth of mainly lactic acid bacteria and/or other mesophilic bacteria was almost completely stopped.

The EU standard for high quality raw milk requires the total count of mesophilic aerobic bacteria to be lower than 30,000 cfu/mL, and the count of psychrotrophic bacteria to be lower than 5,000 cfu/mL (Kumarsan et al., 2007).

However, the extended storage times of raw milk at low temperatures (2-6 °C) have a significant effect on the composition of the natural microbial population present in milk. Thus, in cooled raw milk, initially dominant Gram-positive mesophilic aerobic bacteria are replaced by psychrotrophic bacteria. For these reasons, psychrotrophic bacteria usually account for more than 90% of the total microbial population in cooled raw milk.

Psychrotrophic bacteria associated with milk and dairy products include *Pseudomonas* spp. and

*Bacillus* spp. These are the most commonly isolated psychrotrophic bacteria associated with milk and dairy products, microorganisms that come from degraded raw milk or heat-treated milk (Meer et al., 1991). The main concern about the *Bacillus* spp. is their spoilage activity effecting product acceptance and product shelf-life. They can form endospores and can thus survive during heat treatments commonly used to process raw milk.

The genus *Bacillus* contains a varied array of Gram-positive, aerobic and facultative anaerobic endospore-forming rods. *Bacillus* spp. are found in a wide range of habitats. *Bacillus* genus belongs to the Bacillaceae family and is gram-positive (some variants are variable), aerobic or facultative anaerobic, spore-forming and rod-shaped bacteria (Kalkan and Halkman, 2006). Spore formation ensures that this bacterium is resistant and is not affected by processes such as pasteurization. Therefore, *Bacillus pumilus*, *B. licheniformis*, *B. subtilis* and *Bacillus megaterium* are found in some of the heat-treated products as the spoilage microflora.

Xu and Côté (2003) reported that predominant spore-formers are *Bacillus* genus with *Paenibacillus* in dairy products.

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*Bacillus pumilus*, *B. licheniformis*, *B. subtilis*, and *B. megaterium* have been related to spoilage of acidic food products (Hanlin, 1998).

*Bacillus licheniformis*, *B. cereus*, *B. subtilis*, *B. Mycoides* and *B. Megaterium* are the most prevalent spore-formers in dairy products (Ledenbach and Marshal, 2009). The ability to form spores, make the genus *Bacillus* an important threat for the dairy industry.

### Raw Milk

Raw milk is the general source of spore-forming bacteria. The basic microflora of raw milk consists of the *Bacillus* genus members (Kable et al., 2016; Magnusson et al., 2007). Among the spore-forming bacteria, the incidence of *Bacillus* species is approximately 95% in raw milk. Griffiths (1990) found that *Bacillus* species may be present at a level of  $10^5$  cfu / mL after 7 days of storage at 6 ° C. Several studies have shown that more than 5000 spores/mL can exist in fresh milk (Mikolajcik et al., 1978).

In another study, Lücking et al. (2013) studied the spoilage flora of milk and they found 43 species. The *B. cereus* group and *B. licheniformis* were the predominant species.

*Bacillus* species can produce extracellular proteolytic enzymes which degrade milk components. These components can cause off-flavor and quality defects in milk (Champagne et al., 1994; Ternstrom et al., 1993).

### Pasteurized Milk

HTST (High Temperature Short Time) pasteurization is an effective method which is performed at 72 °C for 15 s. Spores of psychrophilic, mesophilic and thermophilic species can survive from this process. Ivy et al. (2012) reported that the genus *Bacillus* has shown the predominance (>30%) among psychrophilic aerobic spore-formers in raw and pasteurized milk. Among the mesophilic species, *Bacillus subtilis* and *Bacillus licheniformis* are the most prevalent strains. The *B. cereus* group is the major one for psychrophilic species.

### Sterilized Milk

Ultra High Temperature (UHT) treatment is generally applied by heating milk between 135-145 °C for 1-10 s. The UHT process is planned to destroy approximately all microorganisms comprising spores. But some of these spores can survive this process and they can proliferate if they find adequate conditions.

*Bacillus licheniformis*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus sporothermodurans* and *Bacillus sphaericus* are the main *Bacillus* species isolated from UHT milk (Pettersson et al., 1996; Rombaut et al., 2002; Aouadhi et al., 2014). In another study carried out in Tunisia, the distribution of *Bacillus* species in UHT milk was 33% and *Bacillus licheniformis* was found to be the predominant strain in UHT milk samples (Kmiha et al., 2016).

### Cheeses

While the occurrence of *Clostridium* species in cheeses from different geographic areas has been widely investigated, there is no adequate research relating to *Bacillus* species.

In the study performed by Iurlina et al. (2006,) *B. pumilus* was identified in both Port Salut and Quattrolo cheeses with an incidence of 50% and 25%, respectively. They reported Port Salut Argentinian cheeses showed an incidence of *B. cereus* in 50% of the 30 samples. Meanwhile, they determined positive proteolytic and lipolytic activities in their isolates that could be associated with the spoilage of cheeses.

Cosentino et al. (1997) studies on 183 cheese samples collected in Sardinia to detect the frequency level of *Bacillus* spp. contamination. They found the incidence of positive samples at a ratio of 78% in Ricotta cheese and 69% in processed cheese. Their results showed that *B. cereus* was the predominant species in the cheese samples. *B. sphaericus* was the second prevalent species in Ricotta cheese samples while *B. brevis* was prevalent in processed cheese samples.

### Milk Powder

The primary bacteria causing the spoilage of milk powder are thermophilic bacilli. Thermophilic bacilli are not pathogenic but when milk powder is used, spores can germinate in the product. This may induce acid production, enzyme production and significant off-flavors in the product (Chopra and Mathur 1984; Chen et al. 2004). Spores can survive at high temperatures during the process and the low water activity, the cleaning-in-place (CIP) system and the long-term storage of the final product.

Rueckert et al. (2004) studied the distribution of thermophilic spores in milk powder for infants in China. *B. licheniformis* was the most frequent species found in the samples. These samples have spore contamination generally less than 1000 CFU g<sup>-1</sup>. Other bacilli that are also found in milk powder are the facultative thermophiles such as *Bacillus licheniformis*, *Bacillus coagulans* and *Bacillus subtilis*. These are usually present at low levels (Crielly et al. 1994; Ronimus et al. 2003).

### Conclusion

Spoilage of milk and dairy products through *Bacillus* spp. causes high economic losses in the dairy industry. The species isolated in dairy product, especially from milk and cheese, have a high spoilage potential.

*Bacillus* spp. have proteolytic and lipolytic activity and this may cause quality losses such as sweet curdling of milk, off-flavors and bitterness in cheese and textural defects in cultured dairy products. In order to prevent and control of contamination, many research studies are focused on the possible contamination sources. Thus, to understand and control the effects of spore forming, spore germination and food-spoilage mechanisms need to be well established.

In conclusion, this review provides food microbiologists with an overview of *Bacillus* species responsible for dairy product spoilage.

### REFERENCES

- [1] A.K., Chopra and D.K., Mathur, 1984. "Isolation, screening and characterization of thermophilic *Bacillus* species isolated from dairy products," *Journal of Applied Bacteriology* vol.57, pp. 263-271
- [2] A., Ternstrom, A.M., Lindberg, G., Molin, 1993. "Classification of the spoilage flora of raw and pasteurized bovine-milk, with special reference to *Pseudomonas* and *Bacillus*," *J. Appl. Bacteriol.*, vol. 75, pp. 25-34
- [3] A., Rueckert, R.S., Ronimus, H.W., Morgan, 2004. "A RAPD based survey of thermophilic bacilli in milk powders from different countries," *International Journal of Food Microbiology* vol. 96, pp. 263-272
- [4] B., Petterson, F. Lembke, P. Hammer, E. Stackebrandt, and F. G. Priest, 1996. "Bacillus sporothermodurans, a new species producing highly heat-resistant endospores," *Int. J. Syst. Bacteriol.*, vol. 46, pp. 759-764
- [5] C., Aouadhi, A., Maaroufi and S., Mejri. 2014. "Incidence and characterization of aerobic spore-forming bacteria originating from dairy milk in Tunisia," *Int. J. Dairy Technol.*, vol. 67, pp. 95-102
- [6] C.P., Champagne, R.R., Laing, D., Roy, A.A., Mafu, M.W., Griffiths, 1994. "Psychrotrophs in dairy-products - their effects and their control," *Crit. Rev. Food Sci.*, vol. 34, pp. 1-3
- [7] D., Xu, J., Côté, 2003. "Phylogenetic relationships between *Bacillus* species and related genera inferred from comparison of 3' end 16S rDNA and 5' end 16S-23S ITS nucleotide sequences," *Int. J. Syst. Evol. Microbiol.*, vol. 53(3), pp. 695-704
- [8] E.M., Mikołajcik, N.T., Simon, 1978. "Heat Resistant Psychrotrophic Bacteria in Raw Milk and Their Growth at 7 °C," *Journal of Food Protection*, vol. 41 No.2, pp. 93-95
- [9] E.M., Crielly, N.A., Logan, A., Anderton, 1994. "Studies on the *Bacillus* flora of milk and milk products," *Journal of Applied Bacteriology*, vol. 77, pp. 256-263

- [10] G., Kumarsan, R., Annalvilli, K., Sivakumar, 2007. "Psychrotrophic spoilage of raw milk at different temperatures of storage," *Journal of Applied Science Research*, vol. 3, pp. 1383-1387
- Ledenbach, H. and R.T. Marshall. 2009. *Microbiological Spoilage of Dairy Products*. W.H. Sperber, M.P. Doyle (eds.), *Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Food Safety*, DOI 10.1007/978-1-4419-0826-1\_2, C Springer Science+Business Media, LLC 2009
- [11] G., Lücking, M., Stoeckel, Z., Atamer, J., Hinrichs, M., Ehling-Schulze, 2013. "Characterization of aerobic spore-forming bacteria associated with industrial dairy processing environments and product spoilage," *International Journal of Food Microbiology*, vol. 166 Issue 2, pp. 270-279
- [12] J.H. Hanlin, 1998. "Spoilage of acidic products by *Bacillus* species," *Dairy foods and environmental sanitation*, vol.18, pp. 655-659
- [13] L., Chen, T., Coolbear, R.M., Daniel, 2004. "Characteristics of proteinases and lipases produced by seven *Bacillus* sp. isolated from milk powder production lines," *International Dairy Journal*, vol.14, pp.495-504
- [14] M.W., Griffiths, 1990. "Toxin production by psychrotrophic *Bacillus* spp. present in milk," *Journal of Food Protection*, vol. 53, pp. 790-792
- [15] M., Magnusson, A., Christiansson, B., Svensson, 2007. "Bacillus cereus spores during housing of dairy cows: factors affecting contamination of raw milk," *J. Dairy Sci.*, vol. 90, pp. 2745-2754
- [16] M.O., Iurlina, A.I., Saiz, S.R., Fuselli, R., Fritz, 2006. "Prevalence of *Bacillus* spp. in different food products collected in Argentina," *LWT*, vol. 39, pp. 105-110
- [17] M.E., Kable, Y., Srisengfa, M., Laird, J., Zaragoza, J., McLeod, J., Heidenreich, M.L., Marco, 2016. "The core and seasonal microbiota of raw bovine milk in tanker trucks and the impact of transfer to a milk processing facility," *mBio*, vol. 7 (4):e00836
- [18] R.R., Meer, J., Baker, F.W., Bodyfelt, M.W., Griffiths, 1991. "Psychrotrophic *Bacillus* spp. in fluid milk products: a review," *Journal of Food Protection* vol. 54, pp. 969-979
- [19] R., Rombaut, K., Dewettinck, G., de Mangelare and A. Huyghebaert, 2002. "Inactivation of heat-resistant spores in bovine milk and lactulose formation," *Milchwissenschaft*, vol. 57, pp. 432-436
- [20] R.S., Ronimus, L.E., Parker, N., Turner, S., Poudel, A., Ruckert, H.W., Morgan H W, 2003. "A RAPD-based comparison of thermophilic bacilli from milk powders," *International Journal of Food Microbiology*, vol.85, pp. 45-61
- [21] S., Cosentino, A.F., Mulargia, B., Pisano, P., Tuveri, F., Palmas, 1997. "Incidence and biochemical characteristics of *Bacillus* flora in Sardinian dairy products," *International Journal of Food Microbiology*, vol. 38, pp. 235-238
- [22] S., Kalkan, K., Halkman, 2006. "Bacillus cereus ve İçme Sütünde Oluşturduğu Sorunlar," *Orlab On-Line Microbiology Journal*, vol. 04/1, pp. 1-11
- [23] S., Kmiha, C., Aouadhi, A. Klibi, A., Jouini, A. Béjaoui, S., Mejri, and A., Maaroufi, 2016. "Seasonal and regional occurrence of heat-resistant spore-forming bacteria in the course of ultra-high temperature milk production in Tunisia," *J. Dairy Sci.*, vol. 100, pp. 1-10

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