Serap KEÇECİ

QUALITATIVE AND QUANTITATIVE DETECTION OF MILK POWDER IN UHT AND PASTEURIZED MILK

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by

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APPROVAL PAGE

This is to certify that I have read this thesis written by Serap KEÇECİ and that in my opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Genetics and Bioengineering.

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Serap KEÇECİ

M.S. Thesis – Genetics and Bioengineering
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Thesis Supervisor: Assoc. Prof. Dr. Mustafa Fatih ABASIYANIK

ABSTRACT

The milk consist nutrients which are required for development of the body right from infancy to old age and thus needed for a good health. Importance on the consumption of milk is increasing with each passing day. But there are some concerns and debates about the drinking milk which are being produced by dairy sectors. Although drinking milk manufactures declare that drinking milk is produced from raw milk while raw milk producer claims that drinking milk contain milk powder and this practice economically damages them. Besides, consumers want be specified on the label if milk powder is used. In this study, a novel analytical HPLC method that used qualitative and quantitative detection of milk powder in UHT and pasteurized milk was developed. The research consists of five steps: sample preparation, protein content determination, thermal treatments, hydrolysis, filtration and HPLC determination. Furosine, a Maillard reaction product, was used as an indicator of milk powder. The results indicated here is a linear interaction with milk powder concentration and mg Furosine/100 g protein.

Keywords: Milk Powder, HPLC, UHT Milk, Pasteurized Milk, Furosine, Maillard Reaction

UHT VE PASTÖRİZE SÜTLERDE NİTEL VE NİCEL SÜT TOZU TAYİNİ

Serap KEÇECİ

Yüksek Lisans Tezi – Genetik ve Biyomühendislik
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Tez Danışmanı: Doç. Dr. Mustafa Fatih ABASIYANIK

ÖZ

Süt vücudun bebeklikten yaşlılığa kadar olan dönemde gelişmesi ve hastalıklardan korunması için gerekli olan besin maddelerini içermektedir. Süt tüketimine verilen önem her geçen gün artmakla birlikte bazı endişe ve tartışmalar da söz konusu olabilmektedir. Çiğ süt üreticileri UHT ve pastörize süt üretiminde ithal süt tozu kullanıldığını ve bunun sonucunda zor durumda kaldıklarını, içme sütü üreticileri süt tozu kullanmadıklarını iddia etmektedirler. Tüketiciler ise süt tozu kullanılıyor ise etiketinde belirtilmesini istemektedirler. Bu çalışmada UHT ve pastörize süt üretiminde süt tozu kullanılıp kullanılmadığının kullanılıyor ise ne oranda kullanıldığının tespit edilebilmesi amacıyla yeni bir analitik HPLC yöntemi geliştirildi. Araştırmamız örneklerin hazırlanması, protein tayini, ısıl işlem, hidroliz, filtrasyon ve HPLC analizleri olmak üzere beş aşamadan oluşmaktadır.Maillard reaksiyonu ürünü olan Furosin amino asidi indikatör olarak kullanılmıştır. Sonuçlar incelendiğinde süt tozu miktarları ile mg Furosin/100 g protein oranı arasında lineer bir ilişki olduğu gözlenmiştir.

Anahtar Kelimeler: Süt Tozu, Pastörize Süt, UHT Süt, Furosin, HPLC, Maillard Reaksiyonu

This thesis is dedicated to my husband Akif KEÇECİ

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LIST OF SYMBOLS AND ABBREVIATIONS

SYMBOL/ABBREVIATION

USDA	United States Department of Agriculture
FAO	Food and Agriculture Organization
TSEK	Turkish Dairy Industry Institution
AGE	Advanced glycated end products
UHT	Ultra high temperature
HPLC	High performance liquid chromatography

CHAPTER 1

INTRODUCTION

Milk is a complex mixture consisting of an oil-in-water type emulsion which is stabilized by phospholipids and proteins that are absorbed on the surface of the fat globule. In addition it contains proteins in colloidal dispersions, lactose, numerous minerals, particularly calcium and phosphorous, fat soluble and water soluble vitamins, enzymes and various organic compounds.

According to the USDA (United States Department of Agriculture) food guide pyramid and Turkey Specific Nutrition Guide which is established by Health Ministry of Turkey with Hacettepe University (Department of Nutrition and Dietetics), there are four food groups of nutrition in order to have an adequate and balanced diet. One of the four food groups in the daily diet is milk and milk replacer such as cheese, yogurt. They are consumed, especially in need of protein and calcium content. It is also an important source of nutrients such as vitamin B_2 (riboflavin), vitamin B_{12}, vitamin A, thiamine, niacin, phosphorus and magnesium. People of all age groups especially women, children and young ones need to consume them each day. Beside the nutritional value consisting of basic proteins, lipids and saccharides, milk also contains numerous biologically active substances, such as immunoglobulins, enzymes, antimicrobial peptides, oligosaccharides, hormones, cytokines and growth factors making them indispensable in diet.

Milk protein is a heterogeneous mixture consisting of casein and whey protein as the basic ingredients, enzymes, and minor amounts of non-protein nitrogen containing compounds. Because of the presence of essential amino acid content, milk protein is known to be high quality protein and is used as standard for estimation of protein

quality in foods. In addition to being key precursors for synthesis of hormones and low-molecular weightnitrogenous substances, amino acids, which compose protein structure, are also cell signaling molecules and regulators of gene expression and the protein phosphorylation cascade.

Lipids, which influence milk appearance, taste, flavor and resistance, are source of energy, essential fatty acids and fat soluble vitamins. Milk contains triglycerides (between 97-98%), phospholipids (between 0.2-1.0 %), free sterols (between 0.22-0.41%) such as cholesterol, waxes, etc., fat soluble vitamins [A, D, E, K] and more than 400 free fatty acid derivatives.

Milk contains all of the fat-soluble vitamins which are A, D, E, K and water-soluble vitamins which are ascorbic acid and the B family (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folacin, biotin, choline, inositol, and vitamin B12). They are essential for balanced nutrition and sound health. Although it is a poor source of ascorbic acid (vitamin C), milk is one of the richest food sources of all vitamins.

Milk contains all of the 14 essential minerals which includes the macro group-calcium, phosphorus, potassium, magnesium, sulfur, sodium, and chlorine; and micro group- iron, copper, cobalt, manganese, iodine, zinc, and fluorine. Milk and dairy products are an excellent source of calcium and a good source of phosphorus, both of which are the two major tooth-and-bone-building elements, but are a poor source of iron and a variable source of iodine.

The energy content of milk varies according to its type. The elements of the macro-nutrients such as carbohydrate, fat and protein generate the energy content of unadulterated milk. On the other hand the organic acid and alcohol affects this value.

Animal derived foods are important for human nutrition, particularly due to their high quality protein, mineral, vitamin and micronutrient content, and also have major importance for optimizing human performance among the moderately malnourished populations. In 2005, the per capita consumption of meat, milk and fish were recorded at 9.10 kg/year, 136.61 kg/year, and 6.93 kg/year respectively in Turkey.

Livestock is a carrier power of food industry which meets the critical needs of the people. With an appreciable value of 15%, dairy industry has an important role in the

food industry. According to the FAO (Food and Agriculture Organization) statistics, cow milk was one of the 20 most important food and agricultural commodities in the world in 2010. According to the same statistics, cow milk is the most valuable food and agricultural commodities in Turkey. According to the Turkish Statistical Institute, total quantity of milk as an animal product is 13,543,674 tons. Same statistic shows that 48 % of the total employment in Turkey is in agricultural sector.

Popular milk products are cheese, yogurt, liquid milk, milk powder etc. According to the final product, types of milk production processes differ. In this study we concerned about liquid milk and milk powder technology.

Adulteration of raw milk by adding reconstituted milk (milk powder with water) or the selling of reconstituted milk as the fresh product can be economically advantageous where either plenty of milk powder prevails or in countries where the importation of milk powder is supported. However adulteration could create unfair competition and market distortions could occur. In the meanwhile such practices are prohibited by legislation, thus the detection of milk powder becomes an analytical problem. Because of prevailing concern, we focused on the detection of milk powder in UHT and pasteurized milk in this study.

CHAPTER 2

REVIEW OF LITERATURE

2.1 MILK

According to the Turkish Food Codex Regulation (consumable) raw milk is the secretion (except colostrums) of mammary glands obtained from cows, goats, sheep, and buffalos by milking without any extra processes including heat treatment (it should be above 40˚C). (Raw milk and heat treated liquid milk notification.

According to the Turkish Standards (TS) 1018 raw milk standards are that raw milk should be a white or crème colored liquid secreted from cow, sheep, goats and buffalos mammary gland and have special taste and consistence, any of its ingredients should not be separated or it should not be mixed with anything.

2.2.1 A Brief History of Milk

Milk was used to feed babies of mammals. But after the domestication of animals during the Neolithic Revolution, human learned to consume milk of other mammals not only to feed young of animals but also to use it as food for human being.

The first domesticated animals were cattle, ships and goats as early as 9000-7000 BC in Southwest Asia and 3500-3000 BC in the Americas. However domestic cattle have been derived from wild aurochs populations several times since (Bellwood, 2005). From Southwest Asia domestic dairy animals spread to Europe around 7000-4000 BC and South Asia 7000-5500 BC (Beja-Pereira et al. 2006). Sheep and goats were introduced to Africa from Southwest Asia, but African cattle may have been domesticated around 7000-6000 BC (Meadow, 1996). In the rest of the world (East and Southeast Asia, Australia) milk and dairy products were historically not a large part of

diet. Milk consumption became common in these regions after the European colonialism.

2.1.2 Composition of Milk

Milk is a complex mixture consisting of an oil-in-water type emulsion which is stabilized by phospholipids and proteins that are absorbed on the surface of the fat globule. In addition it contains proteins in colloidal dispersions, lactose, numerous minerals, particularly calcium and phosphorous, fat soluble and water soluble vitamins, enzymes and various organic compounds (Louis et al., 1970). Composition of milk differs according to the species (Table 2.1).

Table 2.1 Average composition (%) of milk of various mammals (FAO, 1992).

Species	***Water***	***Fat***	***Protein***	***Lactose***	***Ash***
Human	87.43	3.75	1.63	6.98	0.21
Cow	87.20	3.70	3.50	4.90	0.70
Goat	87.00	4.25	3.52	4.27	0.86
Sheep	80.71	7.90	5.23	4.81	0.90
Indian buffalo	82.76	7.38	3.60	5.48	0.78
Camel	87.61	5.38	2.98	3.26	0.70
Horse	89.04	1.59	2.69	6.14	0.51
Llama	86.55	3.15	3.90	5.60	0.80

Composition of milk differs according to the countries, too. The largest differences are observed in total fat content as shown Table 2.2 (Scheonfeldt et al. 2012).

Table 2.2 Approximate composition of whole bovine milk from different countries.

Whole Bovine Milk per 100 g		South Africa	USA	UK	Denmark	Australia
Moisture	g	88.0	88.3	87.8	87.8	87.5
Energy	kj	260	252	275	269	278
Protein(N*6.38)	g	3.25	3.22	3.20	3.40	3.30
Lactose	g	4.80	5.26	4.80	4.64	4.70
Total Fat	g	3.43	3.25	3.90	3.50	4.00

One of the factors influencing the composition of milk is genetic characteristic. Each dairy breed produces nutrients in specific proportions (Soyeurt and Gengler, 2008). Another factor is volume of milk and stage of lactation. An increase in milk volume that is produced per animal results in composition alters e.g. changed fat and protein profiles and decreased nutrient density. The last factor is feeding regime. Milk lactose or proteins have sensitivity to the dietary manipulation but milk fat has greatest sensitivity (Jenkins and McGuire, 2006).

Amount of vitamins and other components in milk is also directly influenced by same factors so composition of them differs according to the countries (Soyeurt and Gengler, 2008), (Table 2.3).

Table 2.3 Vitamin and mineral composition of whole bovine milk from different countries (mg or μg /100g).

		South Africa	USA	UK	Denmark	Australia
Vitamins						
Retinol	μg	43.4	28.0	52.0	29.2	36.0
Beta carotene	μg	-	5.00	21.0	16.0	19.0
Vitamin B_1	mg	0.02	0.04	0.04	0.04	0.03
Vitamin B_2	mg	0.16	0.18	0.17	0.17	0.18
Vitamin B_3	mg	-	0.11	0.08	0.86	0.70
Vitamin B_6	μg	34.5	36.0	60.0	47.0	20.0
Folic acid	μg	-	5.00	6.00	11.0	7.00
Vitamin B_{12}	μg	-	0.44	0.40	0.45	-
Biotin	μg	-	-	1.90	1.40	3.90
Vitamin C	mg	-	-	1.00	1.20	1.00
Minerals						
Calcium	mg	120	113	115	116	117
Iron	mg	-	0.03	0.05	0.04	-
Magnesium	mg	11.7	10.0	11.0	11.1	11.0
Phosphorus	mg	90.3	91.0	92.0	93.0	92.0
Potassium	mg	157	143	140	144	155
Sodium	mg	48.3	40.0	55.0	45.4	-
Zinc	mg	-	0.40	0.40	0.42	0.40
Copper	mg	-	0.01	Tr	0.01	0.003
Manganese	μg	-	0.003	-	0.01	0.0007
Selenium	μg	-	3.70	1.00	1.61	1.00
Iodine	μg	-	-	15.0	24.3	13.3

Milk and milk products have been used as important foods throughout the civilizations. They have extreme nutritional value and include more essential nutrients then another single food. Because they contain supplement those lacking in meat,

vegetables, fruit, and cereals, anybody who wishes gain (or lose) weight should have dairy products in the daily diet for well-being and health.

2.1.2.1 Proteins and Amino acids

Milk proteins are heterogeneous mixtures which consist of casein and whey protein as the basis, enzymes, and minor amounts of non-protein nitrogen containing compounds. 73% of protein is casein and %8 of its whey protein (Fox and McWeeney, 2003). Because of the essential amino acid content, milk protein is known as high quality protein and is used as standard for estimation of protein quality in foods (Baysal, 2004).

Amino acids, which compose protein structure, are defined as organic substances containing both amino and acid groups. Amino acids have enormous biological importance. In addition to being key precursors for syntheses of hormones and low-molecular weight nitrogenous substances, amino acids are cell signaling molecules and regulators of gene expression and the protein phosphorylation cascade.

On the basis of needs from the diet for nitrogen balance or growth, amino acids were traditionally classified as nutritionally essential (indispensable) or non-essential (dispensable) for humans and animals. Essential amino acids for humans are Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine. Non-essential amino acids for humans are Alanine, Asparagine, Aspartate, Cysteine, Glutamate, Glutamine, Glycine, Proline, Serine, Taurine and Tyrosine (Guoyao, 2009).

2.1.2.2 Carbohydrates

Lactose, which is the fundament carbohydrate of milk forms 4,7% of milk and 54% of milk-solids-non-fat (McCance and Widdowson, 1988). Its concentration represents a balance between the high nutrient requirements of the infant and the constraints of carbohydrate concentration in milk due to osmolarity. Most milk contains small amounts of glucose and galactose, the biosynthetic precursors of lactose. The oligosaccharide content varies greatly among species, and within human populations oligosaccharides also manifest great heterogeneity both qualitatively and quantitatively (Jensen, 1995).

Many people suffer from a condition known as 'lactose intolerance'. This means that they are unable to digest the milk fat (lactose). Such people can, however, tolerate milk if it is fermented to produce foods such as yoghurt. During fermentation, lactic acid producing bacteria break down lactose, and in doing so eliminate the cause of irritation (FAO, 1992).

2.1.2.3 Lipids

Lipids, which influence milk appearance, taste, flavor and resistance, are a source of energy, essential fatty acids and fat soluble vitamins. Milk contains triglycerides (between 97-98%), phospholipids (between 0.2-1.0 %), free sterols (between 0.22-0.41%) such as cholesterol, waxes, etc., fat soluble vitamins [A, D, E, K] and more than 400 free fatty acid derivatives (Gehardt et al. 2006).

Although milk fat contains 5% saturated fatty acids, it is important in terms of health for the positive events in the chronic diseases since contains conjugated linoleic acid, sifingomiyelin, butyric acid and myristic acid (Ebringer et al. 2008).

2.1.2.4 Vitamins

Milk contains all of the fat-soluble vitamins which are A, D, E, K and water-soluble vitamins which are ascorbic acid and the B family (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folacin, biotin, choline, inositol, and vitamin B12). They are essential human nutrition and health. Although it is a poor source of ascorbic acid (vitamin C), milk is a one of the richest food sources of all vitamins (Louis, 1970).

2.1.2.5 Minerals

Milk contains all of the 14 essential minerals which are major group-calcium, phosphorus, potassium, magnesium, sulfur, sodium, and chlorine; microgroup-iron, copper, cobalt, manganese, iodine, zinc, and fluorine. Milk and dairy products are an excellent source of calcium and a good source of phos-phorus that are the two major tooth-and-bone-building elements, but are a poor source of iron and a variable source of iodine (Louis, 1970)

2.1.2.6 Energy

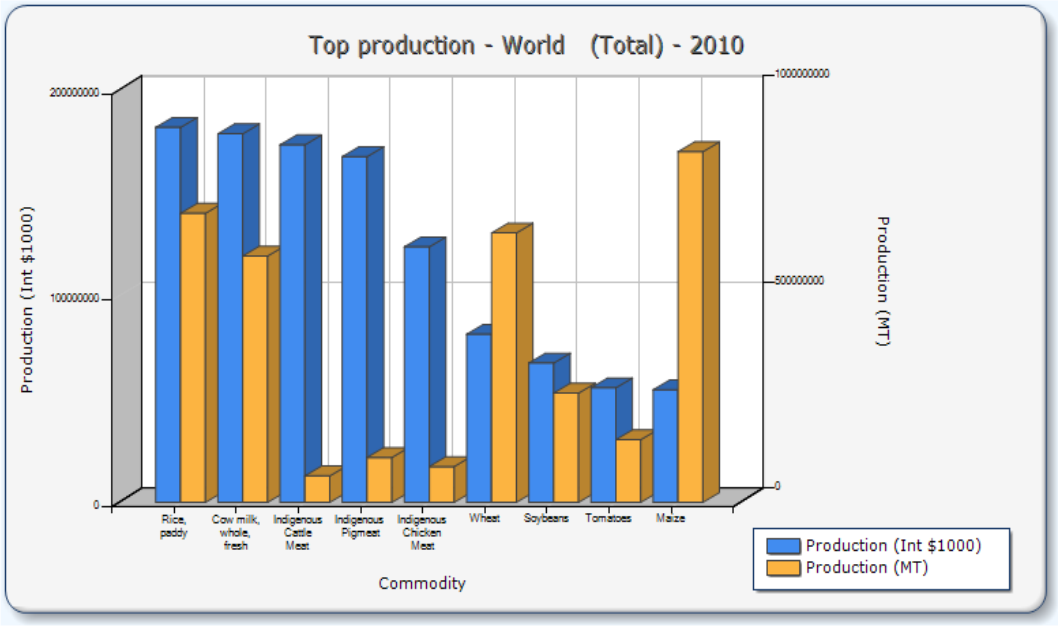
The energy content of milk varies according to the type. The elements of the macro-nutrients such as carbohydrate, fat and protein generate the energy content of unadulterated milk. Additionally the organic acid and alcohol affect this value.

2.1.3 Nutritional Value of Milk

According to the USDA food guide pyramid and Turkey Specific Nutrition Guide which is established by Health Ministry of Turkey with Hacettepe University, Department of Nutrition and Dietetics there are four food groups of nutrition in order to adequate and balanced diet. One of the four food groups in the daily diet is milk and milk replacer such as cheese, yogurt. They are consumed, especially in terms of protein and calcium content. Otherwise it is an important source of nutrients such as vitamin B_2 (riboflavin), vitamin B_{12}, vitamin A, thiamine, niacin, phosphorus and magnesium. People of all age groups especially women, children and young need to consume each day. Aside from the nutritious value consisting of basic proteins, lipids and saccharides, milk also contains numerous biologically active substances, such as immunoglobulins, enzymes, antimicrobial peptides, oligosaccharides, hormones, cytokines and growth factors (Ebringer et al. 2008).

2.1.4 Dairy Sector by Numbers

Livestock is a carrier power of food industry which meets the critical needs of the people. With a value of 15% dairy industry has an important role in the food industry. According to the FAO statistics, cow milk is one of the 20 most important food and agricultural commodities in the world for 2010 and production value of it is 178.7 billion dollar (see Figure 2.1).

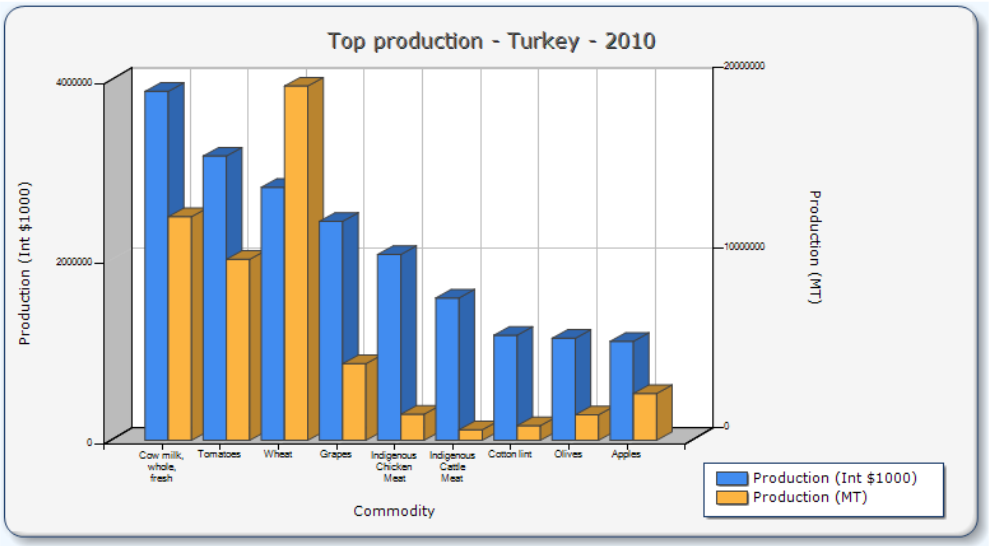


Rank	Commodity	Production (Int $1000)	Flag	Production (MT)	Flag
1	Rice, paddy	181912414	*	701127975	A
2	Cow milk, whole, fresh	178787744	*	597608883	A
3	Indigenous Cattle Meat	173436446	*	64203001	A
4	Indigenous Pigmeat	167700746	*	109092033	A
5	Indigenous Chicken Meat	123949279	*	87018162	A
6	Wheat	81554403	*	653355358	A
7	Soybeans	67601682	*	264972690	A
8	Tomatoes	55581485	*	152055325	A
9	Maize	54617721	*	850445143	A
10	Sugar cane	53528915	*	1694505010	A
11	Hen eggs, in shell	52826294	*	63753024	A
12	Potatoes	45307909	*	334262523	A
13	Vegetables fresh nes	44966170	*	262244103	A
14	Grapes	38307995	*	67016892	A
15	Buffalo milk, whole, fresh	36266933	*	92236864	A
16	Cotton lint	33896489	*	23717040	A
17	Apples	29382111	*	70035744	A
18	Bananas	29256235	*	105213003	A
19	Cassava	23005753	*	236707262	A
20	Indigenous Sheep Meat	22649675	*	8318526	A

* : Unofficial figure
A : Aggregate, may include official, semi-official or estimated data

Figure 2.1 Production of the 20 most important food and agricultural commodities (ranked by value) in the world for 2010 (FAO, 2010).

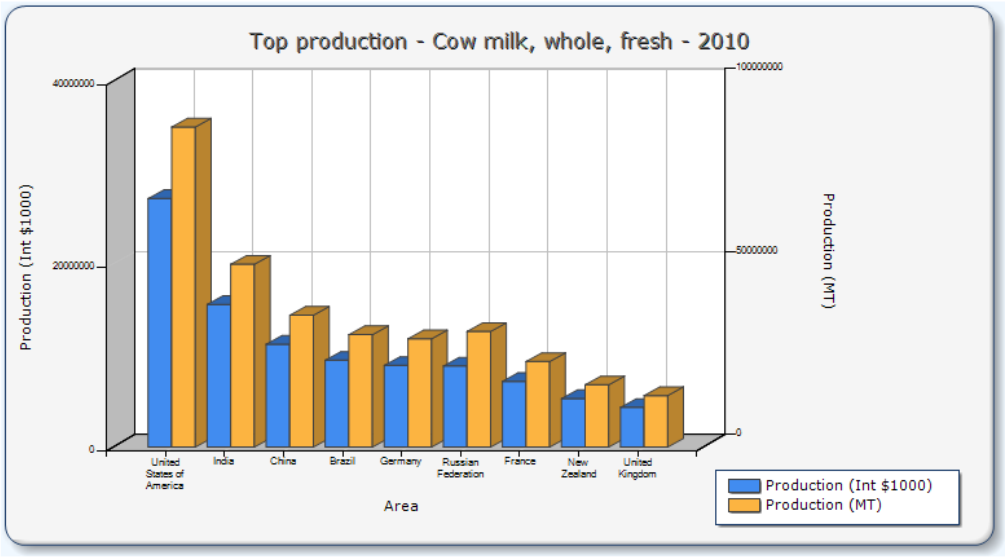
According to the same statistics, cow milk is the most valuable food and agricultural commodities in Turkey for 2010 and production value of cow milk are 3.8 billion dollar (see Figure 2.2). With this production value Turkey is the tenth of 20 highest producing countries of cow milk for 2010 (see Figure 2.3).



Rank	Commodity	Production (Int $1000)	Flag	Production (MT)	Flag
1	Cow milk, whole, fresh	3875330	*	12418500	
2	Tomatoes	3157603	*	10052000	
3	Wheat	2810247	*	19674000	
4	Grapes	2432230	*	4255000	
5	Indigenous Chicken Meat	2064251	*	1449200	Fc
6	Indigenous Cattle Meat	1581026	*	585267	Fc
7	Cotton lint	1167238	*	816705	
8	Olives	1132995	*	1415000	
9	Apples	1099576	*	2600000	
10	Hazelnuts, with shell	961745	*	600000	
11	Chillies and peppers, green	935255	*	1986700	
12	Sugar beet	771761	*	17942100	
13	Potatoes	720204	*	4548090	
14	Indigenous Sheep Meat	643242	*	236243	Fc
15	Hen eggs, in shell	613768	*	740024	
16	Cherries	531271	*	417905	
17	Pistachios	420369	*	128000	
18	Cottonseed	409337	*	1272800	
19	Strawberries	407102	*	299940	
20	Onions, dry	399063	*	1900000	

* : Unofficial figure
[ ]: Official data
Fc: Calculated data

Figure 2.2 Production of the 20 most important food and agricultural commodities (ranked by value) in Turkey for 2010 (FAO, 2010).



Rank	Area	Production (Int $1000)	Flag	Production (MT)	Flag
1	United States of America	27161602	*	87474400	
2	India	15590567	*	49960000	
3	China	11245443	*	36036043	
4	Brazil	9489243	*	30715500	
5	Germany	8926161	*	29593900	
6	Russian Federation	8855245	*	31585200	
7	France	7178744	*	23374300	
8	New Zealand	5292710	*	17010500	
9	United Kingdom	4331718	*	14081000	
10	Turkey	3875329	*	12418500	
11	Poland	3706879	*	12278700	
12	Pakistan	3687047	*	12437000	
13	Netherlands	3628052	*	11626100	
14	Mexico	3331781	*	10676700	
15	Argentina	3277233	*	10501900	
16	Italy	3267278	*	10500000	
17	Ukraine	2926258	*	10977200	
18	Australia	2803243	*	9023000	
19	Canada	2572318	*	8243000	*
20	Japan	2396459	*	7720460	

* : Unofficial figure
[ ]: Official data

Figure 2.3 Highest 20 producing countries of cow milk for 2010(FAO, 2010).

According to the Turkish Statistical Institute, total quantity of milk as an animal product is 13,543,674 tons. %91.7 of it is cow milk, %6 of it is ship milk %2 of it is goat milk and %0.3 of it is buffalo milk (Turkish Statistical Institute, 2012).

2.1.4 An Overview of Dairy Sector in Turkey

The livestock sector and dairy subsector have great importance for Turkey, in terms of both nutrition and economy. The share of the livestock sector in the total agricultural production value has varied between 25% and 30% in the last three decades. Raw milk production is characterized by small-scale farms, with an average of

three heads of dairy cattle per farm. Turkey's dairy industry was established and developed by the State with the opening of the Turkish Dairy Industry Institution (TSEK) in 1963 as a state-owned enterprise. TSEK facilitated modernization of the sector, created a dairy market and played a role in stabilizing the consumer and producer prices. In 1995, a liberalization movement in the agricultural sector started in Turkey and the TSEK enterprises were privatized. After privatization, producer prices decreased between 11.51% and 18.45% and consumer prices of dairy products increased in general. Today, the dairy processing sector has a dual structure that on one hand comprises many small- and medium-sized enterprises, while on the other features seven large holding companies that hold the largest market share (Gönenç and Tanrıvermiş, 2008).

Animal-sourced foods are important for human nutrition, particularly due to their high quality protein, mineral, vitamin and micronutrient content, and have also major importance for optimizing human performance among the moderately malnourished populations. In 2005, the per capita consumption of meat, milk and fish was recorded at 9.10 kg/year 136.61 kg/year, and 6.93 kg/year in Turkey, respectively (FAO, 2007).

According to the results of an agricultural census, in 2001 there were 3.1 million farms in Turkey, with an average of 6.1 hectares of land. Of these farms, those engaged in both crop production and livestock constituted the largest share, accounting for 67.43% of the total. Of the remaining, 30.21% produced only crops, while 2.36% kept only livestock. The average number of large ruminants per farm decreased from 6.0 in 1980 to 4.6 in 1990, and further to 3.6 in 2001; while the average of small ruminants increased from 39.8 in 1980 to 42.1 in 1990, falling to 12.8 in 2003 (State Institute, 2004).

Turkey's annual milk production is approximately 11.5 million tone, of which around 90% was cow's milk (10 million tone) in 2005. Raw milk production data for Turkey are given in Table 2.4. Since 1961, while total raw milk production has increased by 71.0%, cow's milk production has increased by 107.6% in accordance with the development of the Turkish dairy sector. The share of cow's milk in total milk production has also risen, from 74% in 1961 to 90% in 2005.

Table 2.4 Raw milk productions in Turkey (1000 tones).

Years	Cow Milk	Sheep Milk	Goat Milk	Others	Total milk Production	Cow milk/ Total milk (%)
1961	4 830.00	785.00	644.00	248.52	6 507.00	74,23
1965	5 434.00	780.00	494.80	293.00	7 001.80	77,61
1970	5 722.60	860.00	481.60	279.00	7 343.20	77,93
1975	6 474.00	993.00	485.70	283.00	8 235.70	78,61
1980	7 710.60	1 147.39	483.0	273.90	9 614.90	80,19
1985	7 994.27	1 072.60	363.40	239.85	9 670.12	82,67
1990	7 960.64	1 145,02	337.53	174.22	9 617.41	82,77
1995	9 275.31	934.00	277.20	114.54	10 601.55	87,49
2000	8 732.04	774.00	220.21	67.33	9 793.96	89,16
2001	8 489.08	723.00	219.79	63.33	9 495.55	89,40
2002	7 490.63	657.00	209.62	50.92	8 408.56	89,08
2003	951431	769.96	278.14	48.78	10 611.19	89,66
2004	9 609.32	771.72	259.09	39.28	10 679.41	89,98
2005	10 026.20	789.88	253.76	38.06	11 107.89	92,26
Change(%) 1961-2005	107.58	0.62	-60.60	-84.69	70.71	

Dairy produce consumption per capita is low in Turkey when compared with developed countries. The per capita consumption figures of the main dairy products are shown in Table 2.5 Annual per capita consumption of milk fell from 174.7 kg in 1960 to 98.0 kg in 2004. After privatization, consumer prices of dairy products increased in general and producer prices decreased in all regions by between 11.5 and 18.45% (Gönenç and Rehber, 2007).

Table 2.5 Per capita consumption of some dairy product (kg/year) (2003).

		Butter	Cheese	Whole milk	Milk excluding butter
World	2003	1.30	2.78	44.20	79.00
	Change 1961-2003 (%)	-25.23	57.50	-15.00	5.33
Developed countries	2003	3.09	11.23	92.90	202.10
	Change 1961-2003 (%)	-30.95	140.78	-23.41	1.49
Developing countries	2003	0.82	0.52	31.00	45.60
	Change 1961-2003 (%)	78.60	1173.24	58.97	62.86
Turkey	2003	1.74	1.82	75.50	98.00
	Change 1961-2003 (%)	-45.72	-23.94	-40.55	-43.68

The consumption of fluid milk is not common in Turkey, as raw milk is generally processed and consumed as cheese or yogurt. The raw milk utilization ratio according to products is shown in Figure 2.4 (calculated based on milk equivalent) (Gönenç and Tanrıvermiş, 2008).

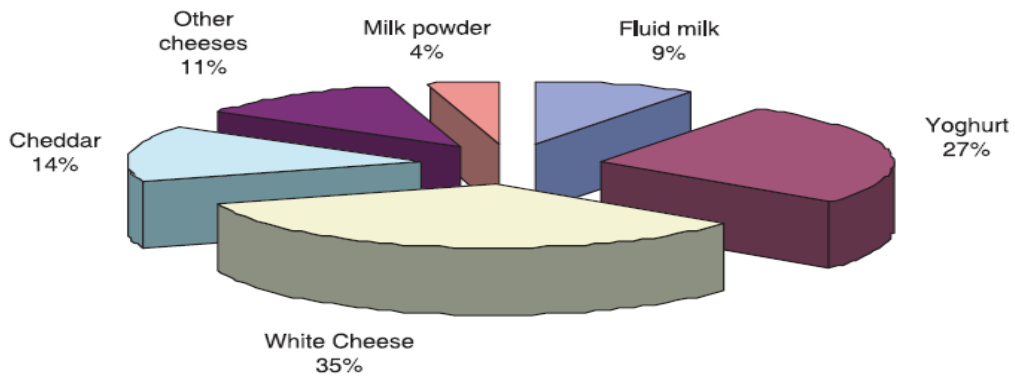


Figure 2.4 Raw milk utilizations according to dairy products (%).

2.2 DAIRY TECHNOLOGY

Popular milk products are cheese, yogurt, liquid milk, milk powder…etc. According to the final product types of milk production process differs. In this study we concerned about liquid milk and milk powder technology.

With the developing technology, new and different methods are used at the stage of milk production, heat treatment, storage and analysis. For the protection of nutrient composition of milk, the collected milk must be cooled as quickly as possible and kept cool during transportation, storage and use. If the cold chain is not provided, number of microorganisms could increase. Before transformation of milk into products different type procedures are used as a heat treatment (Rysstad and Kolstad, 2006).

2.2.1 Heat Treatment of Milk

Heat treatments such as pasteurization and sterilization are used to improve milk quality by destroying pathogenic and other organisms. In this heat treatment the aim is both to destroy pathogenic microorganisms which can be found in the milk and to protect nutritional value of milk

2.2.1.1 Effects of Heat Treatment

Physical and chemical changes occur when the heat treatment of milk. These changes differ according to the temperature and the time. A rapid temperature increase has an effect in altering the chemical composition of milk, such as vitamin destruction, protein denaturation and enzyme inactivation.

In dairy sector common procedures for the heat treatments are pasteurization and sterilization. The first one involves heating milk at 75˚C for 15 second and the second one involves heating at high temperatures (135˚C -150˚C).

Especially nutritional quality of proteins of milk after different heat treatments is important. According to the literature among the three different thermal processes applied to milk such as pasteurization, sterilization and domestic boiling only sterilized milk have an adverse effect on the growth of weanling rats comparison to raw milk (Efigenia, 1997).

2.2.1.2 Maillard Reaction and Furosine

Maillard reaction is one of the well-known modifications induced in food by heating. This reaction occurs even at very low temperature and in every system containing free amino groups and reducing sugars. It has also been identified in human blood and has also been related to the ageing of some tissues such as the retina of eyes (Resmini and Pellegrino, 1994).

For as long as food has been cooked, Maillard reaction which is related to aroma, taste and colour has played an important role in improving the appearance and taste of foods in particularly in traditional processes such as the roasting of coffee and cocoa beans, the baking of bread and cakes the toasting of cereals and the cooking of meat (Martins et al. 2001).

Hodge is put forward the first coherent scheme in 1953 but this reaction has been named by French chemist Louis Maillard (Hodge, 1953).

Maillard reaction is a complex chemical reaction between an amine group and a carbonhyl group and it can be decomposed into three stages (Figure 2.5).In the early stages of Maillard reaction a reducing sugar (lactose in milk and milk products) condenses with an amine base (lysine in milk and milk products) to form Amodri compounds.(mainly lactulosyllysine in milk and milk products) which are aminoketoses. After this stage Amodri products turn into advanced glycated end products (AGE) such as carboxymethyllysine. During the final stage of this reaction some advanced glycated end products can polymerise to form melanoidins, brown nitrogenous compounds which are easily monitored by colorimetry (Roux et al. 2009).

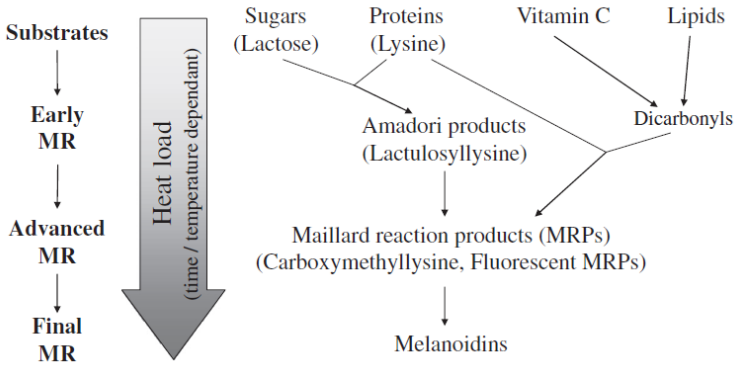


Figure 2.5 Simplified diagram of Maillard reaction.

After the Maillard reaction products are well-understood, beneficial or detrimental biological activities of these products are investigated. Especially relation between oral absorption of this products and accumulation in some tissues has been well studied.

Glycation is the Maillard reaction at 37C˚ in biological systems and it is revealed that some Maillard reaction products also called advanced glyction end products. When advenced glyction end products formed in vivo which accumulate in some tissues or are present in the systemic circulation, play a fundamental physiopathological role in cases of metabolic and aging disorders. For instance, the progression of cardiovascular diseases and non-insulin-dependent diabetes has been associated with the accumulation of Ne-carboxymethyl-lysine which is the first advanced glyction end products to be isolated and characterized from glycated proteins in vivo and is used as a marker of both dietary and in vivo Maillard reaction products (Knecht et al. 1991). Therefore these products suspected by the authors of containing reactive products and named glycotoxins (Ahmed et al. 2005).

The question is whether an elevated exposure to Maillard reaction products could contribute to the systemic level of advenced glyction end products and their deposition in tissues, and thus accelerate oxidative stress and chronic diseases. According to the literature a diet rich in Maillard reaction products, may accelerate oxidative stress, inflammation, and mechanisms related to glucose regulation especially for some vulnerable groups such as diabetics and renal failure patients. Few studies have evaluated the risk associated with the intake of dietary Maillard reaction products in

healthy subjects. The recent results of the International Cancer Alliance for Research and Education (ICARE) study in healthy adults confirmed the effective deleterious effects of a diet mainly composed of highly thermally processed foods (Tessier et al. 2012).

However, several studies suggest that some Maillard reaction products present in foods could have beneficial effects on health. For instance, the melanoidins are brown Maillard polymers which seem to have functional properties in food products (Rufian et al. 2007). They are also capable of inhibiting growth of a tumor cell line in culture (Marko et al. 2005). In addition, it was also found recently that a selection of foods rich in Maillard reaction products could inhibit the oxidation of low density lipoprotein in vitro (Dittrich et al. 2009).

In addition the potential health effects, Maillard reaction related to the aroma, taste and colour of food products. According to the dairy sector this relation is significant role for quality. Especially third stage is not preferred because of the brown colour of milk products Both to avoid potential health effects of maillard reaction and to keep from quality deficiencies of final products, monitoring the stage of this reaction is a considerable practice.

Melanoidins and brown nitrogenous compounds are used as an indicator of third stage and can be detected by colorimetry. These compounds can be formed by polimerisation of second stage products during the thermal treatment or storage Because of that monitoring the second stage products is necessary.

Second stage indicators are advanced glyction end products. Some of these products can be studied by using the FAST method which interests two fluorescence signals. The first one of the signals is fluorescence of trytophane which can be correlated to protein content and therefore to protein thermal denaturation. The second one of the signals is fluorescence of advenced Maillard reaction products. FAST index is given by the ratio of these two signals. Because of second one cannot be calibrated with any standard solution (Birlouez et al. 2001). Carboxymethyllysine which is an advenced glycation end product and which is a terminal product that accumulates during the reaction can also be used to follow the reaction via the fluorescence property (Ferrer et al. 2005).

Amodri compounds are the indicator of the first stage. Since the reducing sugar is lactose and amine base is lysine Lactulosyllysine is used as an amodri compounds to monitor first stage of Maillard reaction in the dairy industry. Lactulosyllysine converses into Furosin by acid hydrolysis and can be measured.

Furosine is a product produced by the Maillard reaction and a global indicator of first stage of this reaction. Furthermore it can be used as a marker of nutritional value of foods and can be used in monitoring the heat treatment of milk products. According to the literature Furosine content increased with increasing temperature from 100C˚ to 140C˚ (Sun et al., 2009). Literature also shows that the furosine content in high pasteurized milk was 7 times higher than that in mildly pasteurized milk (Feinberg et al. 2006).

2.2.2 Liquid Milk Technology

The use of high quality raw materials are required to obtain high-quality final products. So process of liquid milk technology starts with the quality assessment of raw milk.

Initially composition of raw milk which is used to produce pasteurized milk should be natural form neither have any additives and contaminants such as antibiotics, pesticides, detergents, disinfectants and radioactive material nor defatted. According to the raw milk and heat treated liquid milk notification of Turkish Food Codex Regulation maximum limit of these contaminants are limited. Later sensory characteristics of raw milk must be perfect, namely color, odor, taste and appearance of the raw milk must be normal. Lastly bacteriological quality of raw milk should be high and acidity must be low. In this case, the number of bacteria, bacterial species, toxins, and especially heat-stable enterotoxin is taken into account. Already the total number of bacteria in raw milk is limited by the Turkish Food Codex and the maximum limit is one hundred thousand bacteria per milliliter raw milk.

Raw milk which is used to produce sterilized milk should have all the features of raw milk which is used to produce pasteurized milk. In addition these features the stability of milk proteins must be high. Since, in the face of the high-temperature of sterilization technology, protection of the stability of the milk is of great importance.

The factors affecting the stability of the milk collected in two groups, including physico-chemical and bacteriological factors

Physico-chemical factors: After a certain level, the temperature norms of the sterilization process leads to deterioration of many of balance. Most important ones of these deteriorations originate from minerals and some of the proteins, especially from the lactalbumin. If there is any mineral salt in milk, protective caseinate will be separated and lactalbumin will be collapsed. So faulty milk which contains milk salts or milk serum proteins especially lactalbumin such as colostrums and mastitis cannot be used in sterilized milk production (Raynal, 2007).

Bacteriological factors: Presence of the spores and heat-resistant microorganism causes serious problems during the sterilized milk production. Most troublesome sports are the species of Bacillus. B. stearothermophilus, B. subtilis, B. licheniformis, Bacillus cereus, B. circulans, B. coagulans sports can be found in sterilized milk. Since the presence of these microorganisms will cause major problems in the production of sterilized milk process, lack of these microorganisms in raw milk is required. To do these sanitary inspections which are started in the barn and continued in the factory should not be neglected (Sorhaug, 1997).

After the quality assessment, liquid milk process continues with pre processing of raw milk. Liquid milks are classified based on the fat content, aroma additives and the method by which the milk is obtained from milk powder. Although the first and the second are used in Turkey, the third one is not used.

According to the Turkish Food Codex Regulation “Raw milk and heat treated liquid milk notification” liquid milk are called whole milk (3.5% fat), fatty milk (3%fat), semi-skimmed milk (1.5%fat), skimmed milk (0.15% fat) based on the amount of milk fat. In the UK they are marketed and labeled as whole milk (4%fat), semi-skimmed milk (1.7%fat), skimmed-milk (between 0.1-0.3 %fat) and in the USA they are marketed as whole milk (3.25%fat), reduced-fat milk (2%fat), low fat milk (1%fat), and fat free milk (0% fat).

Flavored milks which are classified based on aroma additives are called according to the aroma additives such as banana milk, strawberry milk and chocolate milk.

Liquid milks which are classified based on the method by which the milk is obtained from milk powder are called, reconstituted milk, recombine milk, toned milk, double toned and filled milk. Reconstituted milk is obtained by the dissolution of milk powder in water. Recombine milk is obtained by mixing skim milk powder, water and milk fat. Toned milk is obtained by mixing whole milk, water and milk powder. Double toned milk is obtained by mixing skim milk powder with whole milk and it must have at least 2% milk fat and 10% milk-solids-not-fat. Filled milk is obtained by mixing skim milk powder, water and hydrogenated vegetable oil (Demirci and Şimşek, 1997).

2.2.2.1 Pasteurized Milk

Pasteurization is a heat treatment aimed at reducing the number of any pathogenic micro-organisms in milk and liquid milk products, if present, to a level at which they donot constitute a significant health hazard. Pasteurization conditions are designed to effectively destroy the organisms *Mycobacterium tuberculosis* and *Coxiella burnetii*.

According to the Turkish Food Codex pasteurized milk is a milk that is obtained without damaging the natural characteristics of the raw milk by applying pasteurization process to destroy vegetative forms of pathogenic microorganisms completely with a large portion of other micro-organisms and is cooled at a temperature not exceeding 6ºC.

According to this codex and validations carried out on whole milk, the minimum pasteurization conditions are those having bactericidal effects equivalent to heating every particle of the milk to 72 ºC for 15 seconds or 63 ºC for 30 minutes. Although the first condition is common to use in continuous flow pasteurization processes, the second condition is common to use in batch pasteurization.

According to the International Food Standards (Codex Alimentarius) Pasteurization process has performance criteria and is designed to achieve at least a 5 log reduction of C.*burnettii* which is the most heat-resistant non-sporulating pathogen likely to be present in whole milk (4% milk fat). There is a logarithmic relation between time and temperature to destroy microorganisms. Similar conditions can be obtained by joining the line connecting these points on a log time versus temperature graph. During the verification of pasteurization process, products should show a negative alkaline

phosphatase reaction instantly after the heat treatment as decided by an acceptable method.

According to the Turkish Codex, products should show a negative alkaline phosphatase reaction and a positive peroxsidase reaction. If they show a negative alkaline phosphatase reaction it means there is a fault about process condition or a contamination of raw milk. Unless the products show positive peroxsidase reaction they should labeled as “pasteurized at high temperature”.

There are two type pasteurization methods. The first one which is used as a batch pasteurization method is low temperature and long time (LTHT). This operation runs in the tanks covered with a jacket. Heating or cooling is providing by circulating steam or hot water through the jacket. The conditions are usually 63 ºC for 30 minutes.

The second one which is used as a continuous pasteurization process is high temperature and low time (HTST). In this method, heat exchangers, operating according to the continuous flow system, are used. The heat exchangers may consist of plates or tubules. The conditions are usually 72 ºC for 15 minutes.

Pasteurized milk is filled tightly into the packing material which does not affect the composition or pasteurization of milk and affected by the milk. The material may be a glass bottle, plastic bag, plastic bottle or plastic coated paper cartons. Pasteurized milk can be stored at 5-7 ºC for about 5 days in unopened packaging.

2.2.2.2 Sterilized Milk

According to the Turkish Food Codex definition; sterilization is a long term heat treatment at high temperature which destroys all microorganisms and spores that can cause deterioration under normal storage conditions in order to obtain a commercially sterile product that can be stored at room temperature. It is applied at appropriate time and temperature combination such as at least 115 ºC for 13 minutes or at 121 °C for 3 minutes.

The methods that are used for sterilization in the long life liquid milk processes divided two groups. The first one is Classic Sterilization and the other is Ultra High Temperature sterilization.

2.2.2.2.1 Classic Sterilization

The conventional technique of milk sterilization is done by heating between at 110-120 °C for 20-40 minutes. Sterilization of milk at more than 100 °C needs pressure more than atmospheric pressure. Changes in the chemical composition of the milk during sterilization due to the application of high temperature occur. Depending on these chemical events the sensory qualities and nutritional value of the milk are imperfect (Baysal, 2004).

Quite a lot of vitamin and mineral loss is occurs in classic sterilization. B1 (thiamine), B6, B9 (folic acid) and vitamin B12. Vitamin loss is by 10% ratio and the lysine loss is by 6-10% ratio. A proportion of the amount of soluble calcium can reach up to 50% is decrease.

2.2.2.2.2 UHT Sterilization

According to the Codex Alimentarius UHT (ultra-high temperature) treatment of milk and liquid milk products is the application of heat to a continuously flowing product using such high temperatures for such time that renders the product commercially sterile at the time of processing. When the UHT treatment is combined with aseptic packaging, it results in a commercially sterile product.

UHT liquid milk is a milk, filled under aseptic condition into the opak packing containers, obtained leading to minimum changes in the physical and sensory properties of raw milk when destruction of all spoilage organisms and their spores by UHT process.

UHT treatment is a continuous operation that can either be carried out by direct mixing of steam with the product to be sterilized, or by indirect heating by means of a heat exchanging surface, followed by further aseptic processing (eventual) and aseptic packaging/filling. Thus the UHT plants are constituted by heating equipment in conjunction with appropriate packaging equipment and, eventually, additional treatment equipment (e.g. homogenization).In-container sterilization may be a batch or continuous process.

UHT treatment is normally in the range of 135 to 150 °C in combination with appropriate holding times necessary to achieve commercial sterility. Problems of classic sterilization such as nutritional value and sensory, physical, chemical properties of milk are minimized by this method. In this method, mostly steam and hot water are used as a heating medium.UHT process is divided two groups based on the application. The first one is direct UHT application by which milk is sterilized with directly steam (via steam injection or infusion into the product). The second one is indirect UHT application by which milk is sterilized in heat exchanger (Varnam and Sutberland, 1994).

Thermal processes which necessary to obtain commercially sterile products are designed to result in the absence of viable micro-organisms and their spores capable of growing in the treated product when kept in a closed container at normal non-refrigerated conditions at which the food is likely to be held during manufacture, distribution and storage.

According to Codex Alimentarius process criteria for products at risk of contamination with *Clostridium botulinum* such as certain composite milk products (as identified as likely to occur by a hazard analysis), the minimum thermal process should be established in consultation with an official or officially recognized authority. Where the risk of contamination with *Clostridium botulinum* is lower, alternative thermal processes may be established by an official or officially recognized authority, provided that the end products are microbiologically shelf stable and verified.

According to Codex Alimentarius verification of UHT process the products subjected to commercial sterilization must be microbiologically stable at room temperature, either measured after storage until end of shelf life or incubated at 55 °C for 7 days (or at 30 °C for 15 days) in accordance with appropriate standards. Other methods could also be used to demonstrate that the appropriate heat treatment has been applied.

2.2.3 Milk Powder Technology

Drying is the first preserving method to protect foods from microorganisms and to make food durable. Known history of powdered milk begins in the middle Ages. Marco Polo in the 13th century reported that soldiers of Kublai Khan carried sun-dried milk on

their expeditions (Yule, 1903). The first usable commercial production process for dried milk was invented by the Russian chemist M. Dirchoff in 1832. T.S. Grimwade took a patent on a dried milk procedure in 1855, though a William Newton had patented a vacuum drying process as early as 1837.

According to the Turkish Food codex definition; milk powder is a solid product, that is obtained by removing the water directly from whole milk, fatty milk, semi-skimmed milk, skimmed milk, cream or mixture of this product, and whom moisture content is maximum 5% by weight of solid product.

2.2.3.1 Manufacture

During milk powder manufacture the water is removed by boiling the milk under reduced pressure at low temperature in a process known as evaporation. The resulting concentrated milk is then sprayed in a fine mist into hot air to remove further moisture and so give a powder. Approximately 13 kg of whole milk powder or 9 kg of skim milk powder can be made from 100 L of whole milk. The milk powder manufacturing process is shown in the following schematic and is described in detail below (Ralph, 1998, Westergaard, 2010).

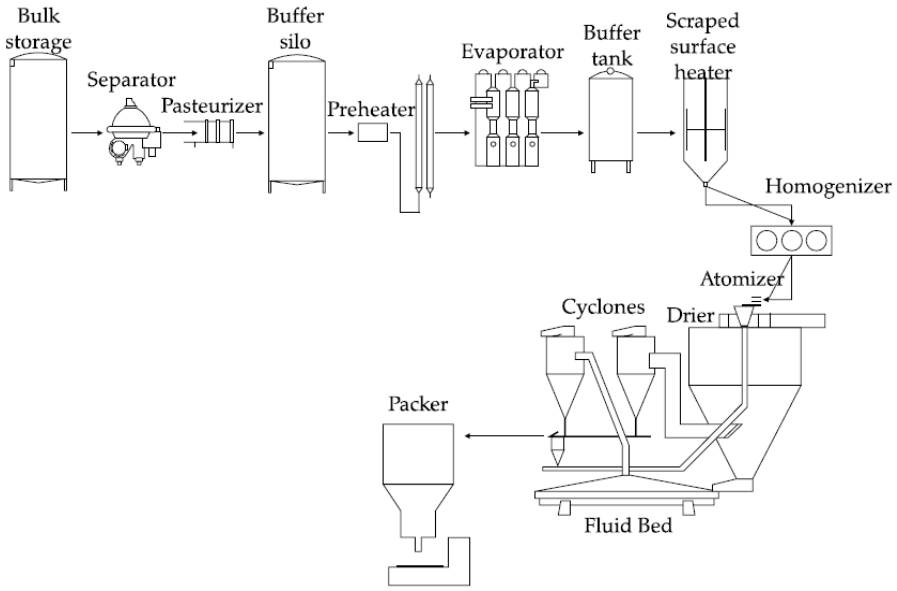


Figure 2.6 Milk powder manufacturing process.

Milk powder production process starts with taking the raw milk that received at the dairy factory and before this process, raw milk is separated from visible impurities and microorganisms by clarification, filtration and bactofugation.

First step of this process is pasteurizing and separating it into skim milk and cream using a centrifugal cream separator. If whole milk powder is to be manufactured, a portion of the cream is added back to the skim milk to produce milk with a standardized fat content (typically 26- 30% fat in the powder). Surplus cream is used to make butter or anhydrous milk fat.

The next step in the process is "preheating" in which the standardized milk is heated to temperatures between 75 and 120°C and held for a specified time from a few seconds up to several minutes. Preheating causes a controlled denaturation of the whey proteins in the milk and it destroys bacteria, inactivates enzymes, generates natural antioxidants and imparts heat stability. Preheating may be either indirect (via heat exchangers), or direct (via steam injection or infusion into the product), or a mixture of the two. Indirect heaters generally use waste heat from other parts of the process as an energy saving measure.

Subsequent step of the process is “evaporation”. In the evaporator the preheated milk is concentrated around 9.0% total solids content for skim milk and 13% for whole milk, up to 45-52% total solids. This is achieved by boiling the milk under a vacuum at temperatures below 72°C in a falling film on the inside of vertical tubes, and removing the water as vapour. More than 85% of the water in the milk may be removed in the evaporator.

The last step of the milk powder production process is “drying”. Milk is commonly dried by application of roller drying or spray drying

Even though roller drying is an old method, nowadays it is used to produce fatty milk powder, especially in chocolate sector. Roller drying is based on drying concentrated milk on the surface of a hot (between 115-130°C) drum. As the water evaporates, a thin layer of dried milk (nearly 0.5mm) is formed on the drum that is continuously removed by a scraper. The product is later pulverized in a hammer mill.

During drying, the milk is in contact with the roller surface for up to 3 seconds, and temperature of the milk reaches almost the temperature of steam. Conditional on the contact with the hot drum surface irreversible changes in the milk occur. Caramelisation of lactose, Maillard's browning reactions, and denaturation of proteins are the most important reactions. Thus, roller dried powder has low solubility in water, decreased nutritional value, and is susceptible to oxidation reactions during storage.

Spray drying is now the predominant method of drying milk. It involves atomising the milk concentrate from the evaporator into fine droplets. This is done inside a large drying chamber in a flow of hot air (up to 200 °C) using either a spinning disk atomiser or a series of high pressure nozzles. The milk droplets are cooled by evaporation and they never reach the temperature of the air. The concentrate may be heated prior to atomisation. Much of the residue water is evaporated in the drying chamber, leaving a fine powder of around 6% moisture content with a mean particle size typically of < 0.1 mm diameter. Final or "secondary" drying takes place in a fluid bed, or in a series of such beds, in which hot air is blown through a layer of fluidised powder removing water to give product with a moisture content of 2-4%.

Milk powder can be packed into either plastic-lined multi-wall bags (25 kg) or bulk bins (600 kg). Since protection from moisture, oxygen, light and heat is needed in order to maintain their quality and shelf life, packaging should be chosen to provide a barrier to moisture, oxygen and light.

2.2.3.2 Usage

Aim of milk powder production;

Increase the durability of milk: However durability period of milk powders ranges from six months to one year with appropriate packaging and storage conditions, durability period of raw milk ranges two or three days on suitable conditions.

Protect the quality and yield of milk production during periods of plenty

Meet the adequate amounts of milk or milk powder needs of sectors that use milk as a raw material in every season.

Meet the need of sectors that cannot use liquid milk as milk such as chocolate sector.

2.2.3.3 Main Concerns of Turkish Media about Milk Powder

In recent years there are some debates concerning about health and economical setbacks as a result of the use of milk powder. Concerns of consumers about negative health effects such as melamine and their demand to know that if the dairy products are produced by raw milk or milk powder are reality. Millions of consumers sent petition to the Ministry of Food, Agriculture and Livestock in Turkey in 2010. In the petition, they expressed their demand about labeling of dairy products produced by raw milk or milk powder (Kanat, 2010). Another debate is related to import of milk powder. According to the Ahmet Aydın, melamine adulterant of milk powder damages kidney, cardiovascular and reproductive systems. Despite import of milk powder adulterated with melamine being banned, UHT milk, yogurts, ice creams and cheeses still includes them (Aydın, 2011). Ankara Stud Dairy Cattle Breeder Association President Cengizhan Yorulmaz stated that 57 thousand tons of milk powder under the name of the animal formula was being imported and used as raw milk in dairies. Since The Europan Union prohibited the milk powder after melamine problems in Chine, milk powder stocks of Europe are transferred to other countries such as Turkey (Pazarcı, 2010). Manavgat Milk Producers Association (MSUB) President Bilal AY claimed that milk powder, imported as the calf and dog food since illegal, is economically advantageous when it is used as raw milk. But it creates falling of raw milk prices and unfair competition and market distortions (Büyükkeskin, 2010). According to the Ali Ekber Yıldırım, animal number of Turkey decreased drastically after cows were let to cut because of falling prices of raw milk. This resulted in import of Livestock. Ministry of Food, Agriculture and Livestock is supporting milk powder production but milk powder producers are themselves using milk powder which is shocking (Yıldırım, 2011).

2.2.3.4 Detection Methods of Milk Powder

Adulteration of raw milk by adding reconstituted milk (milk powder with water) or the selling of reconstituted milk as the fresh product can be economically advantageous when either a overplus of milk powder consist or in countries where the importation of milk powder is supported. However adulteration could create unfair

competition and market distortions could be occur. In the meanwhile such practice are prohibited by legislation, the detection of milk powder becomes an analytical problem.

According to the literature detection methods have depended on changes in the molecular structure of milk constituted, resulted from heat treatments and changes resulted from drying of milk powder (Guan et al., 2005). One of these methods includes fluorimeter to obtain Fluorescence of advanced Maillard products and Soluble Tryptophan (FAST index). The others are as follows: the determination of their ultra violet and visible spectra (700 to 240 nm) (Madkour et al., 1989), the detection of hydroxymethylfurfural by spectrophotometer (Rehman et al., 2000), measurements of δD and ^{18}O stable isotope ratios by mass spectrometry (Lin et al., 2003).

After all Furosine, the most popular indicator of Maillard reaction, is used to detection of milk powder. Several analytical techniques are used to measure the Furosine level in dairy products such as gas–liquid chromatography (Büser and Erbersdobler, 1985), ionexchange chromatography, capillary electrophoresis (Tirelli and Pellegrino, 1995). Recently, the popular technique to detect Furosine is High performance liquid chromatography (HPLC) which is not time consuming and provides a good detection limit (Ferrer et al., 2000). For instance it has been used to detect the illegal addition of reconstituted whey and milk powder to fresh cheese (Resmini et al., 1993). Other instances, it has been used to characterize the authenticity of mozzarella cheese (Pellegrino et al., 1996), and the detection of furosine as an indicator of milk powder that presences in raw and in pasteurized milk (Resmini et al., 1992), Quantitative determination of furosine in cow's milk containing reconstituted skim milk (Ohta et al., 2002).

There are no literature on the quantitative detection of milk powder in dairy products. In our study we practiced to use ion-pair reversed phase HPLC for the quantitative determination of milk powder in UHT and pasteurized milk by using Furosin as an indicator.

2.2.4 Chromatography

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction (Ettre, 1991).

2.2.4.1 Classification of Chromatography

Different methods were attempted for classification of chromatography. As shown below classification fall into three categories based on the chromatographic methods (Ettre, 1993).

According to the shape of the chromatographic bed:

Column chromatography is a separation technique in which the stationary bed is within a tube and planner chromatography is a separation technique in which the stationary phase is present as or on a plane

According to the physical state of the mobile phase;

Gas Chromatography(GC), in which the mobile phase is a gas, is always carried out in a column and liquid chromatography (LC), in which the mobile phase is a liquid, can be carried out either in a column or on a plane. Supercritical-Fluid Chromatography (SFC) is a separation technique in which the mobile phase is a fluid above and relatively close to its critical temperature and pressure.

According to the mechanism of separation.

Adsorption Chromatography: Separation is based mainly on differences between the adsorption affinities of the sample components for the surface of an active solid.

Partition Chromatography: Separation is based mainly on differences between the solubilities of the sample components in the stationary phase (gas chromatography), or on differences between the solubilities of the components in the mobile and stationsuy phases (liquid chromatography).

Ion Exchange Chromatography: Separation is based mainly on differences in the ion exchange affinities of the sample components.

Exclusion Chromatography: Separation is based mainly on exclusion effects, such as differences in molecular size and/or shape or in charge.

Affinity Chromatography: This expression characterizes the particular variant of chromatography in which the unique biological specificity of the component and ligand interaction is utilized for the separation.

2.2.4.2 High Performance Liquid Chromatography

High-performance liquid chromatography (or High pressure liquid chromatography, HPLC), which is a specific form of column chromatography, used in biochemistry and analysis to separate, identify, and quantify the active compounds (Martin et al., 2005).

HPLC mainly make use of a column that holds packing material as a stationary phase, a pump to move the mobile phase(s) through the column, and a detector to show the retention times of the molecules. Retention time depends on the interactions between the stationary phases, the molecules being analyzed, and the solvent(s) used (Liu et al., 2006).

The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retained by specific chemical or physical interactions with the stationary phase. The amount of retention depends on the nature of the component and composition of both stationary and mobile phase. The time at which a specific component elutes (comes out of the end of the column) is called the retention time (Hearn, 1990).

Separation has been done to vary the mobile phase composition during the analysis; this is known as gradient elution (Abidi, 1991). The gradient separates the component mixtures as a function of the affinity of the component for the current mobile phase. The choice of solvents, additives and gradient depend on the nature of the stationary phase and the component.

Generally phase system used in the process determines the types of HPLC. Following types of HPLC generally used in analysis (Hearn, 1990 and Xiang et al. 2006).

Normal phase chromatography (Normal Phase HPLC): In this method components are separated based on polarity. NP-HPLC uses a polar stationary phase and a non-polar mobile phase. The polar component interacted with and is retained by the polar stationary phase. Adsorption strengths increase with increased component polarity, and the interaction between the polar component and the polar stationary phase increases the elution time.

Reversed phase chromatography (Reversed phase HPLC): (RP-HPLC or RPC): In this method there are a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC works on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar component, and the non-polar stationary phase. The binding of the component to the stationary phase is proportional to the contact surface area around the non-polar segment of the component molecule upon association with the ligand in the aqueous eluent.

Size exclusion chromatography (Size exclusion chromatography), In this method particles are mainly separated on the basis of size. It is useful for determining the tertiary structure and quaternary structure of proteins and amino acids. This technique is widely used for the molecular weight determination of polysaccharides.

Ion exchange chromatography: In this method retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. This form of chromatography is widely used in purifying water,

Bio-affinity chromatography: In this method separation based on specific reversible interaction of proteins with ligands. They are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands.

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Equipments

The list of the machine and equipments used in the study was as shown: HPLC Equipment (Shimadzu CTA-10AS), UV-Detector (Shimadzu SPD-M20A), C18 Column (ThermaScientific ODS Column 250-4,6-5), HPLC software (LC Solution), Microfilters (RC 0,45µ), Analytical Balance (Sartorius CP 323S), Oven , Sealing Vials (glass vials), Paper filters, Glass Syringe (Shimadsu), Kjeldahl Apparatus (Fisons and Buchi K-314), Vortex (IKA LABORTECHNICK), Water Purification System (Millipore Water Purification System), Pipettes (Nichipet EX), Tips, Refrigerator (Philips +4, -20).

3.1.2 Samples

During the research whole bovine milk and skim milk powder samples were obtained from a dairy factory. As soon as samples were gotten, they were directly transported in a heat resistant box to keep from damage or change to Molecular Biology and Genetic Research Laboratory of Fatih University at the Buyukcekmece Campus, Istanbul. Then they were used for making drinking milk samples in our laboratory

3.1.3 Chemicals

Hydrochloric Acid (%37), Glacial Acetic Acid, Potassium Chloride, Methanol were supplied from MERCK (Germany). Furosine was purchased from Poly Peptide Laboratories(France).

3.2 METHODS

3.2.1 Sample Preparing

Research was started preparing of Furosine standard solution. Final concentration of Furosine standard solution was 0,0003mg/ml.

Preparation:

Furosine-0: 10 mg of Furosine was dissolved in 16.666 ml of hydrochloride solution (3mol/l).

Furosine-1: 5 ml of Furosine-0 was diluted in 100 ml of hydrochloride solution (3mol/l).

Furosine-2: 1 ml of Furosine-1 was diluted in 100 ml of hydrochloride solution (3mol/l).

For the standard curve Furosine 2 was diluted in 10ml and 5ml. Finally we had standard solutions as below: 0,00003mg/ml, 0,00015mg/ml and 0,0003mg/ml.

During the research reconstituted milk and raw milk were used to prepare desired sample groups. Reconstituted skim milk samples were prepared by dissolving skim milk powder in distilled water (weight/volume percent 10). Volume percent were used when the eight sample groups were preparing by mixing homogenized whole bovine milk and reconstituted milk as shown Table 3.1.

Table 3.1 Samples.

Name	Concentration
Group-0	100% Homogenized Whole Bovine Milk
Group-1	10/90 Reconstituted milk / Homogenized Whole Bovine Milk
Group-2:	20/80 Reconstituted milk / Homogenized Whole Bovine Milk
Group-3:	30/70 Reconstituted milk / Homogenized Whole Bovine Milk
Group-4	40/60 Reconstituted milk / Homogenized Whole Bovine Milk
Group-5	50/50 Reconstituted milk / Homogenized Whole Bovine Milk
Group-6	60/40 Reconstituted milk / Homogenized Whole Bovine Milk
Group-10	100% Reconstituted milk

3.2.2 Thermal Treatment

Digital Block Heater equipped with exchangeable holders was used as a heater. Sample groups were thermally treated as described in Table 3.2.

Table 3.2 Thermal treatments.

	No Heat Treatment	**63 ºC for 30 minutes (LTHT)**	**72 ºC for 15 seconds (HTST)**	**135 ºC for 2 seconds (UHT)**
Group-0	Sample A0	Sample B0	Sample C0	Sample D0
Group-1	Sample A1	Sample B1	Sample C1	Sample D1
Group-2	Sample A2	Sample B2	Sample C2	Sample D2
Group-3	Sample A3	Sample B3	Sample C3	Sample D3
Group-4	Sample A4	Sample B4	Sample C4	Sample D4
Group-5	Sample A5	Sample B5	Sample C5	Sample D5
Group-6	Sample A6	Sample B6	Sample C6	Sample D6
Group-10	Sample A10	Sample B10	Sample C10	Sample D10

3.2.3 Determination of Protein Content

Nitrogen contents of 2ml of samples were determined by Kjeldahl Method and protein contents of them calculated by multiplying the nitrogen content by 6.38.

3.2.4 Hydrolysis

2ml of test samples were pipetted into a screw-cap Pyrex-vial and added 9ml of hydrochloric acid solution (37%). The vial mixed and closed tightly. Then their contents were heated for 12 hour in the oven set at 110˚C. At the end of the time the hydrolysate were cooled and filtered with 1 ml water through a paper filter. Filtered hydrolysates were stored under -20˚C until the Solid Phase Extraction.

3.2.5 Microfiltration of Filtered Hydrolysate

0,45µ syringe filter was mounted onto the glass syringe and 1ml of filtered hydrolysate was taken up into the syringe. It was injected into the cartridge discarding the displaced liquid. Introducing air was avoided.

3 ml of Hydrochloride solution (3mol/l) was pipetted into the syringe. System was slowly eluted until complete drying of the syringe occurs, and then elute was collected.

3.2.6 HPLC Determination

3.2.6.1 Elution Solvents

Dilute acetic acid was used as elution solvent A. To prepare solvent A 4ml of glacial acetic acid was diluted with water to 1000ml.

Potassium chloride solution was used as elution solvent B. To prepare solvent B 3gr of potassium chloride was dissolved in 1000ml of solvent A (dilute acetic acid).

3.2.6.2 Elution Gradients

The elution gradient of mobile phases that used in this research was shown in Table 3.3.

Table 3.3 Elution gradient.

Time (min)	Solvent A %	Solvent B %
0	100	0
2.5	100	0
10.0	50	50
22.0	50	50
24.0	100	0
30.0	100	0

3.2.6.3 Column Temperature and Flow Rate

During the research column temperature kept at constant value. The temperature of column was 30˚C.

Best performance of the research was kept by the 1,2 ml/min. flow rate of the elution solvents.

3.2.6.4 Determination

At the beginning chromatographic system was equilibrated by flushing the column for 30 minutes with a mixture of solvent A and solvent B in the ratio 50/50 and flow rate 1.2ml/min. Then initial condition was set until a stable base line observed

A blank run was obtained by injecting 20 µl of hydrochloric acid solution (3mol/l) in order to check the purity of the solvents. After the final equilibration step, the detector response was stabilized to 280 nm.

20 µl of Furosine standard solution was injected to the chromatographic separation. Then 0,1X, 0,5X, and X of Furosine standard solution was injected and standard curve has obtained.

20 µl of each sample was injected and Furosine concentration was calculated. According to this concentration; milk powder content curve was obtained.

Finally commercial UHT and pasteurized milk samples were injected and milk powder contents were calculated.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Protein Content of Samples

Proportion of protein in samples that calculated from nitrogen content from Kjeldahl method are shown below in Table 4.1.

Table 4.1 Protein contents of samples (mg/100ml milk).

Sample	**Protein(mg/100ml milk)**
Group-0	3.23
Group-1	3.52
Group-2	3.19
Group-3	3.18
Group-4	3.45
Group-5	3.61
Group-6	3.71
Group-10	3.30

4.1.2 Results of Standard Solutions

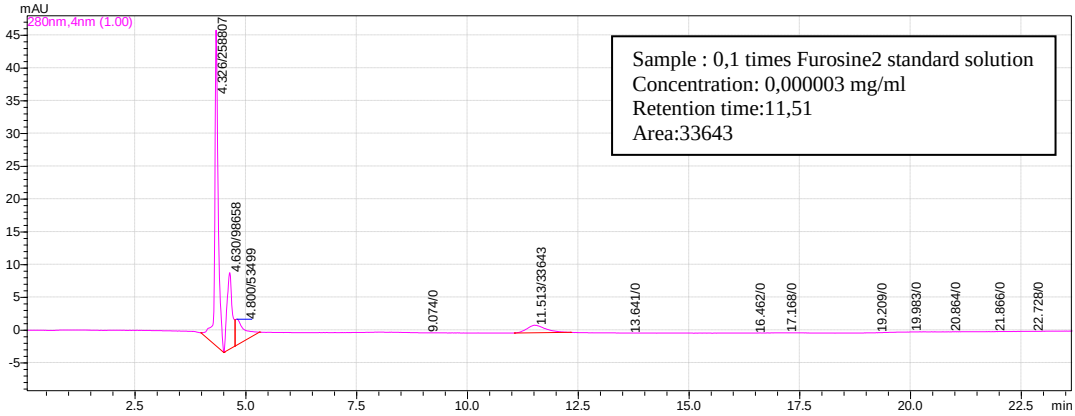
Retention times and the numerical values of the peak area of Furosine obtained for the Furosine standard solutions are shown below in Table 4.2.

Table 4.2 The peak area and the retention times of Furosine in standard solutions.

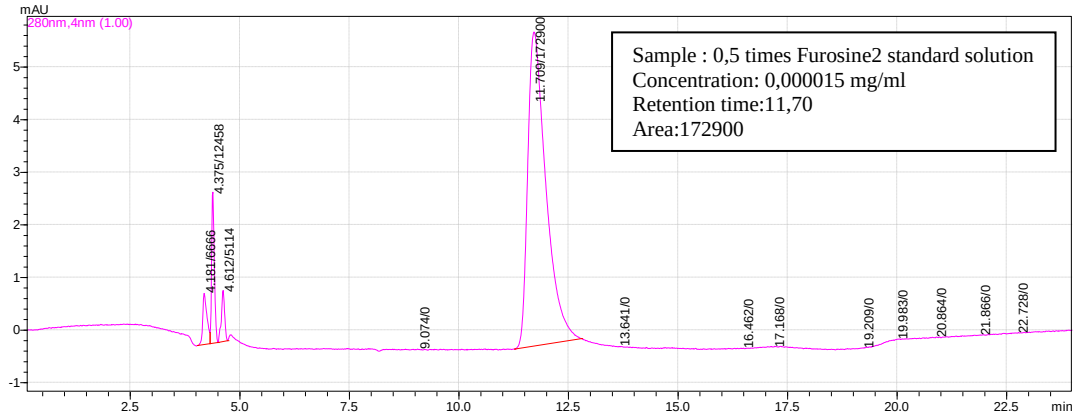
	Concentration mg/ml	**Area**	**Retention Time (min)**
2,5* Furosine2	0,000075	750839	11,557
0,5* Furosine2	0,000015	172900	11,513
0,1* Furosine2	0,000003	33643	11,709

The HPLC chromatogram of standard solutions that we used to prepare calibration curve are shown in Figure 4.1.

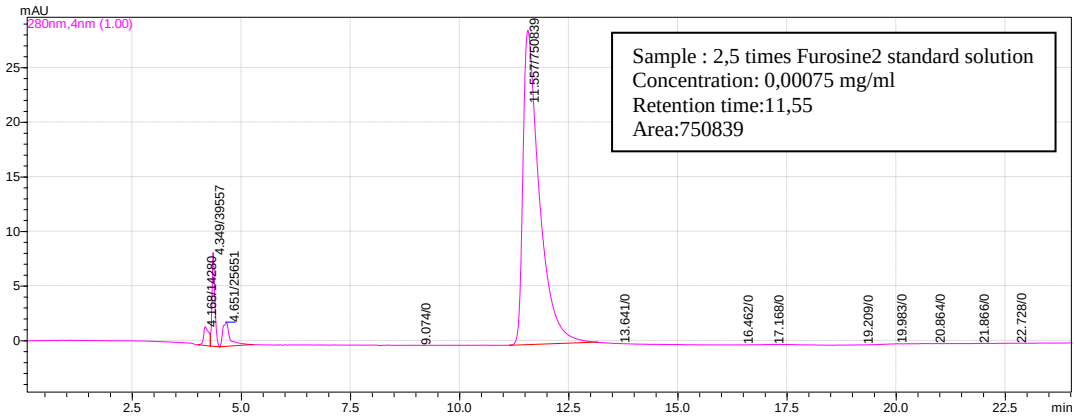
The calibration curve that we prepared according to the standard solutions results is given below in Figure 4.2. The x-axis on the graph is concentration and the y-axis is pick area.



(a)



(b)



(c)

Figure 4.1 The HPLC Chromatograms of standard solutions: (a) 0,1 times Furosine2 standard solution, (b) 0,5 times Furosine2 standard solution, (c) 2,5 times Furosine2.

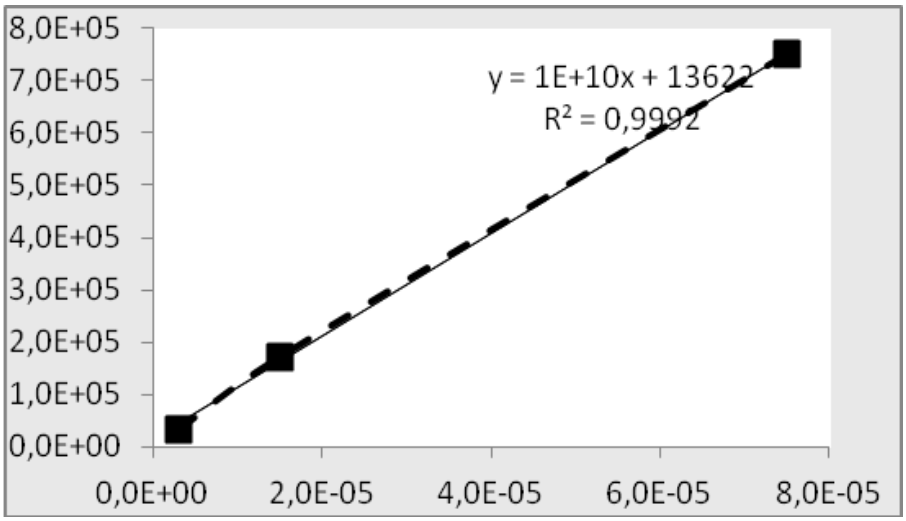


Figure 4.2 The calibration curve that prepared according to the standard solution results.

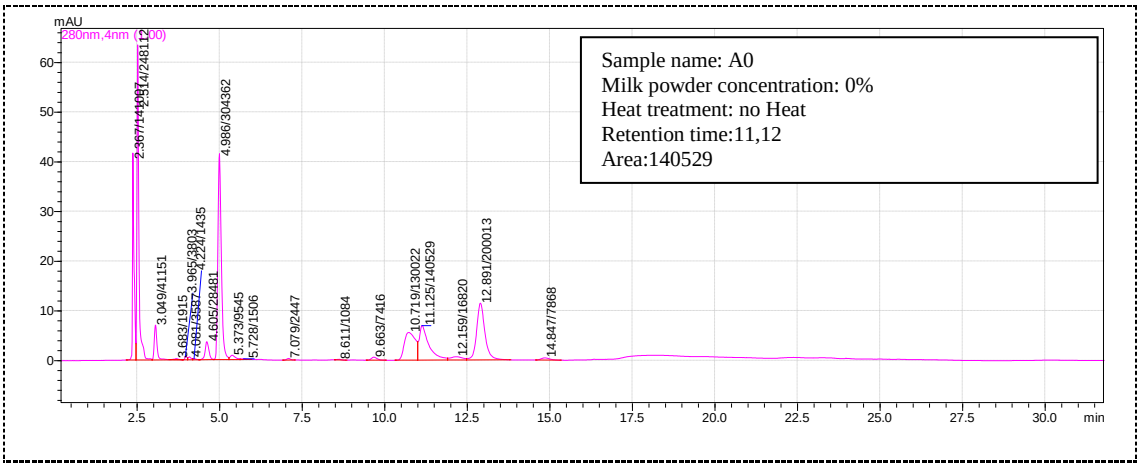
4.1.3 Furosine Content of Samples

The numerical values of the peak area of Furosine obtained for the positive control samples are shown below in Table 4.3. Retention times of these samples were between minute 10,998 and minute 11,316.

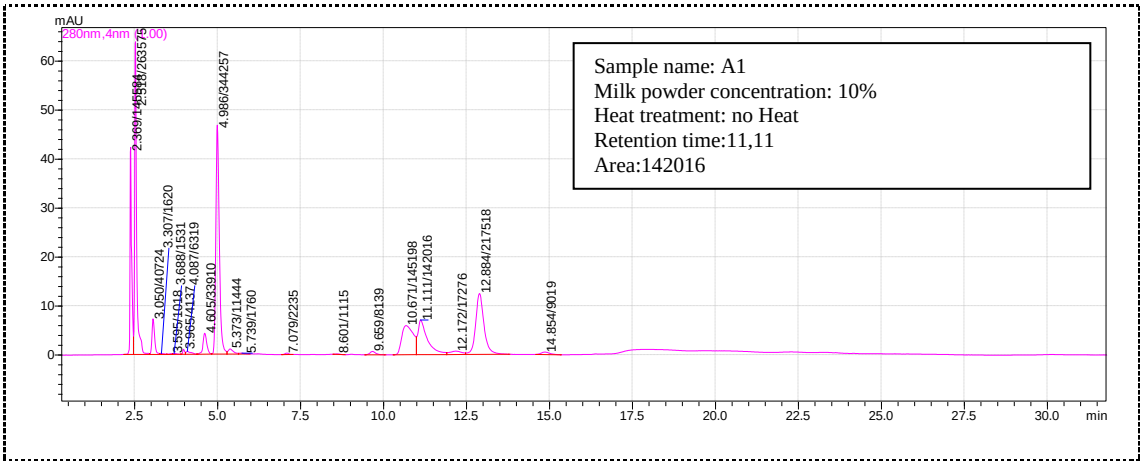
Table 4.3 The peak area for Furosine in 20 µl positive control samples.

	No Heat Treatment **Peak Area(unit)**		**63 ºC for 30 minutes (LTHT)** **Peak Area(unit)**		**72 ºC for 15 seconds (HTST)** **Peak Area(unit)**		**135 ºC for 2 seconds (UHT)** **Peak Area(unit)**	
Group-0	A0	**140529**	B0	**158541**	C0	**174624**	D0	**221728**
Group-1	A1	**142016**	B1	**158667**	C1	**179454**	D1	**232557**
Group-2	A2	**145627**	B2	**162431**	C2	**180229**	D2	**241032**
Group-3	A3	**146320**	B3	**164490**	C3	**182925**	D3	**258168**
Group-4	A4	**146871**	B4	**165335**	C4	**183336**	D4	**267951**
Group-5	A5	**148284**	B5	**166549**	C5	**185935**	D5	**281780**
Group-6	A6	**150389**	B6	**171576**	C6	**188336**	D6	**313907**
Group-10	A10	**151542**	B10	**172795**	C10	**203737**	D10	**397535**

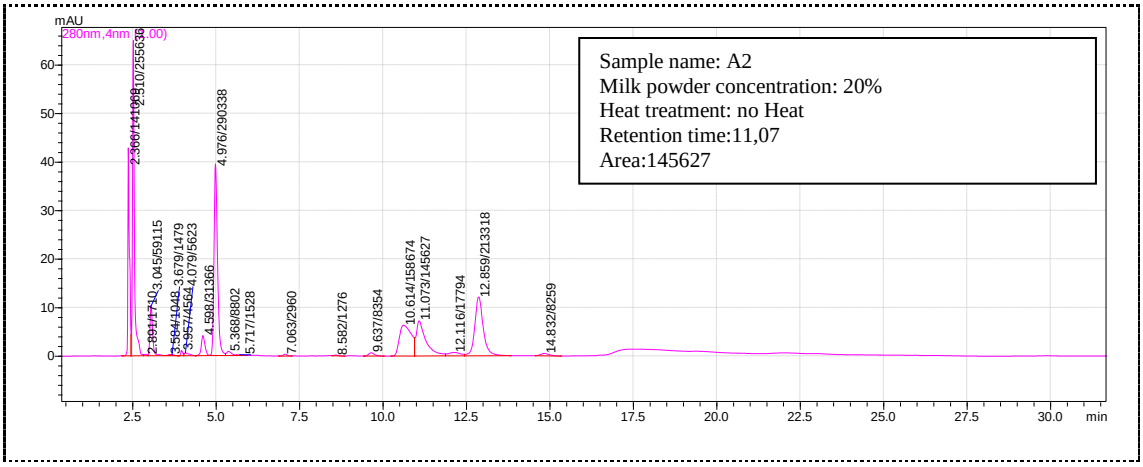
The HPLC chromatogram of positive control samples that we used to prepare milk powder detection curve are shown in Figure 4.3.



A0

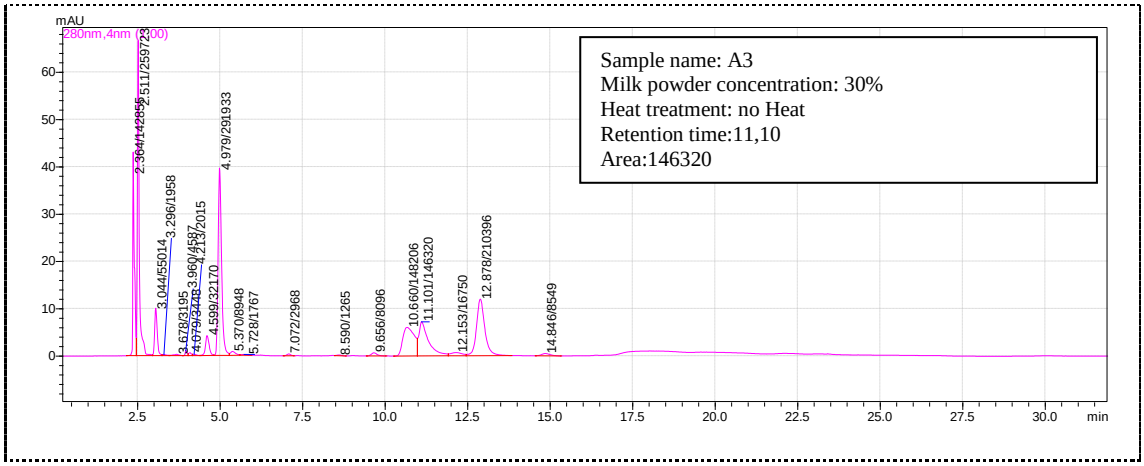


A1

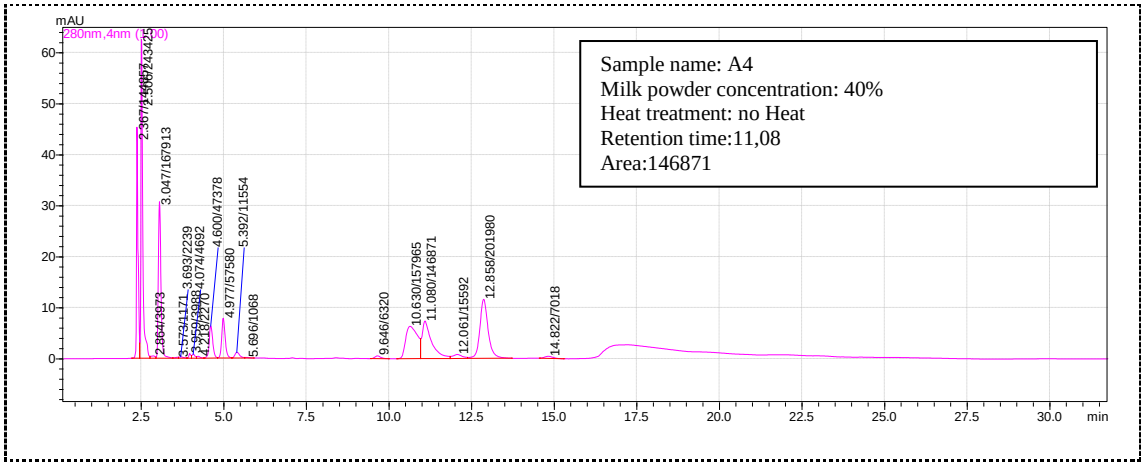


A2

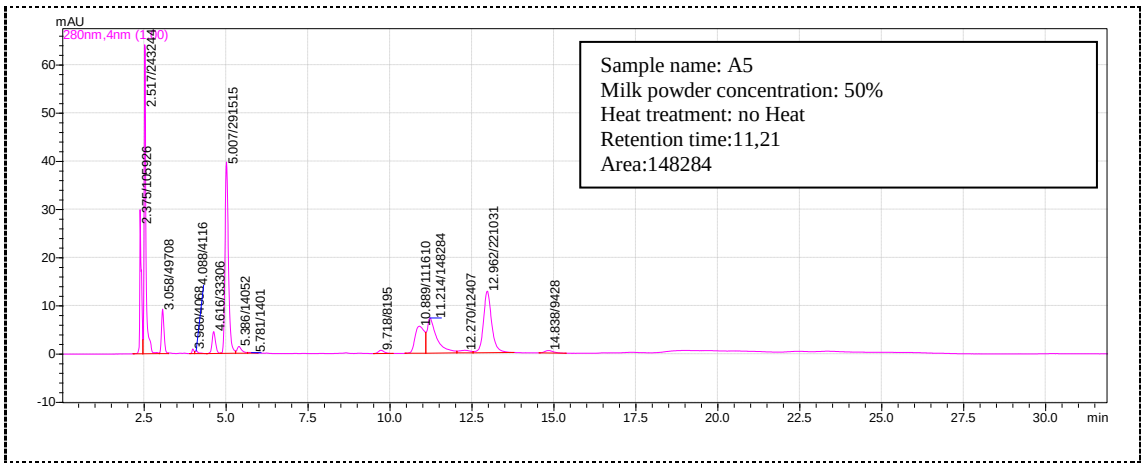
Figure 4.3 The HPLC chromatogram of positive control samples.



A3

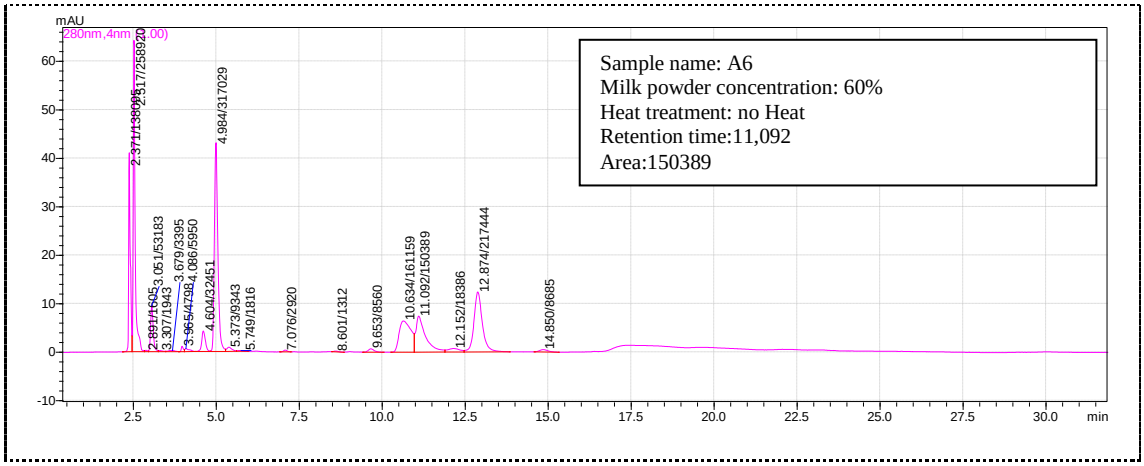


A4

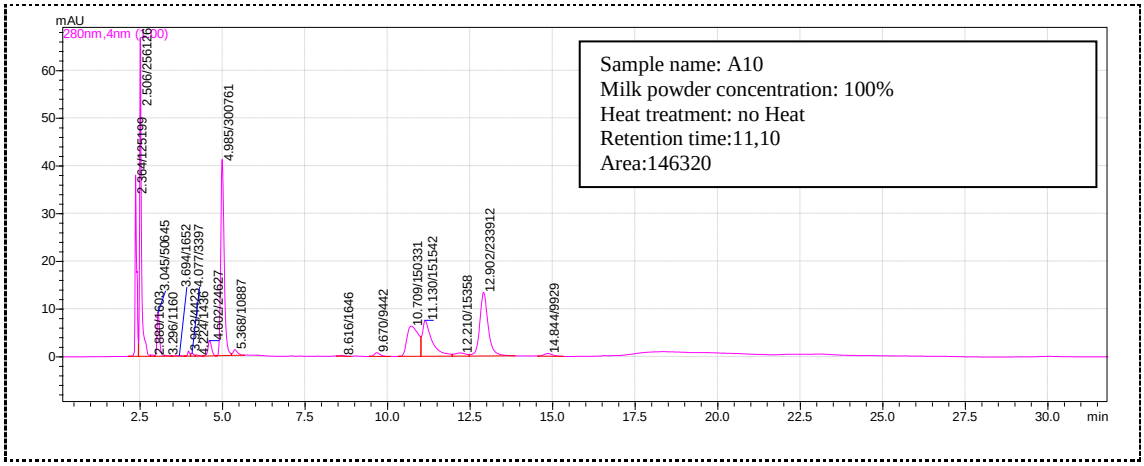


A5

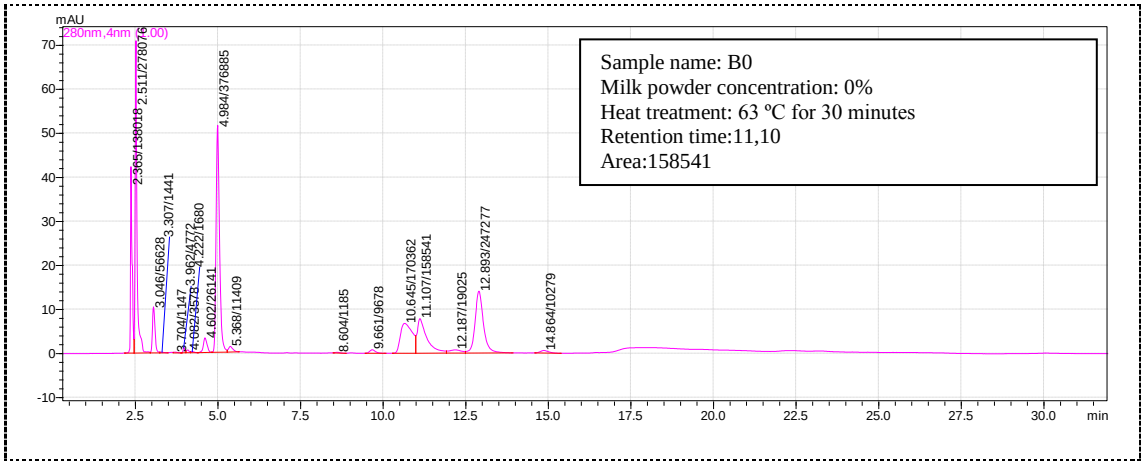
Figure 4.3 (cont.)



A6

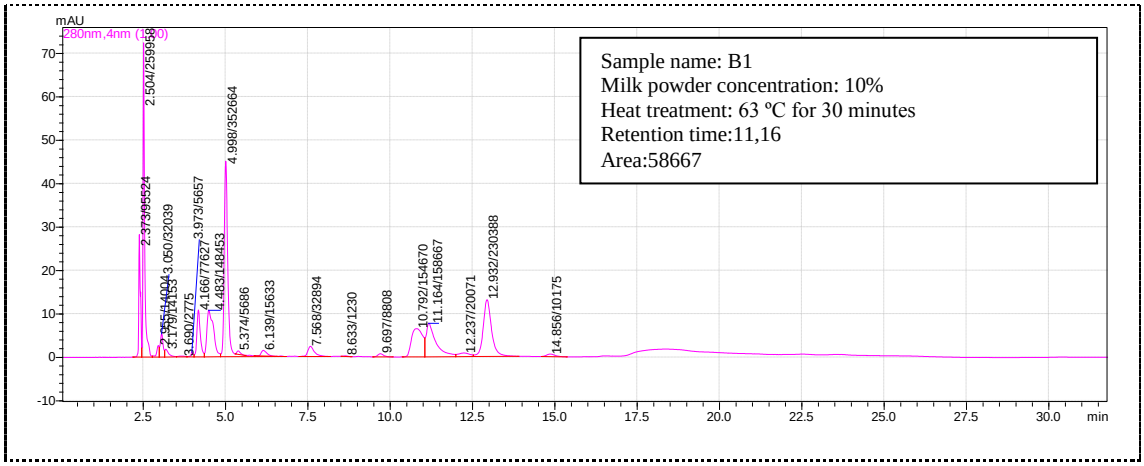


A10

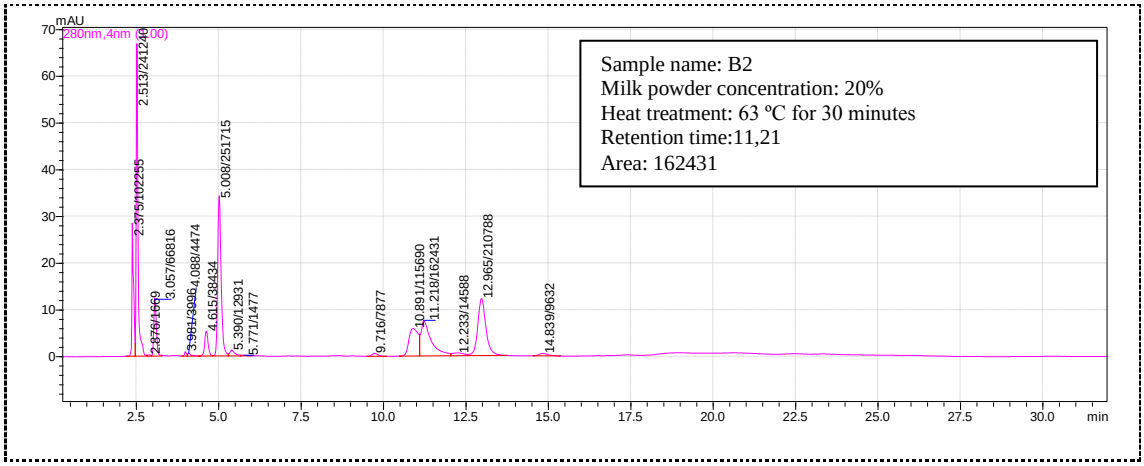


B0

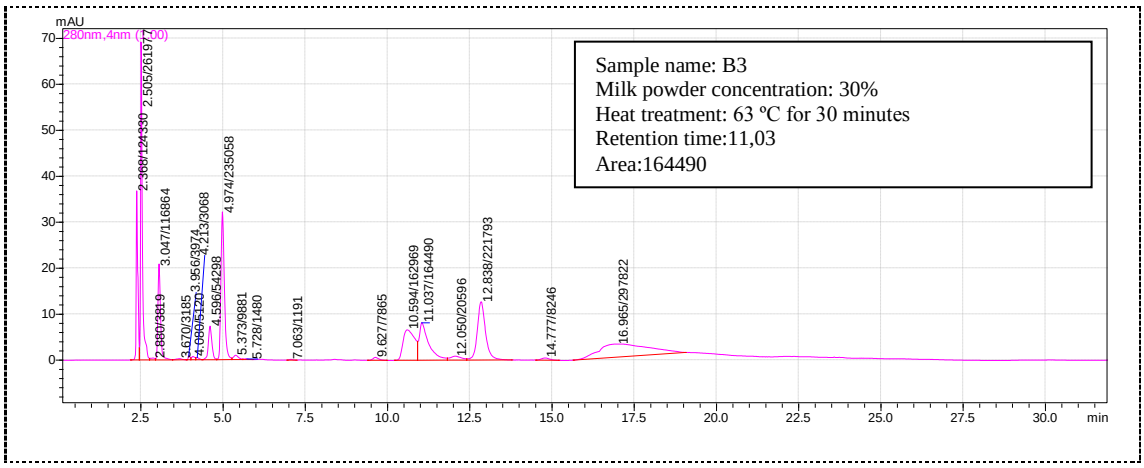
Figure 4.3 (cont.)



B1



B2



B3

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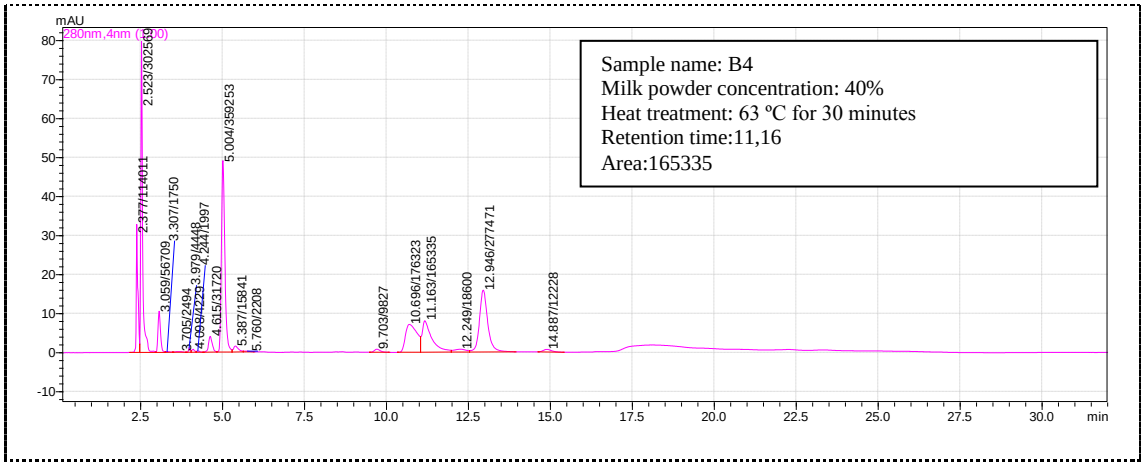
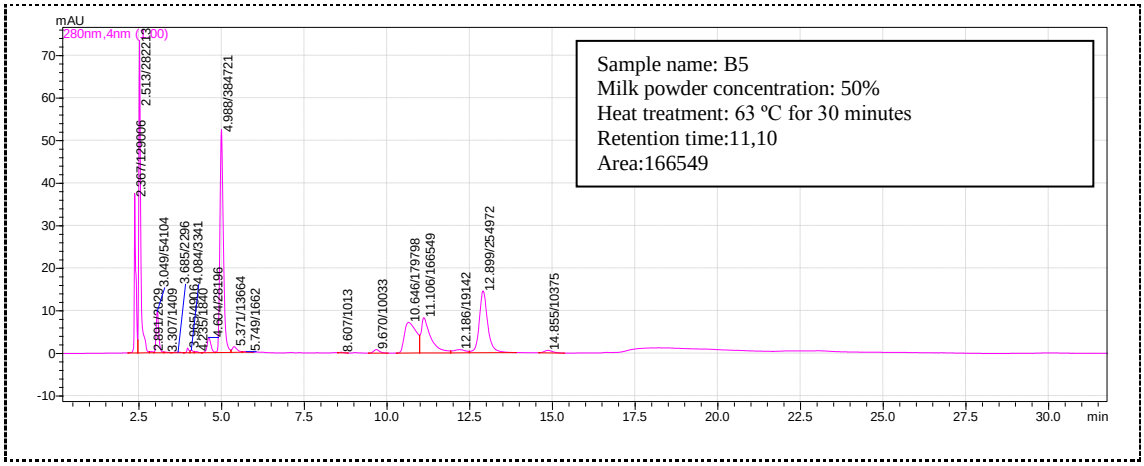
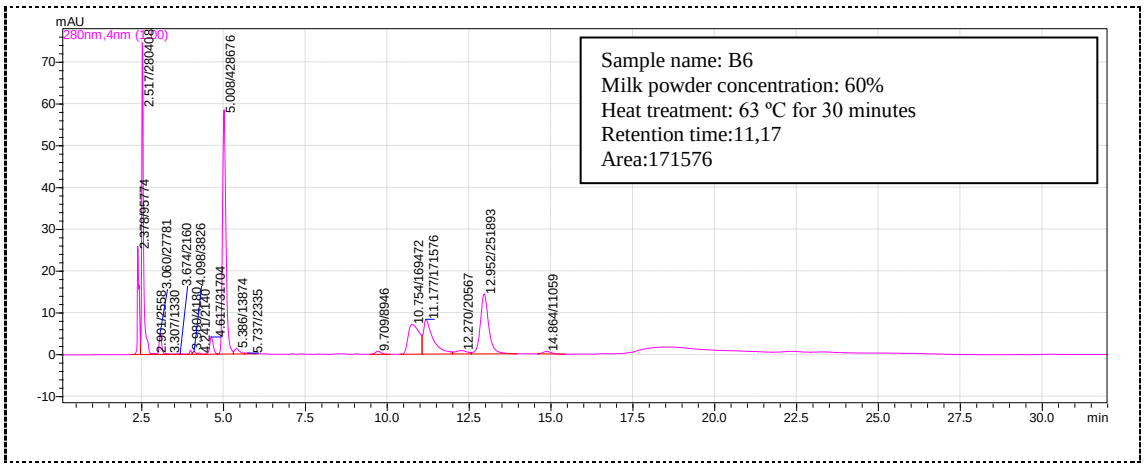


B4



B5



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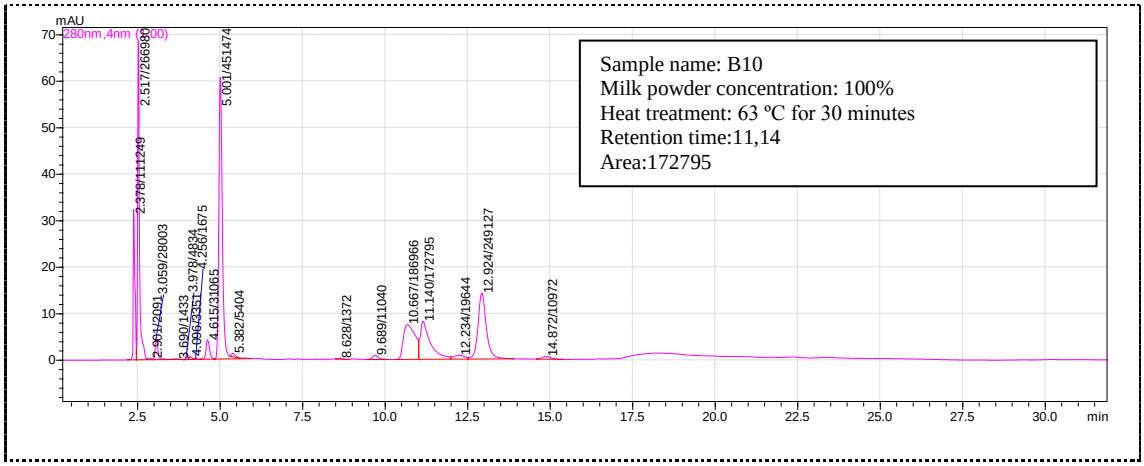

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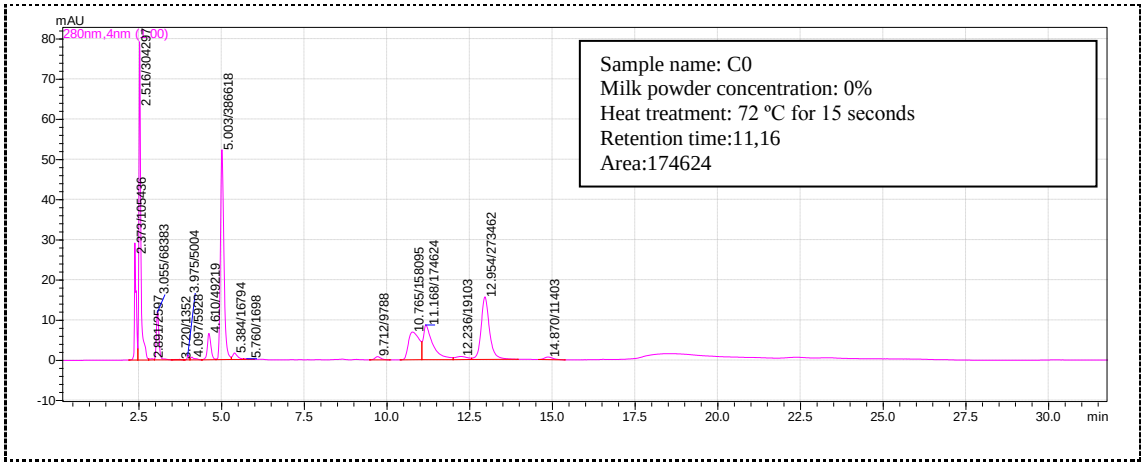
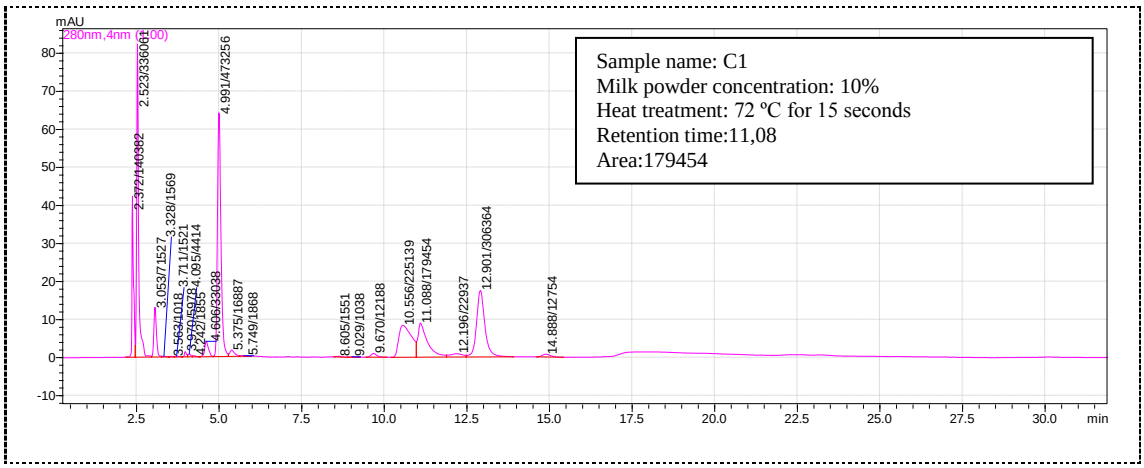


C0



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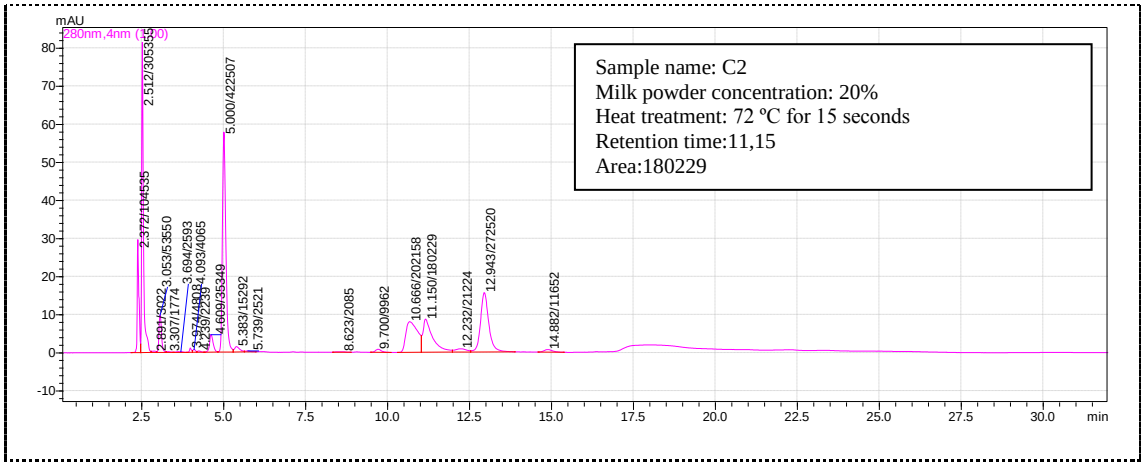

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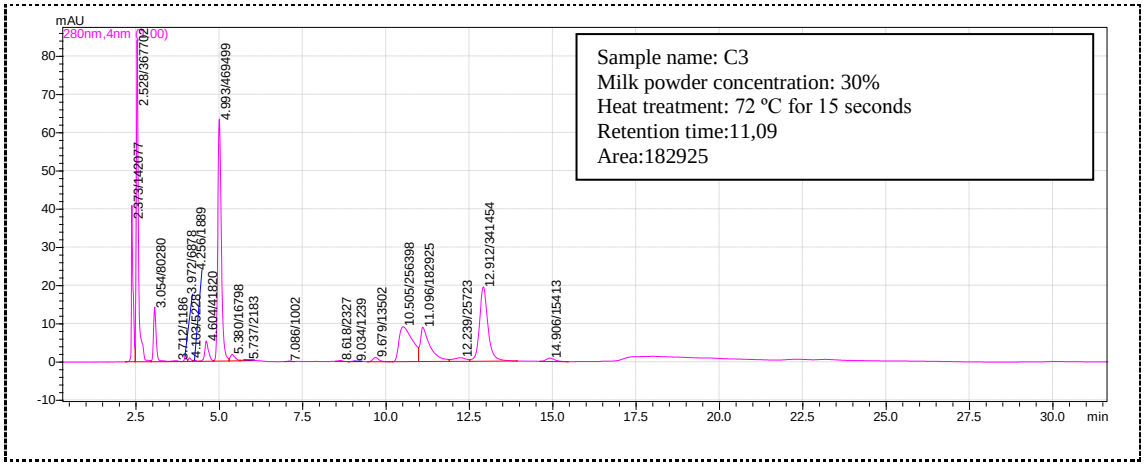

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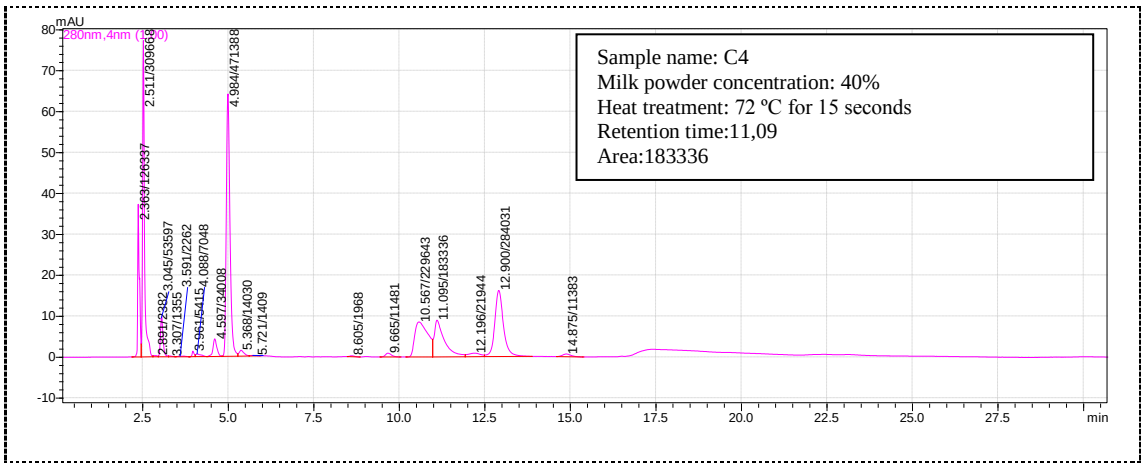

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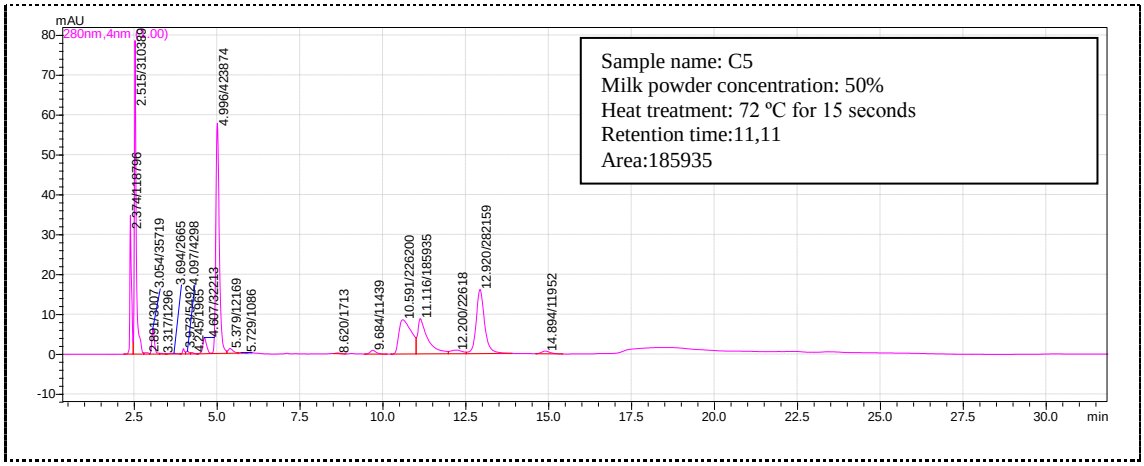

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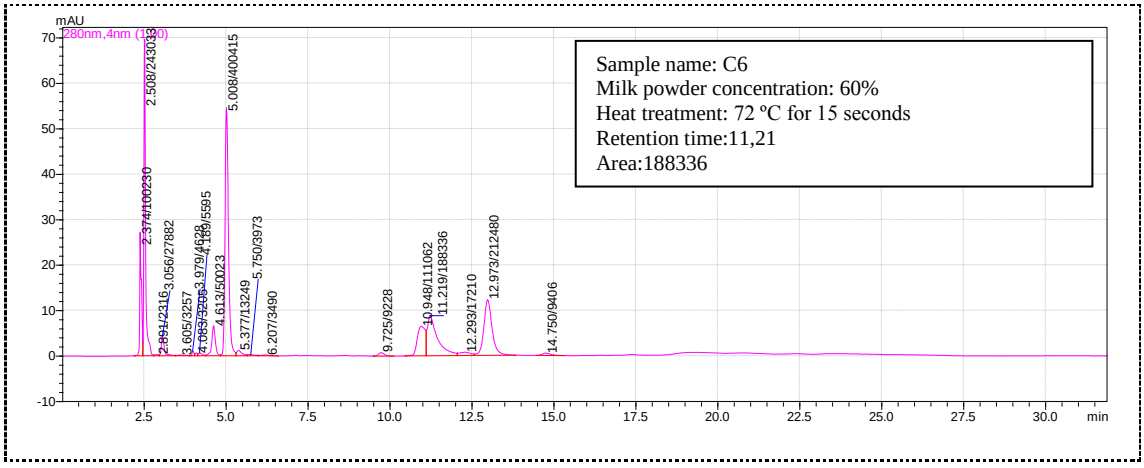
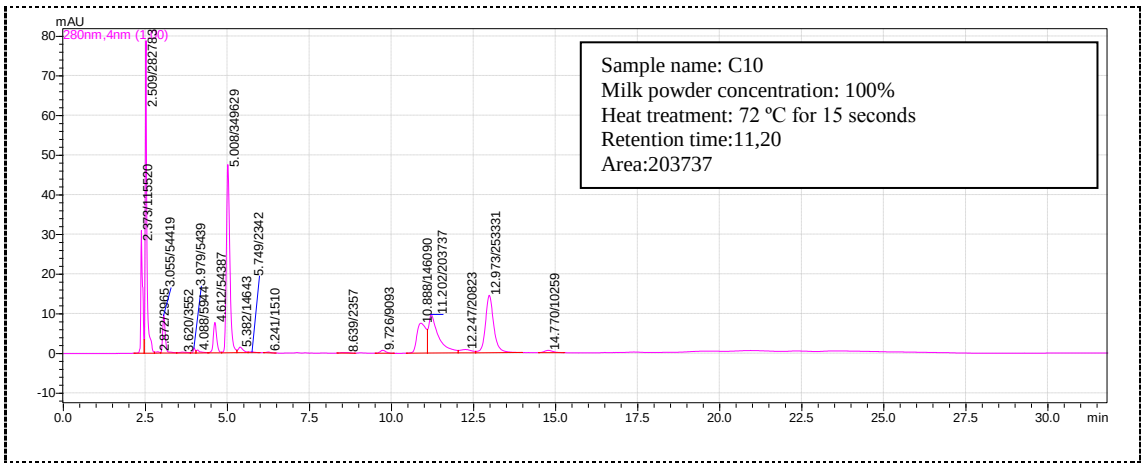
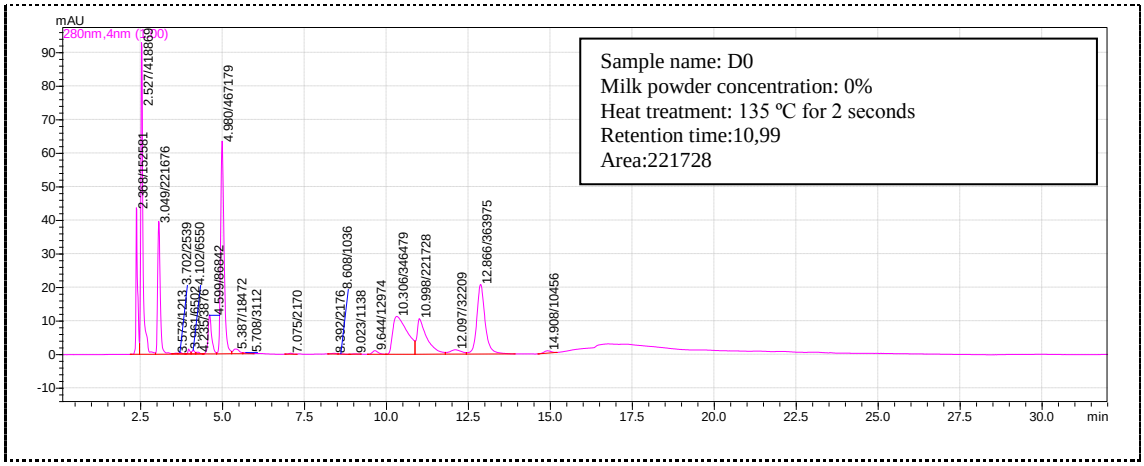


C6

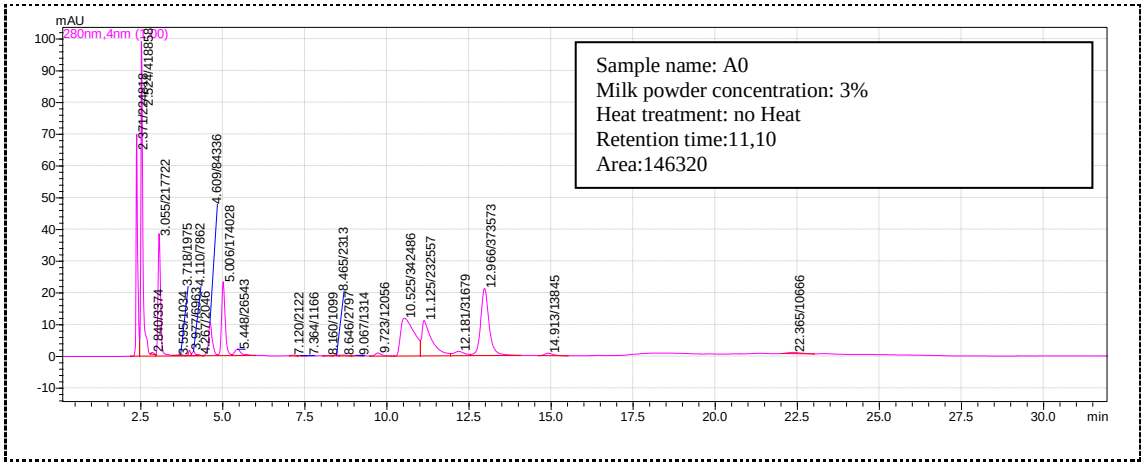


C10

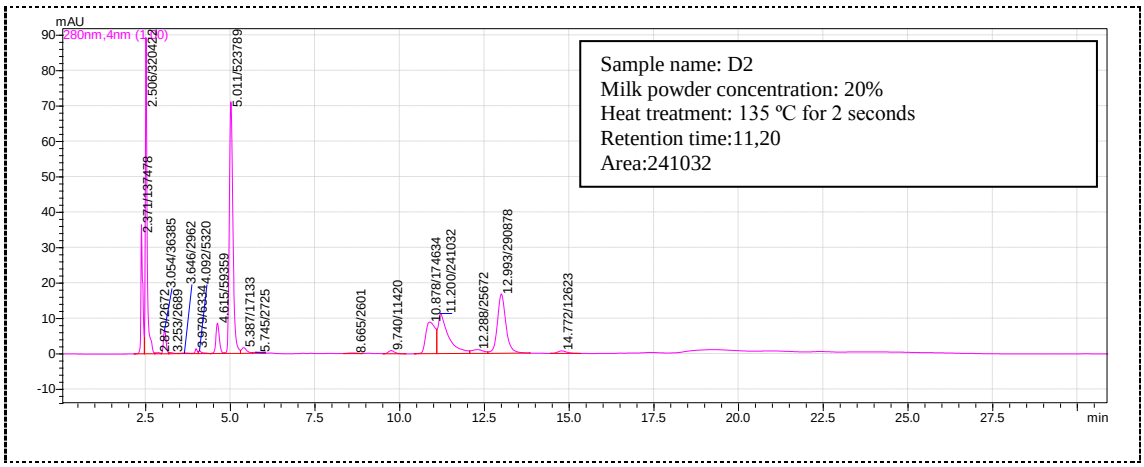
Figure 4.3 (cont.)



D0

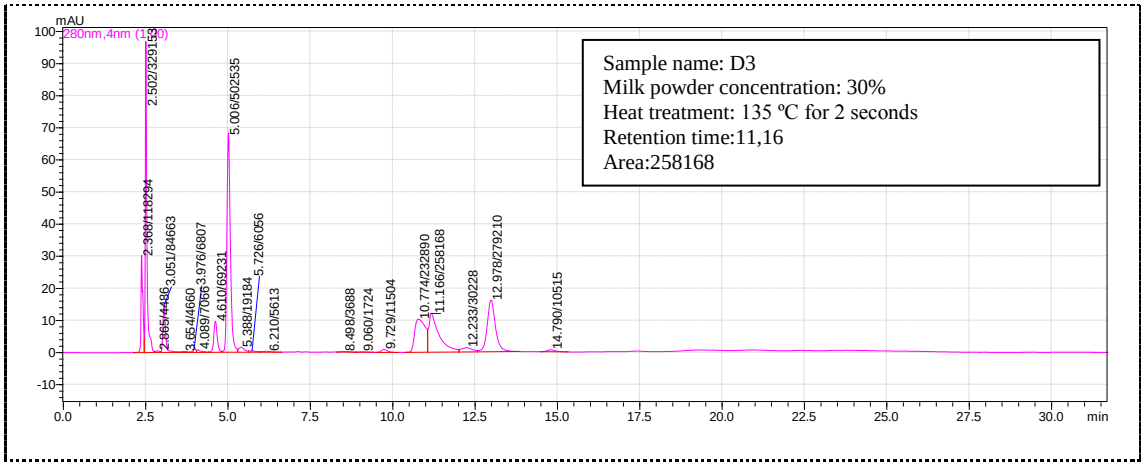


D1

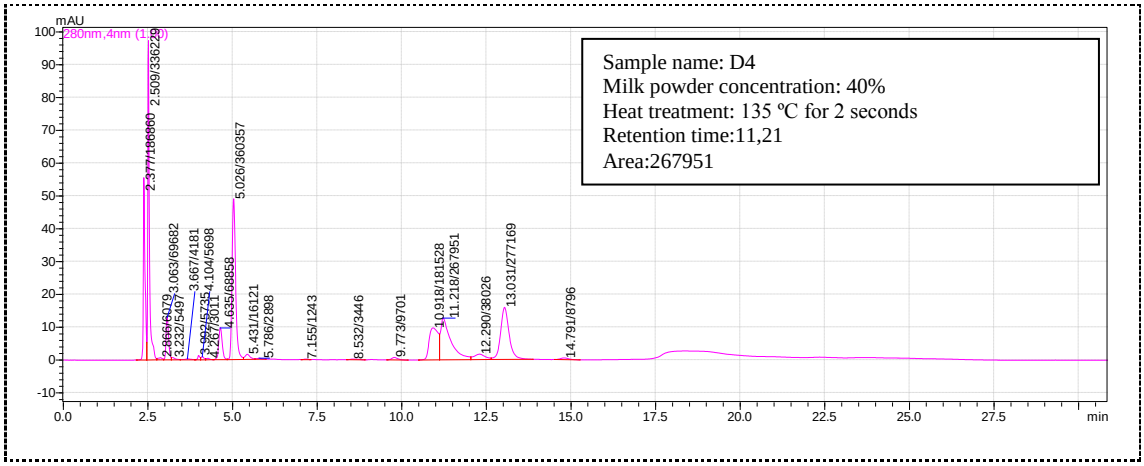


D2

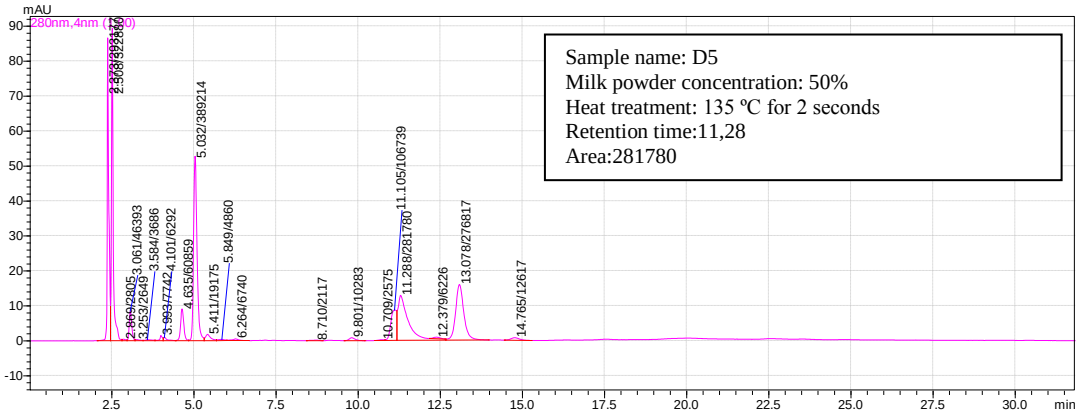
Figure 4.3 (cont.)



D3

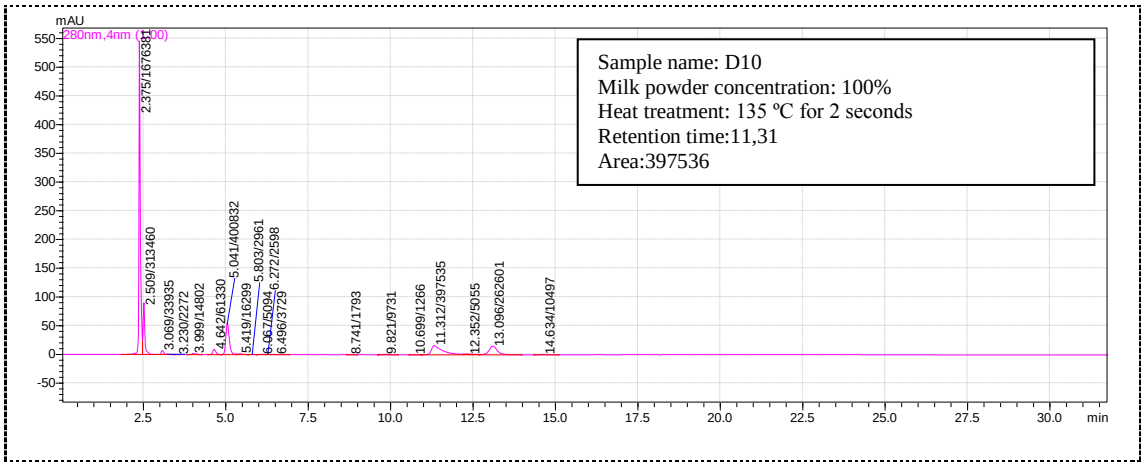


D4



D5

Figure 4.3 (cont.)



D10

Figure 4.3 (cont.)

Furosine contents of positive control samples were calculated by using peak area of positive control samples and standard solution calibration curve. The results are shown in Table 4.4.

Table 4.4 Furosine(mg) contents of positive control samples in ml milk.

	No Heat Treatment **mgFurosine / mlMilk**		**63 ºC for 30 minutes (LTHT)** **mgFurosine/mlMilk**		**72 ºC for 15 seconds (HTST)** **mgFurosine/mlMilk**		**135 ºC for 2 seconds (UHT)** **mgFurosine/mlMilk**	
Group-0	A0	3,75E-04	B0	4,19E-04	C0	4,58E-04	D0	5,73E-04
Group-1	A1	3,79E-04	B1	4,20E-04	C1	4,70E-04	D1	6,00E-04
Group-2	A2	3,88E-04	B2	4,29E-04	C2	4,72E-04	D2	6,20E-04
Group-3	A3	3,90E-04	B3	4,34E-04	C3	4,79E-04	D3	6,62E-04
Group-4	A4	3,91E-04	B4	4,36E-04	C4	4,80E-04	D4	6,86E-04
Group-5	A5	3,94E-04	B5	4,39E-04	C5	4,86E-04	D5	7,19E-04
Group-6	A6	3,99E-04	B6	4,51E-04	C6	4,92E-04	D6	7,98E-04
Group-10	A10	4,02E-04	B10	4,54E-04	C10	5,29E-04	D10	10,2E-04

The calibration curve that we prepared according to the Furosine content of positive control samples is given below in Figure 4.4. The x-axis on the graph is milk powder concentration and the y-axis is mg Furosine in 1 ml sample

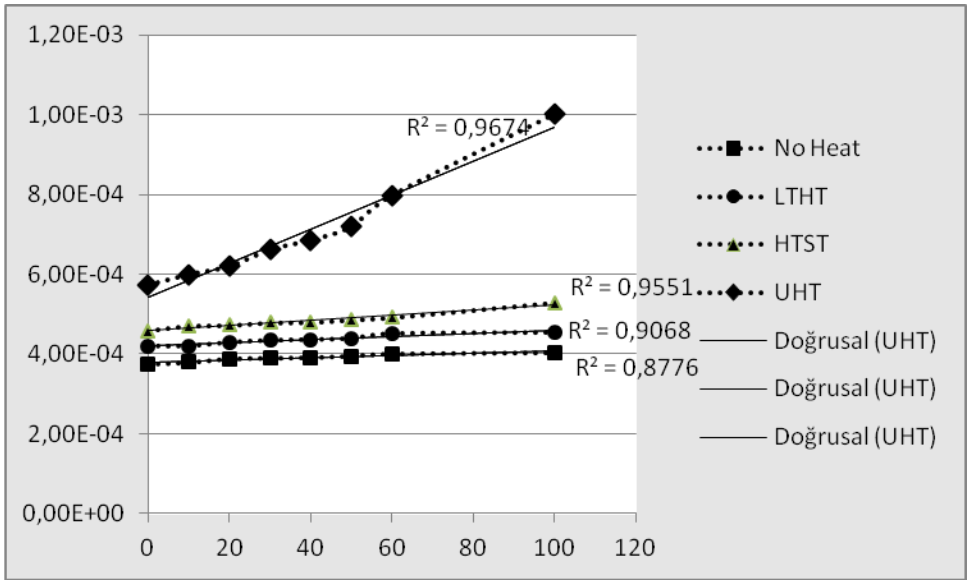


Figure 4.4 The calibration curve that prepared according to the Furosine content of positive control samples.

The Furosine contents per 100 g of protein in positive control samples are calculated by using proportion of proteins and Furosine contents of positive control sapmles. The results are given as ratio of mg Furosine to 100 g protein in Table 4.5.

Table 4.5 Furosine contents per 100 g of protein in positive control samples.

	No Heat Treatment mgFurosine/ 100gProtein		**63 ºC for 30 minutes (LTHT) mgFurosine/ 100gProtein**		**72 ºC for 15 seconds (HTST) mgFurosine/ 100gProtein**		**135 ºC for 2 seconds (UHT) mgFurosine/ 100gProtein**	
Group-0	A0	**1,13**	B0	**1,26**	C0	**1,38**	D0	**1,72**
Group-1	A1	**1,13**	B1	**1,25**	C1	**1,40**	D1	**1,79**
Group-2	A2	**1,22**	B2	**1,34**	C2	**1,48**	D2	**1,94**
Group-3	A3	**1,22**	B3	**1,36**	C3	**1,51**	D3	**2,08**
Group-4	A4	**1,13**	B4	**1,26**	C4	**1,39**	D4	**1,99**
Group-5	A5	**1,09**	B5	**1,22**	C5	**1,35**	D5	**1,99**
Group-6	A6	**1,08**	B6	**1,22**	C6	**1,33**	D6	**2,15**
Group-10	A10	**1,22**	B10	**1,38**	C10	**1,60**	D10	**3,03**

The calibration curve that we prepared according to the ratio of mg Furosine to 100 g protein of positive control samples is given below in Figure 4.5. The x-axis on the graph is milk powder concentration and the y-axis is mg Furosine / 100 g protein.

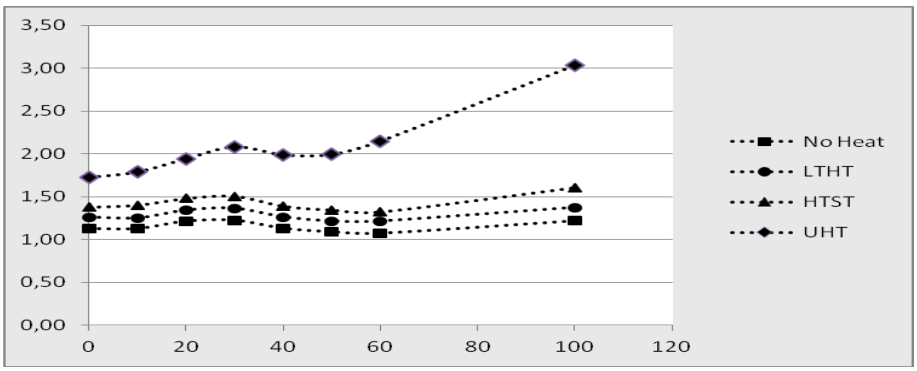


Figure 4.5 Calibration curves that prepared according to the ratio of mg Furosine to 100 g protein.

4.1.4 Protein Contents of Commercial Milks

Proportion of protein in commercial milk samples that calculated from nitrogen content from Kjeldahl method are shown below in Table 4.6.

Table 4.6 Protein contents of samples (g/100ml milk).

Sample	**Protein(g/ml milk)**	**Sample**	**Protein(g/ml milk)**
UHT-1	3,11	**Pasteurized-1**	3,21
UHT-2	3,10	**Pasteurized-2**	3,38
UHT-3	3,13	**Pasteurized-3**	3,29
UHT-4	3,12	**Pasteurized-4**	3,31
UHT-5	2,98	**Pasteurized-5**	3,45
UHT-6	3,21		
UHT-7	3,16		
UHT-8	3,12		
UHT-9	3,30		

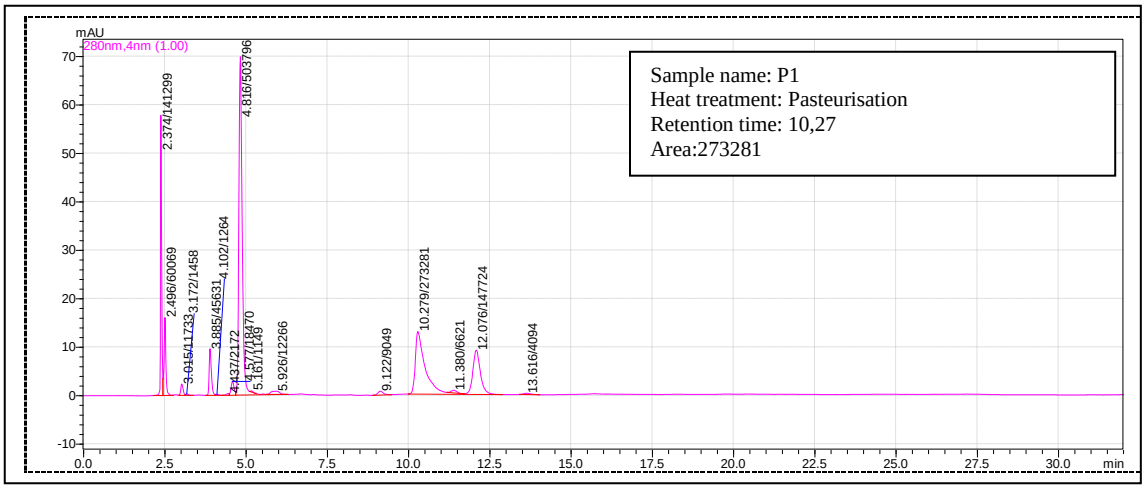
4.1.5 Furosine Concentration of Commercial Milks

The numerical values of the peak area of Furosine obtained for the commercial milks are shown below in Table 4.7. Retention times of these samples were between minute 10,998 and minute 11,316.

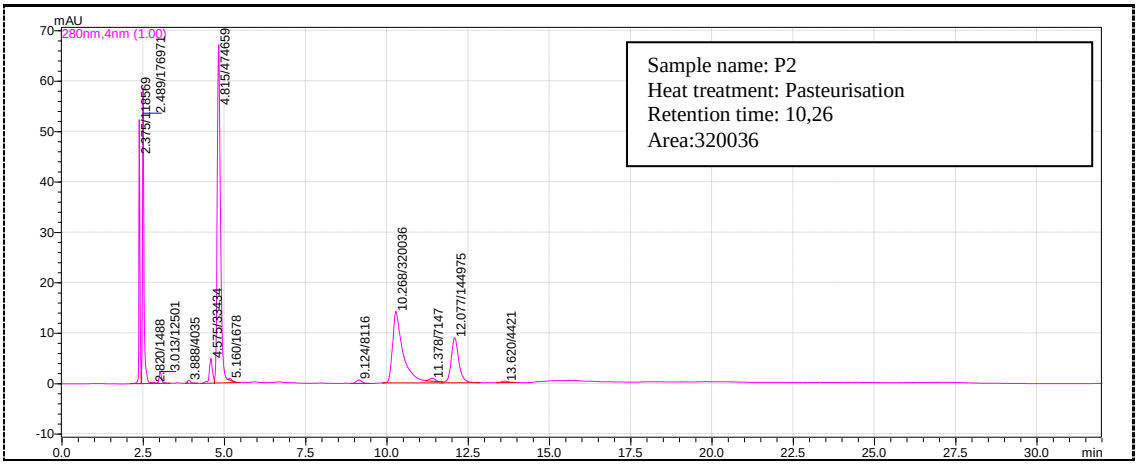
Table 4.7 The peak area for Furosine in 20 µl commercial milk samples.

Sample	**Peak Area**	**Sample**	**Peak Area**
UHT-1	**379017**	**Pasteurized-1**	**273281**
UHT-2	**381228**	**Pasteurized-1**	**320036**
UHT-3	**388228**	**Pasteurized-1**	**326061**
UHT-4	**390749**	**Pasteurized-1**	**346843**
UHT-5	**395817**	**Pasteurized-1**	**367936**
UHT-6	**404812**		
UHT-7	**443962**		
UHT-8	**446238**		
UHT-9	**557490**		

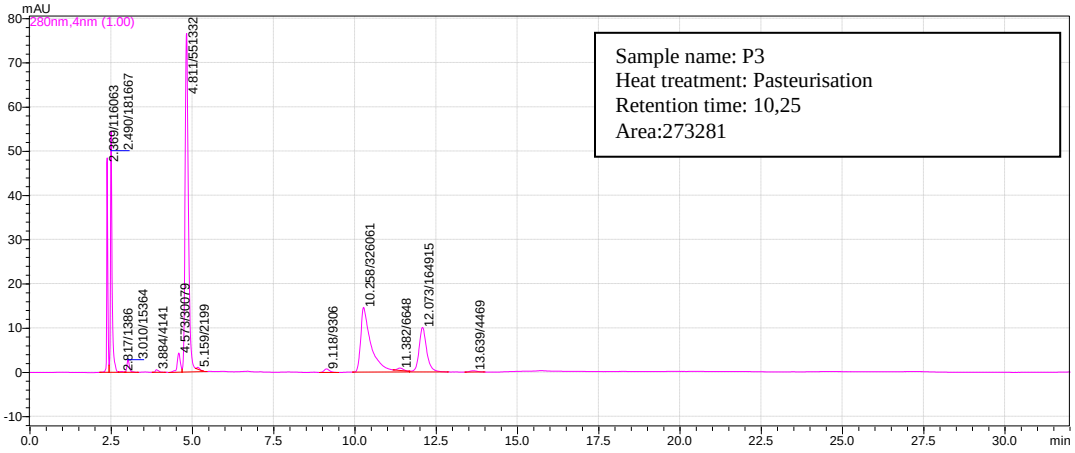
The HPLC chromatogram of commercial milk samples (UHT and Pasturized) are shown in Figure 4.6.



P1

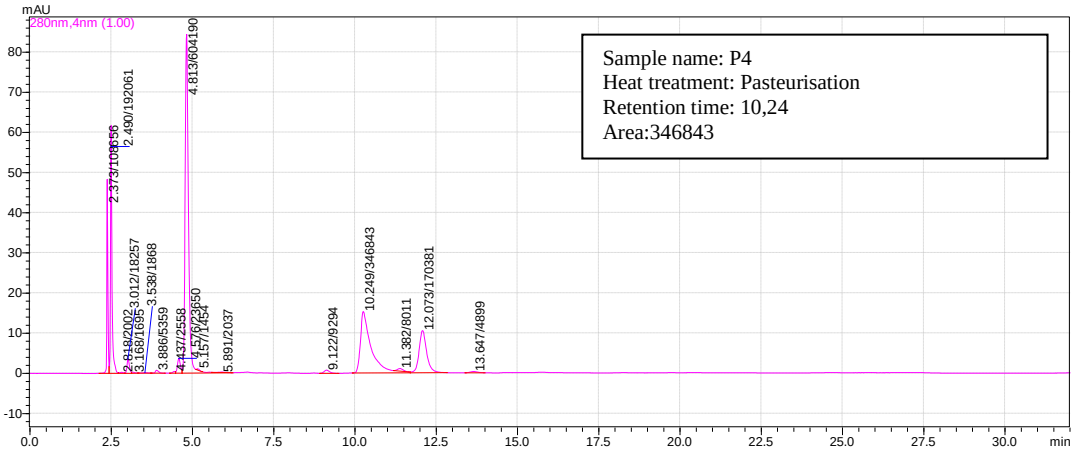


P2

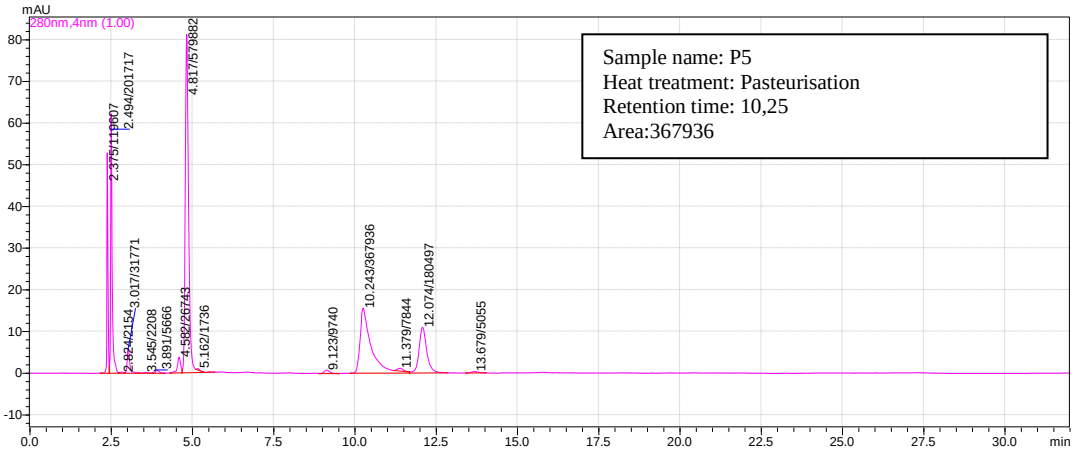


P3

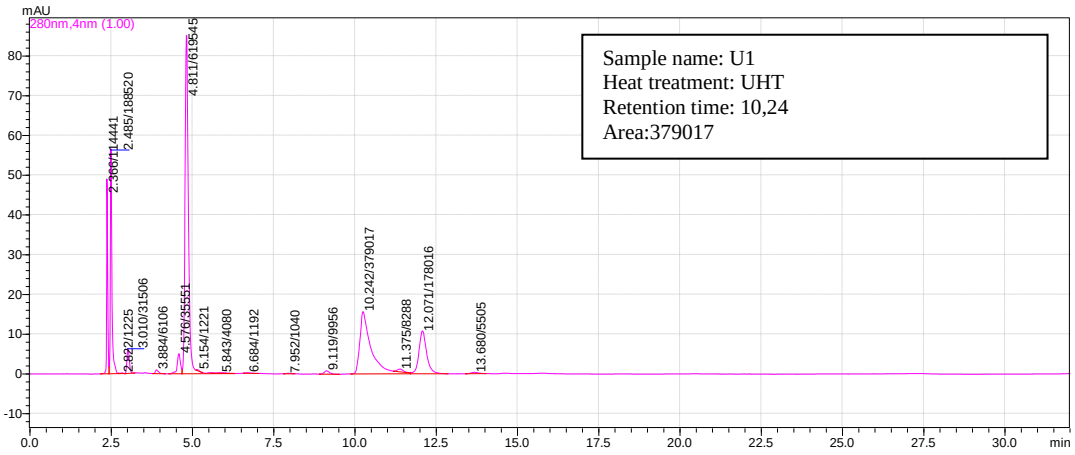
Figure 4.6 The HPLC chromatogram of commercial milk samples.



P4

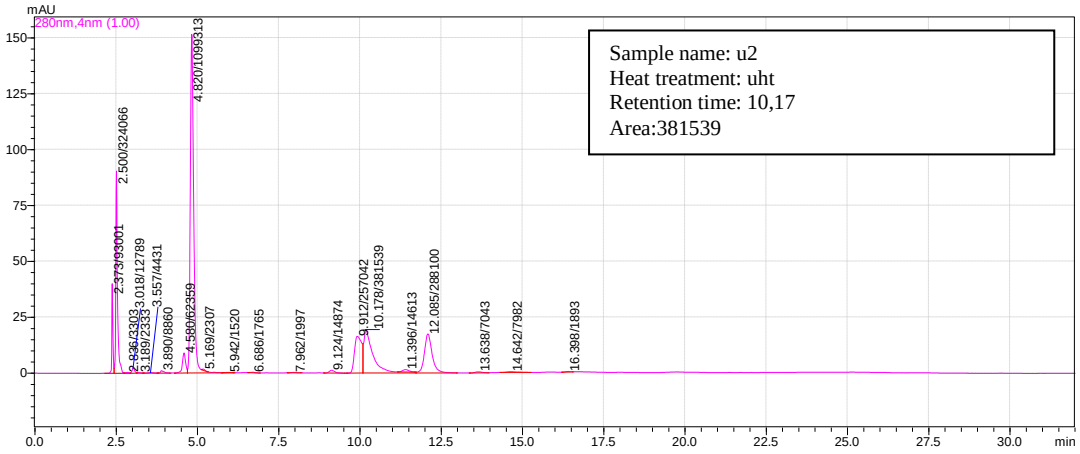


P5

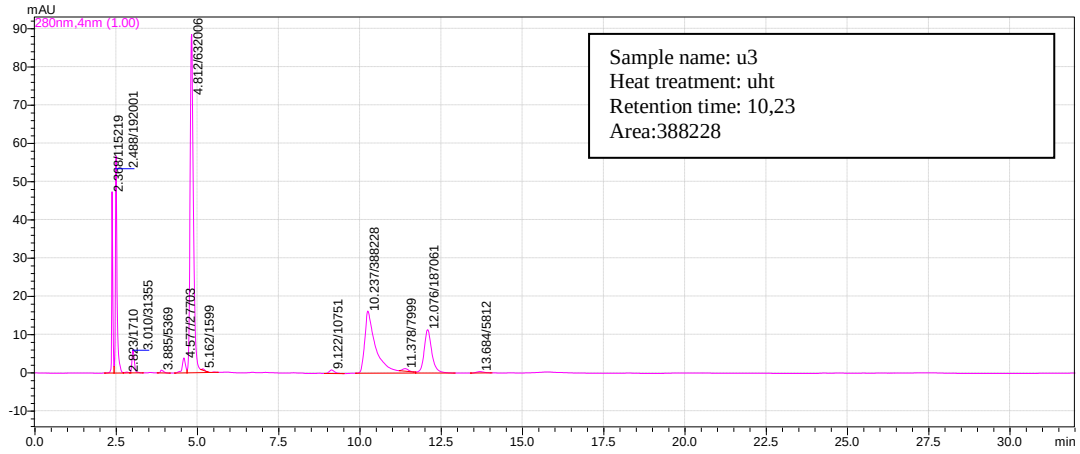


U1

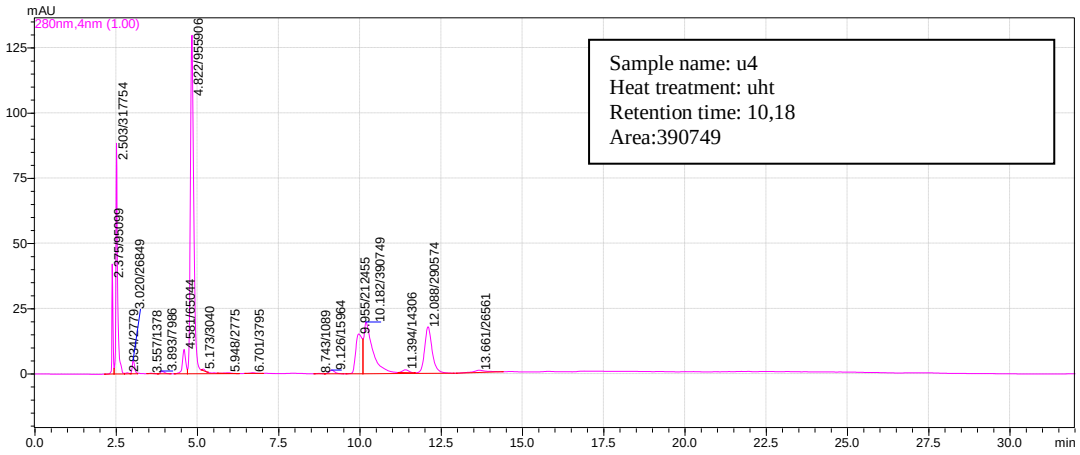
Figure 4.6 (cont.)



U2

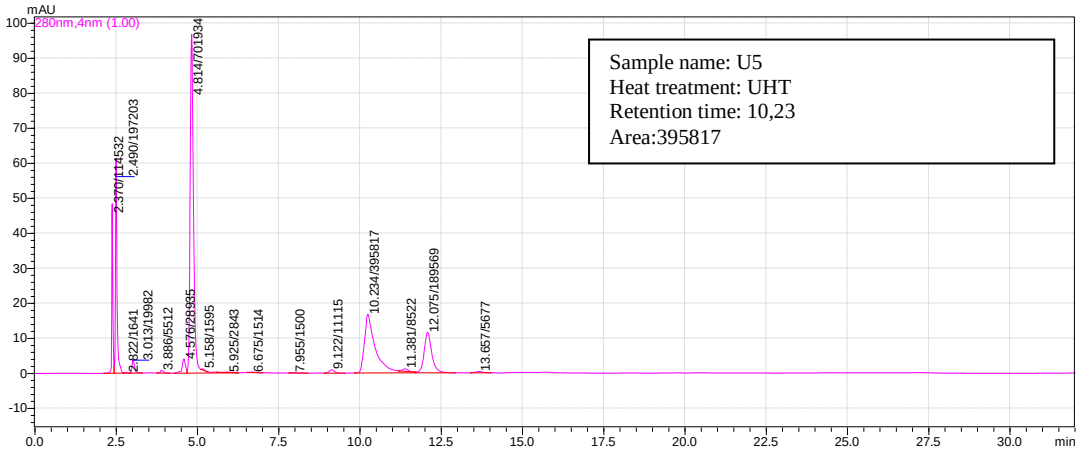


U3

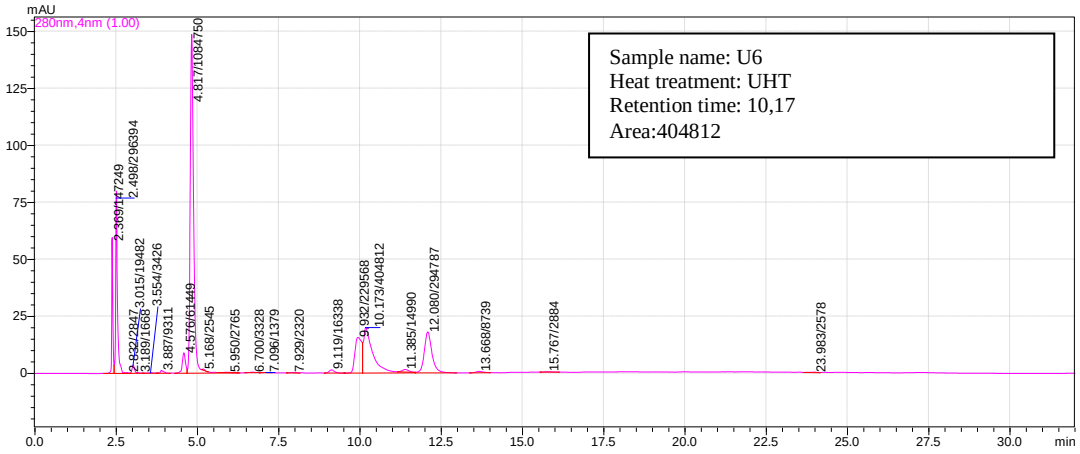


U4

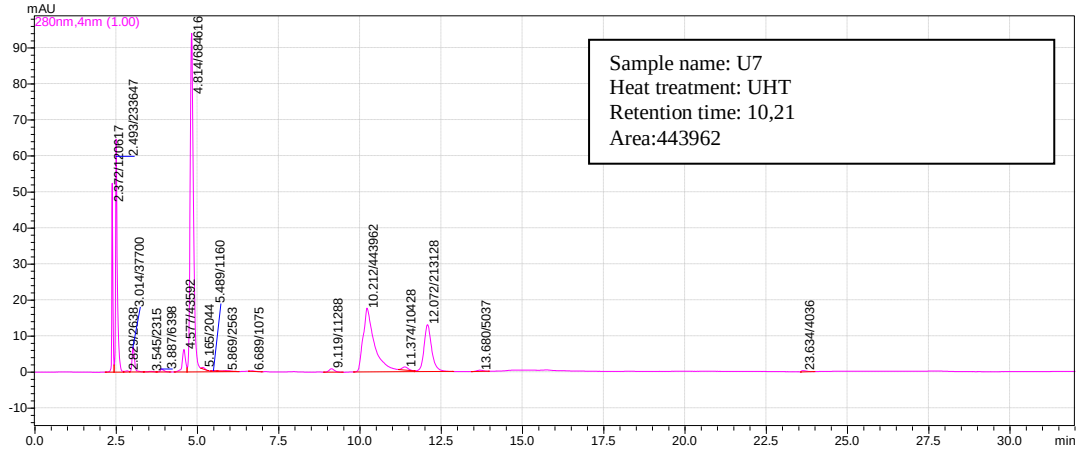
Figure 4.6 (cont.)



U5

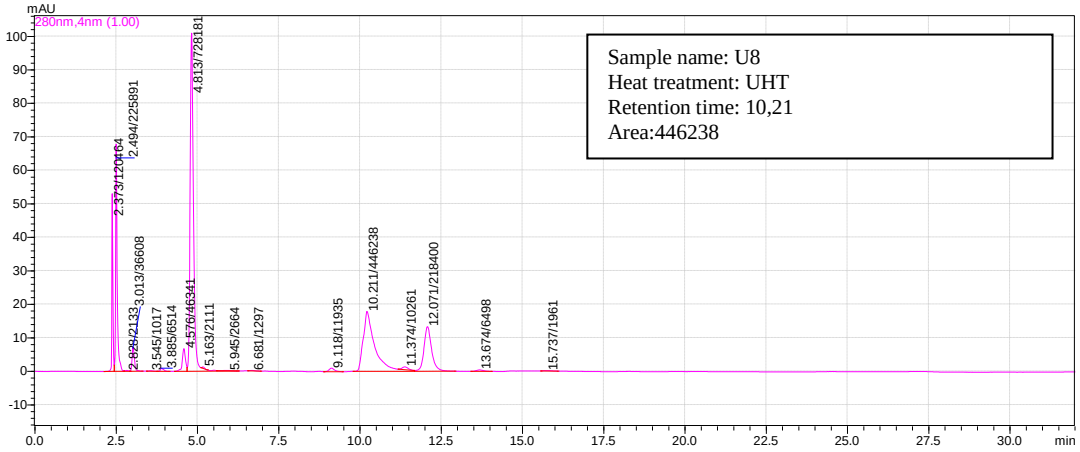


U6

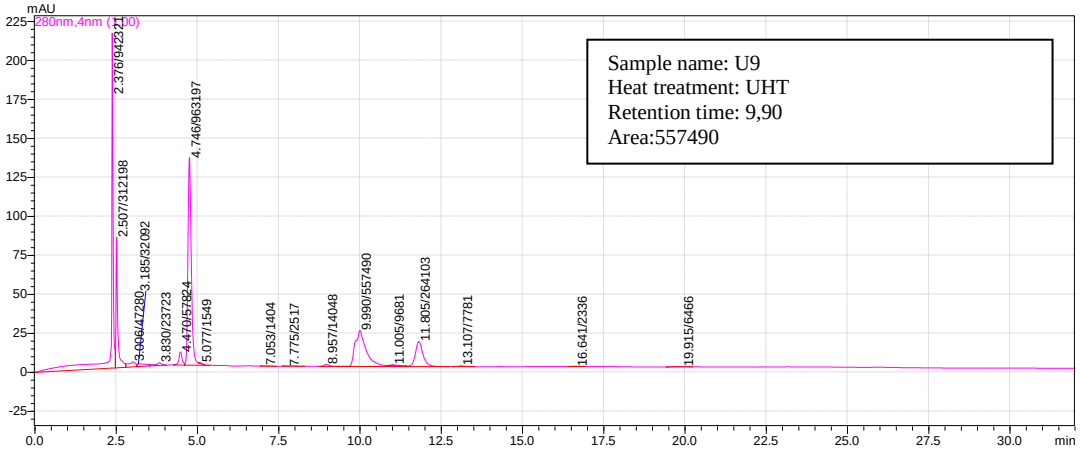


U7

Figure 4.6 (cont.)



U8



U9

Figure 4.6 (cont.)

Furosine contents of commercial milk samples were calculated by using peak area of standard solution calibration curve. The results are shown in Table 4.8.

Table 4.8 Furosine contents of commercial samples.

Sample	**mgFurosine/mlMilk**	**Sample**	**mgFurosine/mlMilk**
UHT-1	0,00096	**Pasteurized-1**	0,00070
UHT-2	0,00096	**Pasteurized-2**	0,00081
UHT-3	0,00098	**Pasteurized-3**	0,00083
UHT-4	0,00100	**Pasteurized-4**	0,00088
UHT-5	0,00102	**Pasteurized-5**	0,00093
UHT-6	0,00111		
UHT-7	0,00112		
UHT-8	0,00139		
UHT-9	0,00003		

The Furosine contents per 100 g of protein in commercial milk samples are calculated by using proportion of proteins and Furosine contents of in commercial milk samples. The results are given as ratio of mg Furosine to 100 g protein in Table 4.9.

Table 4.9 Furosine contents per 100 g of protein in commercial milk samples.

Sample	mgFurosine/100grProtein	**Sample**	mgFurosine/100grProtein
UHT-1	3,0745	**Pasteurized-1**	2,1766
UHT-2	3,1043	**Pasteurized-2**	2,4040
UHT-3	3,1266	**Pasteurized-3**	2,5143
UHT-4	3,1563	**Pasteurized-4**	2,6520
UHT-5	3,3460	**Pasteurized-5**	2,6933
UHT-6	3,1745		
UHT-7	3,5264		
UHT-8	3,5894		
UHT-9	4,2146		

4.1.6 Milk Powder Concentration of Commercial Milks

Milk powder concentration of commercial milks (both UHT and pasteurized) are displayed in Figure 4.7

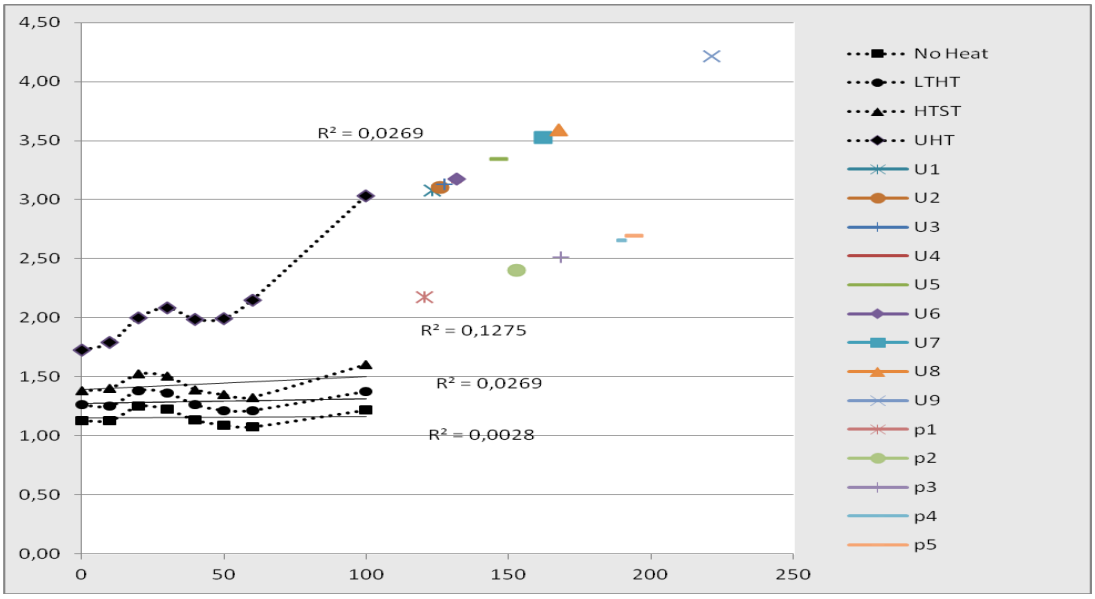


Figure 4.7 Display of commercial milk samples on the calibration curve of positive control samples.

4.1 DISCUSSION

Milk is a food rich in nutrients. It is considered to be a complete nutrient source for humans and is widely marketed and consumed around the world. Milk powder also has an important place in human diets as well as in the world economy. But, there are some concerns and debates about fresh milk and powder. Although drinking milk manufactures declare that drinking milk is produced from raw milk, while raw milk producers claim that drinking milks contain milk powder and this practice economically damages raw milk producers. Besides, consumers also want label specification if milk powder is being used. Challenges in this area attracted us to research an analytical HPLC method for qualitative and quantitative detection of milk powder in UHT and Pasteurized milk.

According to the different literature studies, detection methods depend on changes in the molecular structure of milk constituent, resulted from heat treatments and changes resulted from drying of milk powder (Guan et al., 2005). One among these methods include fluorimeter to obtain Fluorescence of advanced Maillard products and Soluble Tryptophan (FAST index). The others are as follows: the determination of their ultra violet and visible spectra (700 to 240 nm) (Madkour et al., 1989), the detection of hydroxymethylfurfural by spectrophotometer (Rehman et al., 2000), measurements of δD and ^{18}O stable isotope ratios by mass spectrometry (Lin et al., 2003).

After all, Furosine the most popular indicator of Maillard reaction, is used for detection of milk powder in dairy products. Several analytical techniques are used to measure the Furosine level in dairy products such as gas–liquid chromatography (Büser and Erbersdobler, 1985), ionexchange chromatography, capillary electrophoresis (Tirelli and Pellegrino, 1995). Recently, a popular technique to detect Furosine came into light i.e High performance liquid chromatography (HPLC) which is time saving and provides a good detection limit (Ferrer et al., 2000). For instance it has been used to detect the illegal addition of reconstituted whey and milk powder in fresh cheese (Resmini et al., 1993). Apart from this, it has also been used to characterize the authenticity of mozzarella cheese (Pellegrino et al., 1996),

In our study, HPLC determination of Furosine was used for detection of milk powder in UHT and pasteurized milk. There are a few literatures about it. One of them

is the detection of furosine as an indicator of milk powder which is present in raw and pasteurized milk (Resmini et al., 1992), other is Quantitative determination of furosine in cow's milk containing reconstituted skim milk (Ohta et al., 2002). When we analyze the HPLC determination of Furosine and protein content of samples as in Table 4.1, Table 4.4, Table 4.5, it is clear that there is a linear interaction between mgFurosine/1ml sample and concentration of milk powder. When we draw a calibration curve as in Figure 4.4, the R^2 are in between 0.96 to 0.87.

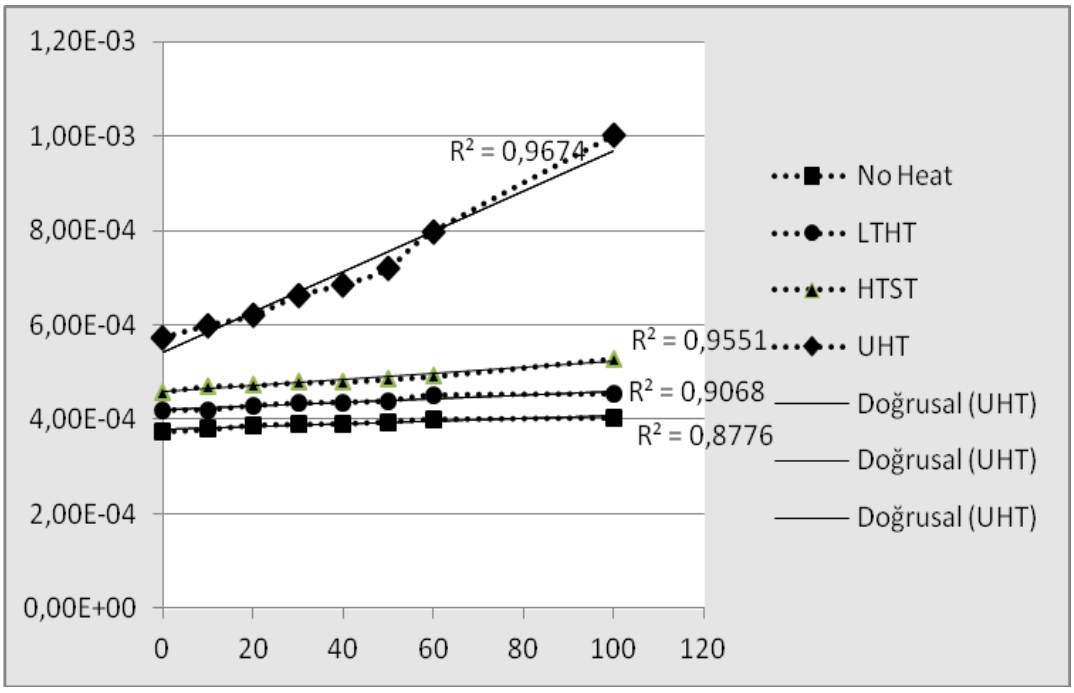


Figure 4.4 The calibration curve that prepared according to the Furosine content of positive control samples

According to the literature studies, the Furosine content in pasteurized milk varies in the range of 1,1- 1,5 mg/L and in UHT milk 17-68 mg/L (Pellegrino et al. 1995, Resmini et al. 1995, Corzo et al. 1994). Our results (Table 4.4) are not in good agreement with the data above.

Furosine content of our samples in pasteurized milk varies in the range of 4,19-5,29 mg/L and in UHT milk 5,73-10,2 mg/L. Since the Furosine formation depends on the time and degree of heat treatment, faults might haveoccurred during heat treatment processes. By using advanced heat treatment equipments the fault can be eliminated or minimized.

Table 4.10 Furosine contents of positive control samples.

	No Heat Treatmenet **mgFurosine / ml milk**		**63 ºC for 30 minutes (LTHT)** **mgFurosine/mlMilk**		**72 ºC for 15 seconds (HTST)** **mgFurosine/mlMilk**		**135 ºC for 2 seconds (UHT)** **mgFurosine/mlMilk**	
Group-0	A0	3,75E-04	B0	4,19E-04	C0	4,58E-04	D0	5,73E-04
Group-1	A1	3,79E-04	B1	4,20E-04	C1	4,70E-04	D1	6,00E-04
Group-2	A2	3,88E-04	B2	4,29E-04	C2	4,72E-04	D2	6,20E-04
Group-3	A3	3,90E-04	B3	4,34E-04	C3	4,79E-04	D3	6,62E-04
Group-4	A4	3,91E-04	B4	4,36E-04	C4	4,80E-04	D4	6,86E-04
Group-5	A5	3,94E-04	B5	4,39E-04	C5	4,86E-04	D5	7,19E-04
Group-6	A6	3,99E-04	B6	4,51E-04	C6	4,92E-04	D6	7,98E-04
Group-10	A10	4,02E-04	B10	4,54E-04	C10	5,29E-04	D10	10,2E-04

CHAPTER 5

CONCLUSIONS

The importance given to the consumption of milk is increasing with each passing day. But, there are some concerns and debates about drinking milk sector. Although drinking milk manufactures declare that drinking milk is produced from raw milk, raw milk producers claim that drinking milks contain milk powder and this practice economically damages them. Besides, consumers also want be specified on the label if milk powder is used. In this study, a novel analytical HPLC method that used qualitative and quantitative detection of milk powder in UHT and pasteurized milk was developed by using Furosine, a Maillard reaction product, as an indicator of milk powder.

When we analyzed the HPLC determination of Furosine and protein content of samples, the results show a linear interaction between mgFurosine/1ml sample and concentration of milk powder. When we draw a calibration curve of this interactions, R^2 are between 0,96 to 0,87. But, our results are not in good agreement with the literature data. Although Furosine content in pasteurized milk varies in the range of 1,1- 1,5 mg/L and in UHT milk 17-68 mg/L, Furosine content of our samples in pasteurized milk varies in the range of 4,19- 5,29 mg/L and in UHT milk 5,73-10,2 mg/L Since the Furosin formation depends on the time and degree of heat treatment, fault might have occurred during heat treatment processes. By using advanced heat treatment equipment the fault can be eliminated or minimized to a greater extent. Furosin can be used as an indicator for milk powder concentration in drinking milk and HPLC which is a useful method to detect furosine can be a golden technique to screen milk powder in commercial UHT and pasteurized milk samples..

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