YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF NUTRITION AND DIETETICS

A RESEARCH ON THE PRESENCE OF GENETICALLY MODIFIED ORGANISMS IN MILK

MASTER THESIS

Ayşegül DEMİRER

ADVISOR
Assist. Prof. Dr. İskender KARALTI

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SAĞLIK BİLİMLERİ ENSTİTÜ MÜDÜRLÜĞÜ'NE

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ÖZET

Tüm dünyanın gıda ihtiyacını karşılayabilmek amacıyla tarım alanlarının büyütülmesine ve mevcut alanların verimliliğinin artırılmasına çalışılıyor. Bu doğrultuda daha çok ürün elde etmenin yolu geleneksel tarım metotlarından uzaklaşılarak modern tarım yöntemlerine geçiştir. Genetik mühendisliği ve moleküler biyoteknolojideki yeni gelişmeler organizmaların genetik yapılarını değiştirilebilmesine olanak sağlamıştır. Bu teknolojiden yararlanılarak canlıların genetik yapılarında değişiklikler yapılmak üzere kaliteli ürün veren ve hastalıklara karşı daha dayanıklı bitki ve hayvan türleri elde edilmeye çalışılmıştır. Dünyada en sık ekimi yapılan GDO'lu ürünler arasında mısır, soya, pamuk ve kanola bitkisi gelmektedir. Küresel hayvancılık popülasyonların da genetiği değiştirilmiş yem bitkilerinin büyük kısmını tüketiciler oluşturmaktadır. Hayvan beslenmesinde genetiği değiştirilmiş yemlerin kullanılması geleneksel yemlere göre daha fazladır. GDO'lu ham madde içeren yemlerle beslenen hayvanlardan elde edilen ürünlerin insan beslenmesinde kullanılması insan sağlığı üzerinde oluşturabileceği olası riskler üzerinde durulmaktadır. Çalışmamızda 20 adet paketli ve 20 adet paketlenmemiş süt örneğinde GDO olup olmadığı araştırıldı. Örneklerden 1'er litre alınıp DNA izolasyonu yapılmıştır. İzole edilen DNA'lar Roche 480 II Real time PCR cihazı ile çalışılmıştır. Testin performansını değerlendirmek için kontrol testi de yapılmıştır. Çalışmamızda incelenen süt örneklerinde GDO varlığı saptanmamıştır. Bu durum umut verici olarak düşünülmektedir.

Anahtar Kelimeler: Genetiği Değiştirilmiş, GDO, Süt

ABSTRACT

It's sought to expand agricultural fields and increase the productivity of current ones in order to fulfil the food need of the entire world. Accordingly, traditional agricultural methods should be laid aside proceeding to contemporary ones in order to obtain more products. In recent years, new developments in genetic engineering and molecular biotechnology have made it possible for organisms' genetic structures to be changed. Making changes in living organisms' genetic structures through utilizing the technology, it has been attempted to acquire plant and animal species stronger against diseases and pests, and yielding more and quality goods. Among the most cultivated GMO goods are corn, cotton and canola. Consumers constitute most of the genetically modified fodder plants also in global stockbreeding population. Genetically modified fodder plants are used more than traditional ones. The potential risks on human health as a result of using the goods provided from animals eating GMO fodder plants containing GMO raw materials are discussed. In our study, 20 packed and 20 non-packed milk was used. 1 litre was received from the samples and DNA isolation was made. Roche Light Cycler 480 II Real Time PCR was used to work on the isolated DNAs. A control test was also done in order to evaluate the performance of the test. No GMO was detected in the milk samples we analysed in the study, which is considered promising.

Key Words: Genetically Modified, GMO, Milk

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ABBREVIATIONS

EFSA : The UN Commission and European Food Safety Authority

rSBH : Recombinant Cattle Growth Hormone

WHO : World Health Organisation

FAO : Food and Agriculture Organisation

EPA : Environmental Protection Agency

AMA: American Medicine Association

FDA : Food and Drug Association

GMO : Genetically Modified Organism

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CHAPTER ONE
INTRODUCTION

It's sought to expand agricultural fields and increase the productivity of current ones in order to fulfil the food need of the entire world. Accordingly, traditional agricultural methods should be laid aside proceeding to contemporary ones in order to obtain more products.

In recent years, new developments in genetic engineering and molecular biotechnology have made it possible for organisms' genetic structures to be changed. Making changes in living organisms' genetic structures through utilizing the technology, it has been attempted to acquire plant and animal species stronger against diseases and pests, and yielding more and quality goods. Living species with new genetic features have been achieved through gene transfer among different living species by using contemporary biotechnological techniques. Living organisms or products obtained through this method are defined as Genetically Modified Organisms (GMO). It has been assumed that the increase in productivity in agricultural production as a consequence of molecular biotechnology applications might resolve the famine in the world; however several potential unfavourable impacts upon human health and environment along with the increase in productivity caused some suspicions to arise (2).

Nowadays, there are different views on GMOs. Even though laws have various dimensions and it seems like health, agricultural, economic and environmental organizations' evaluations seem to be limited with their own scientists, they basically attempt to maintain discussions with a point of view in favour of public health. Public health seeks to hold the benefits of the health of society at the maximum level (3).

Contemporary gene Technologies provide significant opportunities in increasing agricultural production in order for the rapidly increasing world population to eat healthily. Not only the implementation of sustainable agricultural techniques, but also the development of productive and quality plant species is of great importance (4).

Genetically Modified Organisms are organisms acquired through gaining new features by changing the present genes in living things (5). Rapidly developing gene technology has been a part of our daily lives not only as a research field, but also with health, food, stuff and pets. As the most outstanding topic of gene technology, GMO is still the main subject of the world's agenda (6).

It is argued that GMO production will reduce agricultural pesticide use, increase the shelf-life and productivity of the product; and enable production in unfavourable climates and resolve the famine problem in the world (7). On the other hand, it has been found out in some studies that GMO products create a resistance against antibiotics, GMO products have nutrition loss, allergic and toxic effects, and however, there is no definite knowledge on its effects on human health in the long run (8-9).

While views on the effect of GMO on human health still vary, no consensus has been reached. Currently, studies on GMO products continue. Potential benefits and harms of GMO products are to be determined thanks to the scientific studies that are being made (4).

According to the World Health Organisation (WHO) and Good Agriculture Organisation (FAO), while generally no advanced allergy test is made in traditionally developed products and convenience foods, GMO products are evaluated through such tests. According to the reports of WHO and FAO, all the GMO products in the market have been tested and no allergic effect has been observed at all. As a consequence of risk assessments, products having allergic effect are prohibited to be sold. WHO states that no general comments will be made on GMO products, each product will be assessed individually (10).

Among the most cultivated GMO goods throughout the world are corn, soy, cotton and canola plant. The first five countries in which GMO goods are cultivated most are The United States of America, Argentina, Brazil, India and China (11).

GMO use in animal forage is not prohibited in our country. Our study aims to determine whether GMO residues contaminate the milk acquired from cows which possibly consume GMO forage.

CHAPTER TWO
GENERAL INFORMATION

2.1 What is GMO?

Genetically modified organism (GMO) is acquired through giving a new character not found in the original structure of a living thing by changing its gene sequence or transferring various bacteria, viruses, genes from animals and plants into it. The 2011/ 18/ EC numbered Directive of the United Nations defines GMO as “any organism, other than human, whose genetic material has been modified unnaturally through intercourse and/ or natural recombination.” Enabling the transfer of selected genes from and organism to another, this technology is called generally “modern biotechnology” or “gene technology”, and sometimes “recombinant DNA technology” or “genetic engineering”. Additionally, various terms such as genetically modified food, transgenic food, biotechnological product, genetically transferred organism or bioengineering plant can be used instead of genetically modified organism (12-13).

2.2 History

Between the years 1940-1960, the Green Revolution emerged with the introduction of hybrid seeds, chemical fertilizers, agricultural machines and irrigation facilities to the agricultural sector. The Green Revolution, which had been regarded as the saver of the time, created serious side effects such as environmental pollution. Since the Green Revolution did not resolve the famine problem, GMO products, whose foundations were laid in the early 1980’s and which started to spread in the midst of the 1990’s, and is regarded as the Second Green Revolution to some, was put into service (14).

In 1972, Paul Berg acquired the first genetically modified DNA molecule. In 1974, Stanley, Cohen, Annie Chang and Herbert Boyer created the first genetically modified organism. In 1976, the National Institutes of Health in the United States of America

determined directives for genetic modification researches. In 1982, sale of insulin which was produced with gene technology by Food and Drug Association of America (FDA) was approved. In 1983, three out of four different groups of scientists added bacteria gene to plants, and one group added bean gene to sunflower in order to acquire genetically modified plans for the very first time (12).

Production of GMO products for commercial purposes began in 1996. The first GMO product which was put on market was a type of tomato having a long shelf life produced by Calgene Company with the name Flavr Savr. Among the GMO products cultivated for commercial purposes are mainly soya, corn, cotton and canola; while there have been genetically gene transfer studies on products such as sugar beet, pepper, potato, tomato, rice, wheat, squash, sunflower, peanut, cassava and daisy. Additionally, gene transfer studies continue on products such as banana, strawberry, cherry, melon, watermelon, raspberry and pineapple (12).

2.3 Methods Used in Gene Transfer

While genes having desired features in a plant species are attempted to be gathered in a species as a consequence of studies of many years in traditional improvement methods, it is possible to transfer the determined gene to a living thing through gene transferring in gene technology. The purpose in gene transferring is to transfer a gene to a target cell. Gene transferring can be made through physical or chemical methods and viral vectors. The most used method in chemical ones is Calcium-phosphate transfection. In this method, the desired gene is precipitated on the host cell along with needed elements for gene expression. Precipitated with Calcium-phosphate, plasmid DNA is received in the target cell through endocytosis or phagocytosis. Therefore, the solution composed of gene and elements is transferred in the target cell and the gene transferring is complete (2).

Microinjection, electroporation and biolistic techniques are physical methods used in gene transferring (2). There is a limited success rate in physical and chemical gene transferring methods despite the fact that they are simple. Transferred genes can be

functioning for some time. Thus, researches in gene technologies basically incline to virus based vectors (2).

The method in which viruses are used as carriers in gene transferring is called viral gene transferring techniques. In this method, viruses and their natural advantages are benefited from. In viral gene transferring method, repairing recombinant genes are placed instead of viruses' gene molecules causing disease. Therefore, viruses carrying repairing genes enter the target cell with their own methods and the protein coded by the gene begins to be produced. Protein coded by the desired gene, namely medicine, is produced and replaces the protein which cannot be produced. The most used viral vectors are retroviruses, adenovıruses and herpesviruses (2).

2.4 Gene Transfer Methods

Gene transferring can be made through either *agrobacterium bacteria* or direct gene transferring method.

2.4.1 Gene Transfer through Agrobacterium

Agrobacterium is a mobile bacillus which lives in soil and produces no negative spores. It has two sorts (*Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*) used in gene transferring. Today's most common instrument used in gene transferring to plants is *A.tumefaciens* plant. It typically enters the plant through the scars developing in root throat and cause tumours as a result of irregular divisions in root throat. Once a tumour begins to form, it can be grown without a bacteria or auxiliary hormone. On the other hand, *A.rhizogenes* is sensitive to phenolic components secreted by injured plant roots. These phenolic compounds stimulate the virulence genes in the bacteria and the plant causes hairy root disease through transferring its own DNA area to the plant genome. They grow faster than typical roots and reproduce easily with no need of hormones due to the fact that they create so many growing points during development. Many cultivated plants such as tobacco, potato, canola and tomato are genetically

transferrable through *A.tumefaciens*. The weak point of this method is that The Graminae is not genetically transferrable and this method fails in several plant species such as The Leguminosae. Despite the fact that the gene transferring with *A.rhizogenes* is a simple method, gene transferring through *A.tumefaciens* and physical methods have made a progress of more importance. However, *A.rhizogenes* for biological studies cannot be used effectively in hairy root production for seconder metabolite production (12).

2.4.2 Direct Gene Transfer Methods

2.4.2.1 Gene Transfer through Electroporation and PEG

The transmittance of membrane is enhanced through creating temporary pores in cell membrane with high voltage electricity (electroporation) or chemical substances (PEG: polyethylene glycol). The DNA part carrying the desired gene is made enter the cell (12).

2.4.2.2 Biolistic

It is carried out through the bombardment of a DNA part attached to metal particles with a particle gun, or directly phage, bacteria or ferment cells with a firing mechanism to target cell and tissues (12).

2.4.2.3 Microinjection

The DNA part carrying the genes which are to be transferred is injected to target cells which are directly immobilized with very thin capillary pipettes or an injector under a microscope. It is usually used in animal cell transformations. Despite the fact that it is a conceptually simple method, its practice is difficult due to the fact that a single cell is transferred at a single time, the application is slow and the operator's hand skills are of importance (12).

2.4.2.4 Sonication

DNA parts are made enter cells through creating gaps between cells and in cell membrane with sound waves (12).

2.4.2.5 Desiccation

It is a method enabling DNA to be received in a cell as a consequence of water intake in an environment where tissues are first faded and the DNA to be transferred are found (12).

2.4.2.6 Transfer through Laser Micro Rays

DNA parts are made enter a cell through opening micro holes in cells with UV laser micro rays (12).

2.4.2.7 Transfer through Fibres

Silicon carbide is a solid ceramic substance and easily forms sharp sides when broke. The plant which is desired to be the target for gene transferring is transferred into a buffer containing DNA and silicon carbide fibres and mixed strongly. Fibres cause a hole in cell wall and membrane as a consequence of a collision between silicon carbide fibres and suspension cells, so DNA entrance is acquired into suspension cells. Products such as corn, tobacco, rice and wheat have been the target of gene transferring through using fibres (12).

2.4.2.8 Transfer through Pollen Tube

Transferring is carried out through the application of DNA onto the stigma surface and the passing of DNA through pollen tube and reaching to ovule. Initially, it has been used for rice, then other species such as wheat, soya and watermelon (12).

2.4.2.9 Gene Transfer through Liposomes

Gene transferring is carried out through the fusion of liposomes carrying DNA with cell membrane of plant protoplasts (12).

2.5 Areas of Use

Nowadays, gene technology applications are used in many areas for different purposes. Modern biotechnology is used in almost every aspect of our lives: health, industry, environment and agriculture etc…

Main areas of use in health are diagnosis and treatment of a disease, organ transferring, organ transplantation, gene treatment and vaccine and medicine production. Substances such as proteins and hormones acquired using genetically modified animals can be used in not only vaccine and medicine production, but also treatment of diseases. With this technique, the insulin hormone is acquired from genetically modified animals and used in diabetes patients, and the clotting factor can be successfully applied on homophile patients. Additionally, livers, kidneys or kidney cells proper for humans can be developed using cloneable animals (Uzogara, 2000) (15).

GMO's are used to produce vitamins, monoclonal antibodies, vaccines, anticancer compounds, antioxidants, plastics, fibres, polyesters, opiates, sleeping pills, interferon, human blood proteins and carotenoid used in medicine industry (Çelik and Balık, 2007) (2-16).

Recombinant DNA technology is beneficial in enzyme production, starter culture production, organic acid, amino acid and vitamin C production in food field. The purpose is to increase the resistance against insects and viral pathogens. Chymosin produced by different recombinant microorganisms is commonly used in food industry (17).

In a study conducted in Denmark, a type of enzyme was produced to be used in drinks as an artificial sweetener and it was found out that it was resistant against pH changes (14). 60% harder cheese production was acquired thanks to an enzyme produced with this technology for rennet (18).

The recombinant DNA technology is used also in the production of some starter cultures. Probiotic lactic acid bacteria are acquired with modern molecular biology techniques. *Leuconostoc spp.* culture is used in order to split acetaldehyde, an undesired compound in butter (17).

Today, glycose recombinant is turned into cetoglyconic acid by an organism in vitamin C production (ascorbic acid), and then cetoglyconic acid is turned into ascorbic acid (19).

As known, starter cultures are used in the production of fermented milk and meat products such as cheese, butter, yoghurt, bologna etc… These cultures give the fermented food a unique flavour and smell through enabling its ripening. *Lactic acid bacteria (LAB)* have been used in food production for many years. LAB are positive bacteria with no spores. As starter cultures, LAB's are commonly used in the fermentation of products such as cheese, yoghurt, kefir, sausage and olive in fermented meat, milk and other food industries. It is known that these bacteria increase the quality, shelf life and nutritional value of the product (17).

The quality of Roquefort cheese has increased using genetically modified *Lactobacillus lactis* strains. Used in yoghurt production, *Lactobacillus delbrueckii* subspecies *bulgaricus* strains have increased the shelf life of the product. Additionally, these strains are used in the production of antibodies and riboflavin and diacetyl enzymes. It is the compound named diacetyl developing during the process of acidity increasing through the multiplying of lactic acid bacteria and creating lactic acid which give the unique flavour and smell to butter. With the same technology, *Leuconostoc spp*. culture has gained a feature of splitting acetaldehyde which is an undesired

compound in butter. *Lactobacillus plantarum* is used in forage industry, which indicated a decrease pH in silage forages (17).

It is assumed that GMO's might reduce environmental pollution through limiting pest use, especially the pesticide use. Moreover, it is claimed that especially the pollution in water resources might be reduced thanks to a decrease in chemical fertilizer use (17).

2.6 Laws, Regulations and Legislations about GMO Products

As GMO products were introduced in the market, some legal arrangements were required to enable both food establishments and consumers to exercise their right of choice about food. However, point of views on GMO products vary greatly, which are basically present between the Organization of Economic Development and Cooperation countries, the USA and the UN countries. While the United States of America has a positive approach towards the use of such products, the United Nations countries are suspicious about it. While there is no legal obligation for the use of GMO products in the United States of America, it is a must in the United Nations countries for such products to be labelled to indicate that they have GMO (2).

As concerns increased, some arrangements were made in order to enable the exportation of such products and most of the developed and developing countries embraced the "Cartagena Biosecurity Protocol" in parallel with this purpose (2).

The Cartagena Protocol states that the Biologic Diversity Agreement, which is approved with the 17.07.2003 dated and 2003/ 5937 numbered decision of the Council of Ministers, supports the idea that human life and health should be prioritized, therefore state parties should act in accordance with the precautionary principle. The said principle supports the idea that if there is any suspicion that a risk is present on the people's health, decision makers should take necessary measures, that is to say, the state should approach such issues with caution and make required arrangements. Prepared in accordance with the Decision of State Parties Conference, the Biosecurity Protocol was

agreed on January 29, 2000 in France by more than 130 countries. Turkey signed the Protocol on May 24, 2000 (20).

Some legal arrangements were made by national and international institutions as GMO products became a part of our lives. The purpose of these arrangements was to protect human and animal health and enable the biosecurity assessment of forage before presented for sale (2).

Main international biosecurity arrangements:

- "Voluntary Direction on the Emission of Organism into the Nature" published by UNIDO (UN Industrial Development Organisation) Secretariat (1991)
- "Plant Biotechnology Directive" published by UN Food and Agricultural Organisation (FAO) (1991)
- "Agenda 21" (1992)
- "Biosecurity Guide" prepared by United nations Environmental Program (NEP) in order to serve as a guide to research the biosecurity capacity of developing countries (1997)
- UN Biological Diversity Agreement (1992)
- UN Cartagena Biosecurity Protocol (2003) (12)

2.7 Legal Arrangements in the USA and the European Union

The Protocol creating the prohibition opportunity of the importation of certain GMO products for importing countries due to health and environmental risks despite the fact that there are no scientific data entered into force on September 11, 2003. On September 20, 2004, importation of genetically modified corn was rejected by member countries in the meeting held in Brussels. No majority was constituted in the voting made for the importation of the corn produced by the company named Monsanto. Examined in depth by a group predominantly made up of French scientists, the corn gained resistance against harmful insects through producing poisonous chemicals. The French Genetic

Engineering Commission determined considerable changes in the kidney weight, albumin/ globulin rates and white blood cell counts of rats eating the GMO corn (2-12).

Supported by Chile, Uruguay and Argentina, the GMO importing countries (the USA, Canada and Australia) adopted a policy due to the free trade of GMO products. Genetically modified products are under the supervision of three institutions which are Food and Drug Administration-FDA, Environmental Protection Agency-EPA and the USA Agricultural Ministry Animal and Plant Health Supervision Service in the USA. Unless there is a change in nutritional values and a warning is required about health, there is no obligation to label GMO products in the USA. There is no moderate approach about labelling GMO products in the USA, because this requires GMO products to be produced separately which results in an economic burden. The UN commission and the European Food Security Authority (EFSA) are two institutions authorized for the implementation of the UN legislation. EFSA carries out the risk assessment on food and forage security, and leaves the rest to the commission for (12).

According to the directive in the UN, there is an obligation for products having more than 0.9% genetically modified substances to be labelled indicating so. While products having glucose syrup which is obtained from genetically modified corn, refined oils, candies, chocolate products, beer and wine are within this classification, there is no obligation for products such as meat, milk and eggs acquired from animals eating genetically modified forage to be labelled (2).

In a research, majority of people living in the United Nations countries desire the genetically modified products to be labelled (21). In a study conducted in England, while there is an objection to genetic studies on human DNA and animals, there is a more moderate approach for genetic studies on plants and microorganism (2). Falk et al. (2002), came to a conclusion that most people in the USA were not aware of GM products, and the number of the aware increased in the last ten years, but most of them consumed such products as they trusted the scientific studies (22).

2.8 Legal Arrangements in our Country or the Approach to GMO Products in Turkey

In the study made by Demir and Pala (2007), it was found out that 41% and 29% of the attendants learnt about GMO from television and during the survey study respectively. While 46% stated that GMO products might cause health problems, 96% supported labelling on GMO products, 86% said they would not buy GMO products (23).

The first legal arrangement in our country was the 1998 dated Directive About Field Attempts of Transgenic Cultivated Plants. The initial international enterprise was Cartagena Biosecurity Protocol approved by the government in 2004 and its definition became legalized. On March 18, 2010, Biosecurity Law came into effect. Accordingly, GMO products' use other than the defined purposes, introduction to the market or use without permission, use in baby food, baby formulas, follow-on formulas and additional baby foods were prohibited (12).

The chronological development of the Turkish GMO legislation (summary):

October 26, 2009 – The Legislation About Genetically Modified Organisms for Food and Forage Purposes and the Importation, Processing, Exportation, Control and Supervision of Such Products was published.

November 2, 2009 – The Ministry of Agriculture and Rural Affairs announced 27 products to be analysed in terms of GMO and instructed for the building of the analysis laboratory in the Provincial Directorate of Agriculture of 4 cities in the same day.

November 9, 2009 – The number of products to be analysed in terms of GMO decreased from 27 to 9 due to the inadequacy of the laboratories capable of GMO analysing.

November 20, 2009 – Five articles in the legislation were amended and a temporary article was added. With the amendment, importers who had received a control document before November 26th were allowed to import GMO products (for both food and forage use) approved by the United Nations until March 1, 2010.

November 20, 2009 – State Council Joint Board of Tenth and Thirteenth Departments decided to halt the execution about the 11th and 20th articles of the regulation on the grounds that "No arrangement can be made in regulation without legislation".

December 24, 2009 – The Chamber for Administrative Cases accepted the objection to the State Council Decision of the Ministry of Agriculture and Rural Affairs.

January 20, 2010 – Regulations on amendment proposing the postponement of the implementation of some articles in the regulation to March 1st, 2010 was promulgated in Official Gazette.

March 18, 2010 – Biosecurity Law was accepted in the Grand National Assembly of Turkey.

March 26, 2010 – Biosecurity Law was promulgated in Official Gazette.

April 28, 2010 – The first GMO regulation dated October 26, 2009 was amended for the third time.

May 11, 2010 – Formed in accordance with the October 26, 2009 dated regulation, Science Commission announced its initial decision that it determined the threshold value of GMO products as 0.9% as in the UN.

August 13, 2010 – Regulation about GMO Products was promulgated in Official Gazette.

August 13, 2010 – Regulation about the Procedures and Principles of Biosecurity Council and Science Commissions was promulgated in Official Gazette..

September 26, 2010 – Biosecurity Law and Application Regulations came into effect..

September 27, 2010 – Ministry of Agriculture and Rural Affairs sent the Application Regulation about GMO and GMO Products to local units.

January 21, 2011 – With the decision of Biosecurity Council promulgated in Official Gazette, 3 sorts of GMO soya were approved to be imported only for forage purposes.

December 24, 2011 - With the decision of Biosecurity Council promulgated in Official Gazette, 13 sorts of GMO corns were approved to be imported only for forage purposes.

May 29, 2014 – In addition to the August 13, 2010 dated regulation, any technically unpreventable, unrestrainable or accidental GMO contamination to a genetically modified or not genetically modified product during production, processing, preparing,

packaging, packing, transportation or protection including primary production stage or due to environmental factors is evaluated as GMO contaminant in case 0.9% and below GMO is determined in the product as a consequence of sampling, analysis and assessment methods through taking Ministry's national and international arrangements into consideration. Any GMO contaminated product can be used suitably for its purpose in case genes determined as contaminant in it are approved by Biosecurity (24).

2.9 Potential Benefits of GMO's

Those supporting genetically modified organisms think that the genetic engineering technology has thrived in the recent years and food and medicine requirement of the gradually increasing population of the world might be pretty much fulfilled (15). Furthermore, they argue that this technology might have potential benefits such as the acquisition of not only herbal products which are resistant against rapidly growing diseases, weather and insects and herbicides, but also more delicious, safe, productive, nutritive, durable and healthy animal and herbal products and organisms contributing to industrial and pharmacological production (16).

It is early to mention the potential benefits of the GMO technology already. The potential benefits of genetically modified organism according to GMO supporters are argued below:

2.9.1 Increasing Food Quality and Benefits on Health

Studies on providing GMO food consumers with more nutrition through having the same amounts of food continue with the nutritional enrichment of GMO products. Potato types having high starch rates, wheat types with increased gluten and bread quality, grain types with increased amino acid rate are produced. It enables fish release more growth hormone with transgenic methods in order to increase the meat productivity. Essential amino acid contents (such as methionine and lysine contents of protein) of food can be enhanced through increasing the protein quality thanks to the gene transferring technology (16-17) Tomatoes to be used in food processing in order to

make ketchup, tomato sauce etc… can be gained dense contents through increasing the carbohydrate contents of GMO's. Potatoes sucking less oil, requiring less cooking time and costing less are produced thanks to the Russert Burbank potatoes with increased starch content developed by Monsanto Company (17).

GMO production is carried out also in order to enhance the benefits of products on health. With gene transferring technology, the amount of anti-oxidant vitamins (carotenoids, flavonoids, vitamin A, C and E) and minerals in products which are harmful compounds resulting in the development of some cancers, hearth diseases and blindness (in case of vitamin A), slowing down or preventing the biological oxidation can be increased. Increasing the amount of the anti-oxidants in food products might decrease the rate of certain cancer and other chronic diseases present in the society. An important anti-oxidant, lycopene is used in genetically modified tomato, tomato products and pepper in great amounts (17).

According to the researches, 14 million children are facing untreatable eye diseases due to vitamin A deficiency and 30% of the world population has iron deficiency. This might result in issues such as a decrease in learning abilities and an increase in infections. Scientists produced a type of rice increasing the amount of vitamin A, and another type of rice multiplying the iron rate twice (17).

Oils high in saturated fat rate are responsible for cholesterol production in the body. The genes of plants can be modified in order to increase the level of unsaturated fat acid in commonly used vegetable oils such as canola, soya, sunflower and peanut. Foods with increased nutritional values help decrease poor nutrition and meet the food needs of developing countries (16).

Additionally, potato types with high rate of starch, wheat types with enhanced gluten and bread quality, grain types with enhanced rate of amino acid are produced. It enables fish release more growth hormone with transgenic methods in order to increase the meat productivity (17).

2.9.2 Increasing the Shelf Life and Organoleptic Quality of Fruits and Vegetables

Produced by Calgene Company, Flavr Savr tomatoes were the initially approved genetically modified products by the USA Food and Drug Administration (US FDA). These tomatoes are plants having a long shelf life by delaying ripening, softening and decaying processes. Ripening and softening depend heavily on ethylene production by fruit cells. Ripening of fruits and vegetables can be delayed through controlling the genes having a role in ethylene production or delaying the pectin destruction by supressing the polygalacturonase enzyme. Therefore, high quality organoleptic features such as smell, flavour, softness/ hardness, and longer shelf lives can be acquired. Moreover, the ripening process of products such as raspberry, strawberry, pineapple and peach can be slowed down as well. Increasing the shelf life of products not only eases the transportation, storage and processing phases for the producer and seller, but also allows the consumer to use the product for a long time without spoiling (12-17).

2.9.3 Increasing the Productivity of Vegetable and Animal Products

Today, there are countries in which deaths occur due to poor nutrition and famine. Concerns arise on fulfilling the food needs with the rapidly growing population. Not only the cultivated areas cannot be expanded, but also the fresh water resources to be used in agriculture are rapidly diminishing. It is required to increase the productivity per unit area in order to provide the sufficient amount of food for the increasing population. Therefore, genetically modified products are thought to be a solution for famine (17).

Genetically modified plants can be used in order to increase the productivity and minimize product loss through gaining resistance against various environmental factors such as insects, weeds, herbicides, viruses, saltiness, pH, heat, frost, dryness and weather. Production can be increased through increasing productivity. Significant annual cultivated cereal products can turn into perennial cultivated ones through gene change. Therefore, solid will be cultivated less (double plowing etc…), erosion might be decreased and a year-long productivity might be acquired. Additionally, that

genetically modified plants have resistance against drought might decrease water use so that these plants can be cultivated in some tropical and dry areas where water resources are inadequate. Increasing the resistance of the products against other environmental factors such as fringe pH, salt, insects, heat etc… helps the inappropriate cultivation fields to be reused for production, therefore pressures on not compensable natural resources lie rain forests lessen. Resistance against environmental factors might be the result of a complex interaction of many genes, that's why it might take some time to gain such features to plants (16).

Cloning in animals led to the production of live stocks in order to fulfil the protein products and meet needs. In 1993, the milk production from milk-giving animals which were given rSBH (recombinant cattle growth hormone) and approved by the USA Food and Drug Administration (USA FDA) increased. Therefore, it is thought that ample production can be acquired in order to export meat and milk products cheap to countries whose resources are not adequate. Genetically modified animals can be used for purposes such as the production of lactose-free milk, low-fat milk, low-fat meat, special protein meat, special quality meat and milk (16).

Animals having resistance against diseases through gene modifications might not only reduce the use of antibodies and other medicines, but also reduce the pathogens in animal proteins and increase the productivity (2). Increasing the forage digestibility and forage use in animals can be optimized. For instance, Golovan et. al made by (2001) new enzymes were developed in order to increase the benefits of forage in animal intestines as an alternative to the enzyme contribution to forages through using biotechnological methods (2).

2.9.4 Edible Vaccine and Medicine Production

Many people become permanently disabled or lose their lives due to preventable health issues. Vaccination is the most effective method in preventing most of these diseases. Many people cannot be vaccinated due to countless reasons such as the high prices of vaccines, application methods, and requirement of trained personnel for application, difficulty of carrying and storing vaccines and social-cultural structure of people. It is worked on to use plants as vaccine by acquiring the particular ones synthesizing some of the proteins of pathogenic microorganisms through the genes to be transferred to vegetables we consume. Genetically modified fruits and vegetables are used for Hepatitis B, diarrhoea, cholera, measles and many other diseases. The major advantage of this method is that the vaccine can be taken orally. With the particular purpose, studies continue on banana, tobacco and lettuce. In a study made with genes transferred to tomato and potato, it was aimed to lower vaccine costs and convey these products to areas like Africa with ease. Additionally, there have been progresses in health area such as banana containing diarrhoea vaccine, potato containing protein, corn containing rabies vaccine and corn types producing monoclonal antibodies (17)

It was aimed to produce insulin plants in order for diabetes patients receive insulin orally instead of injection (17). Some human genes were added to plant chromosome in order to produce large amounts of experimental biomedicines. While tobacco and potato are changed in order to produce serum albumin, rapeseed oil and Arabidopsis are changed in order to produce neurotransmitter, leuenkephalin and monoclonal antibodies (16).

2.9.5 Treatment of Human Diseases

The decrease in the requirement of herbicides and pesticides leads to food safety, human health, and a decrease in allergy and sensitivities. Containing ovokinin having an antihypertensive effect, soya is used as low-lactose milk treatment for individuals having lactose intolerance. Produced to prevent cholera and diarrhoea, genetically modified potatoes have been effective. Solutions are sought and vaccines are prepared

for many diseases ranging from cholera to AIDS. Such developments have a positive effect on human health and save lives (16-17).

As cloned animals serve as models for many human diseases, scientists can study on untreatable human diseases effectively such as cystic fibrosis. Genetically modified animals can be used in order to produce pharmacological proteins such as clotting factor used by homophile patients or insulin used by diabetes patients (16).

2.9.6 Environmental Benefits

In conventional agriculture, chemical fertilizers and drugs used to fight plant diseases and pests create serious negative impacts on environment. It is said that biotechnological methods used in agricultural production have a great contribution on both product increase and environmental protection. It is assumed that the decrease in chemical use in transgenic plants acquired through gene modification instead of using chemicals for diseases and pests resulted also in a great decrease in environmental pollution. Biofuels produced from GMO products can be used for power purposes. It is assumed that these products decrease the environmental pollution as they emit less carbohydrate to the atmosphere (2).

Most agricultural plants can be genetically modified and gained resistance against viruses, insects, weeds, herbicides, diseases and various environmental factors. For instance, *Bt* plants resistant against insects were acquired through transferring a gene having ta insecticidal potential (insect killing potential) from *Bacillus thuringiensis (Bt)* to most vegetable products such as potato, soya and corn. Being toxic for insects such as corn borer and potato beetle, *Bt* protein is not toxic for humans and is split with stomach acid (16).

2.10 Potential Risks of GMO's

The potential negative impacts of GMO products on human health have been discussed for a long time. The potential negative impacts and risks of GMO products are poisoning possibility, allergic reaction possibility, antibody resistance, and side effects of gene addition, incidence possibility of specific toxic contents, nutritional effects and stability of the cloned gene.

2.10.1 Changes in Food Quality and Food Safety

Transgenes transferred to food products might unpredictably change the nutritional features of foods through increasing and decreasing the nutrition of some foods. There is no adequate information about the impact of the changes in the contents of vegetable and animal foods on nutritional interactions, food-gene interaction, and nutritional existence in living things, nutritional power and nutritional metabolism (16).

There is no definite information about the long-term impacts of genetically modified products on health. Therefore, it is thought that consumers should have the right of choice and gain information through assessing the risks of GMO products (16).

When GMO products are assessed in terms of food safety, the horizontal gene transfer of recombinant DNA to humans and its consequences about human health are important issues. Whether the genes transferred to food products nest in human intestine microflora or the genome of human or animal and the consequences are important issues as well. DNA of food products enters the body and is split and digested as it is subjected to various parameters such as heat, pH, pressure, reactive chemicals (radicals) and enzymatic activities (exonuclease and endonuclease such as DNAazI and DNAazII) and then discharged from the body. DNA is usually split rapidly in mammal intestine, which occurs not always instantly and completely, sometimes DNA might stay stable. If DNA reaches the gastrointestinal system where DNA splitting activity is minimum and microflora amount is high such as the final end of the small intestine, caecum and colon before it is split, it has the possibility to receive the bare DNA in the cell. Despite

the fact that the bacteria in microflora also have a mechanism preventing the foreign DNA received in the cell from joining its genomes, it might be possible for bacterial rooted genes to be received in the structure. Used as marker genes during GMO production, antibody resistance genes are usually bacterial rooted which is the most controversial possibility. These antibody resistance genes might be transferred to human intestine microflora or pathogenic microorganisms due to GMO consumption which is already a common phenomenon in the nature might cause an increase in the resistance level of microorganisms against antibody. This might pose a risk to human and animal health through removing therapeutic values of antibodies for the treatment of pathogenic microorganisms (16).

Transfer of the DNA in the consumed GMO products to mammals' cells and the contamination of the horizontal gene transfer to humans is another important issue taken into consideration in terms of food safety. It is assumed that the somatic epythel cells in the intestinal mucosa subjected to DNA particles (for instance: plant, animal, microorganism, virus) is to be discharged from body due to the their constant renewal and it should not pose an important risk. However, studies indicate that chloroplast DNA of corn enters various tissues of cattle and chickens eating corn (16).

According to the World Health Organization, every genetically modified food and its safety should be assessed separately. No general assessment is possible. The World Health Organization states that the GMO products in the market passed the risk assessments and they pose no risk for human health (12).

2.10.2 Allergic Reactions and Toxic Effects

Features of the new gene placed in the organism through gene transfer technology might cause allergic reactions or increase the existing ones. For instance, it has been proved that the gene modification acquired through transferring a gene found in Brazil nut to soya causes allergic reactions in consumers who are allergic to Brazil nut. One of the claims is that genes do not act independently and a gene or genes transferred to an organism might always have unexpected and undesired side effects. Expression and

genetic function of a transgene transferred to genetically modified organisms might lead to unforeseeable differences and therefore might cause unexpected reactions and the development of potential toxins. Allergic reactions occurred as a consequence of genetically modified soya and corn consumption. For instance, a nut gene transferred to soya caused allergic reactions to those allergic to nut. The USA allows the production of 'StarLink' corn only for purposes other than human consumption. Because, a slow digestion of a type of Bt protein found in 'StarLink' was observed to cause allergic reactions in some people (17).

Furthermore, it is stated that transgenes might have an impact upon the arrangement area of a natural toxin on genome and lead to toxin production (16). In 1967, Lenapo potato in the USA was developed and introduced to the market to be used in chips production. Two years later, it was withdrawn from the market by the American Ministry of Agriculture due to the fact that it created solanine toxin (17).

37 people died and 1000 people got sick due to Eosinophile-Miyalgia Syndrome (EMS) between the years 1988-1989. Researchers claimed that foods containing L-Tryptophan were the result of the incidence. Almost 70% of GMO products are produced in order to have resistance against dryness and insects. Claiming these products contain pesticide, Anti-GMO groups argue the consumers of this corn might suffer toxic effects (17)

The World Health Organisation (WHO) does not recommend the application of gene transfer with a potential allergenic substance. While traditional foods are not tested in terms of allergenicity, test protocols for GMO products are assessed by the United Nations Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) (12).

2.10.3 Impact of Gene Patenting and Terminator Technology

Biotechnology companies might aspire to keep important genes under control through patenting. However, gene patenting is seen as an obstacle in terms of the studies conducted by researchers working in public sector. Critics object gene patenting due to the fact that it is not ethical and prevents researchers from making researches about these genes (16).

Biotechnology companies might develop a terminator technology in order to take the seeds of genetically modified plants under control. The terminator technology is an unproductive plant production technology developed in order to prevent the reproduction of the GMO agricultural products belonging to biotechnology companies through collecting the seeds of particular products. Having various applications, terminator technology has three main steps: A terminator gene is added to a genetically modified product, seed companies start the termination process through adding an inductive before selling the seed, and farmers cultivate the plant seed and yield products. However, seeds or products acquired are unproductive (16).

Terminator technology will cause farmers to buy expensive seeds from international companies annually and create a dependency on such companies. Furthermore, seed companies might not only patent genes or take seeds under control, but also produce specific drugs for specific GMO's and oblige farmers to buy these (16).

2.10.4 Concerns about Labelling Genetically Modified Foods

The United Nations regulations states that a product must be labelled GMO in case it differs from its kinds. In the USA, the USA Food and Drug Association arranges the safety and health of food resources (excluding meat and poultry) and it is against the labelling of GMO products. While Environmental Protection Agency states that the protection of consumers against GMO's should be emphasized, American Medicine Association argues that such products must be labelled and it must be indicated that the consumer safety is not open yet for genetically modified foods (16).

Critics argues that labelling GMO products enables consumers willing to stay away from such products due to cultural and religious reasons to do so, product qualities such as long shelf life and resistance against insects which are appealing to the consumer to be emphasized and let the identity of the product be known. Those objecting to labelling argue that it might create not only a bad image, irritation and misunderstanding that GMO products have different effects since such organisms might also be contained in food mixtures, but also high prices due to labelling costs and that labelling must keep on through every step of the food chain (16).

2.10.5 Environmental Concerns

Direct and indirect negative impacts on environment and especially the risks to be caused due to gene escape among species in the natural ecosystem are controversial. As gene transfer is easier between plants than that of animals, gene escape is the ultimate risk. Environmentalists concern there might be environmental risks as a consequence of the cultivation of genetically modified organisms in a large area. Furthermore, genetically modified plants might led to the extinction of natural species through competition. Additionally, new genetic features might escape to natural species, weeds and insects during cross-pollination. Soil microorganisms might receive various resistance genes through collapsed plant DNA's during the decaying of genetically modified plants (16).

While genetically modified plants are expected to reduce herbicide, pesticide and artificial fertilizer use in near future, it might also lead to the emergence of resistant weeds and insects in the long run, which is assumed to cause an increase in environmental pollution through an increase in the dependency on agricultural chemicals such as herbicides, pesticides and fertilizers (16).

2.10.6 Biological and Genetic Diversity Threats

One serious environmental threat is that genetically modified plants might lead to a natural evolutionary deviation of weeds creating the genetic resources, through a loss in genetic diversity, decay in the distribution and balance of species in the ecosystem when released. There, the gene resources of countries having rich genetic resource are under threat. GMO critics argue that with the agricultural applications supporting the embracing a few product types internationally, the genetic diversity of products will be in danger and the trading of GMO products might pose a new threat to genetic diversity which is already in danger. That natural species which cannot fight genetically modified plant species rapidly disappear poses a threat to biological diversity as well. That almost 80% of the terrestrial biodiversity in the world belongs to the countries which are capable of providing the raw materials required for gene transfer technology is another dimension of the threat (16).

A study indicated that GMO's decrease the gene escape risk, harming off-target living things and biodiversity considerably (25). New genetic features might escape to natural species, weeds and insects during cross-pollination (16). The risk of losing balance between populations increase owing to the fact that organism lose their genetic genuineness in time and the use of agricultural drugs due to the emergence of weeds and insects in the long run and the decay in species sociology (26).

2.10.7 Concerns of Some Groups and Religious, Cultural and Ethical Concerns

Animal rights groups strongly object to any type of genetic engineering and cloning in animals and the use of animals in researches (16). On the other hand, organic farmers are afraid that it might be harder for people to reach organic foods since there is not labelling application (15).

Some people object not only to the violation of consumer's right of choice and that GMO's cannot be distinguished from their natural kinds, but also to genetically modified foods due to personal, ethical, cultural and aesthetic reasons. For instance,

some religious groups such as the Muslims, the Hindus and the Jews wish to avoid fruits and vegetables containing genes of insects, animals and humans. Having special religious food rules, the Muslims and the Jews demand to be sure about whether genetically modified foods are against their religious limitations. For instance, both the Muslims and the Jews object to grains containing pig genes. Similarly, some vegetarians might object to fruits and vegetables containing animal genes (16).

2.10.8 Unknown Fears

Consumers also have literally "unknown fears" that lethal microorganisms or super plants might break free during field trials and accidents in biotechnology laboratories might lead to the release of toxic agents, poisons or biological toxins which pose a threat to human and animal population (16).

2.11 GMO Products

2.11.1 Corn

Its most important purpose of use is that it removes the requirement of insecticide use. Therefore, it is aimed to decrease environmental pollution and ease the workload of farmers.

Genetically modified corn is called "*Bt*-corn" due to the fact that its gene synthesising the protein killing insects comes from the bacteria *Basillus thuringiensis*. *Bt* toxin is typically used as spraying in ecological agriculture and disappears in a short time in the nature once applied. However, *Bt* toxin does not collapse and other potential effects are not known too. In a study conducted on rats, GMO corn production was determined to have an effect especially on kidney and liver (27).

Genetically modified corn is used basically in oil, flour, starch, glucose syrup and saccharose; therefore it is added to products such as biscuit, cracker, coated nuts, pudding, vegetable oil, formula, candies, chocolate, waffle and instant soup.

Additionally, it is consumed through animals eating genetically modified corns such as chickens, and similar animal foods (27).

2.11.2 Cotton

As the corn pulp remaining after the cotton seed is used in vegetable oil production has high amounts of protein, it is used as animal forage (added to animal foods). It is an important material in textiles and oil industries. Its oil is richer than that of vitamin E. Short fibres which are inappropriate for textiles are cellulose-and-methylcellulose-rich and used as food additives. It functions as an emulsive and stabilizing filling material. These short fibres are also used in paper production (27).

2.11.3 Soya

The fundamental purpose of using the GMO technique in soya is to use it as biodiesel fuel. Use areas of soya are coffee creamer, cooking oil, filling oil, margarine, mayonnaise, medicines, forage, pharmaceutical insecticides, rubber, oil, anticorrosion materials, antistatic materials, paste compounds, construction materials, maintenance oils, ink, printing substances and analytic chemicals (27).

2.11.4 Papaya

Papaya has become resistant to ringspot virus through the addition of a new gene to the plant genome (27).

2.11.5 Tomato

After tomato is picked, an antisense copy of the gene synthesizing the polygalacturonase (Pg) enzyme in order to prevent softening, therefore enzyme is suppressed. (It is not used as it has been successful.) (27).

2.11.6 Canola

It has gained resistance to herbicides through a new gene transfer to the plant genome (27).

2.11.7 Sugar Cane

It has gained resistance to some pesticides and sucrose-rich through a new gene transfer to the plant genome (27).

2.11.8 Rice

Three different genes were implanted from two different organisms. Two genes were acquired from daffodil genome; one gene was acquired from bacteria gene and "golden rice" was produced. It is Vitamin-A-rich (27).

2.12 Consumer Perspective on GMO Products

It is determined that the perspectives on the production and introduction to the market of GMO products vary among the European Union countries. According to the "Society and Science Association Eurbarometer Survey" attended by 16.078 people from 15 countries in Europe, women are less likely to buy GMO products compared to men. Furthermore, it was found out that whether GMO products had special labels on did not matter for those thinking of buying such products (42). In a research conducted in Germany, it was determined that while 56% of women preferred to consume ecological products, only 39% of men preferred to do so. Additionally, 48% of the attendants stated that they preferred to consume not modified fruits and vegetables (43). In the European Public Concerted Action Group Survey conducted in 1999, it was found out that 74%, 60% and 53% of the attendants believed in the necessity of GMO product labelling, demanded that the public opinion should also be taken into consideration, and believed that the existing arrangements are inadequate to protect individuals respectively (43). In another study conducted in England in 1998, 77% and

61% of the attendants believed that GMO fruits and vegetables must be prohibited, and did not prefer to consume GMO products respectively (43). According to a survey conducted about the members of the National Women Institutes Federation, 98% and 93% of the attendants demanded that GMO products should be discussed more and GMO products should be labelled respectively (43). In a study involving Spain, Italy and Greece, it was determined that the consumers in those countries had different perspectives on GMO products. Therefore, it was emphasized that communication strategies about GMO product should be different in every culture (44). In the study conducted by Roe and Teisl, it was determined that labelling GMO products was of importance for consumers (20).

Despite the fact that GMO products were cultivated in Southern Africa, it was determined that the consumers had little knowledge about GMO and most importantly, there was no legal arrangement for consumers' right of choice (43).

Similarly, while the consumers in Turkey did not have much knowledge about GMO products until today, some organizations, farmers and voluntary agencies bring up the issue to the agenda through universities and various instruments of the local and national press, thanks to which millions of people including producers and consumers have the opportunity to get to know the GMO concept with its both environmental, ecological and health risks and potential benefits (45). Some studies were conducted also in our country in order to determine the perspective and knowledge of consumers about genetically modified products. As a consequence of a survey attended by 408 people between ages 18-60 in various cities, especially those in Marmara Region, it was determined that 60% of the attendants believed that GMO products were not safe and healthy. With the data acquired, it was determined that the society needs to be informed more of genetically modified products and a proper risk communication was required (46). In a survey study conducted in Turkey in 2004, 91% of the attendants stated that labelling GMO products was beneficial (43). In another study attended by 913 people (439 women and 474 men), while 41% pointed out that they heard the term GMO from the television, 28,92% stated that they hear it from the particular survey for the very first time. 45,73%, 95,62% and 85,76% of the attendants stated that GMO products might lead to health problems, GMO labelling must be applied, and they would never

buy a product labelled as GMO respectively. While higher the age, higher the rate of label reading, the approach to GMO foods has become more negative. Women are more sceptic about GMO products than men are (22).

2.13 Genetically Modified Organism and their Use in Feeding Animals

Acquired in order to enable farm animals gain the optimum benefit from forage, transgenic products provided progresses such as an increase in forage performance through a decrease in its non-nutritional contents such as protease inhibitors and lectin etc… and an improvement in protein amount and quality and especially improvement in the starch and oil composition in terms of lysine and methionine, and vitamin and mineral (28). While improvement works proceed on many products, so do the discussions of scientists about it. While some scientists assume such products might have negative impacts (allergic or toxic impacts etc…) on human and animal health in the upcoming years, some believe that unpreventable environmental issues might emerge, the rest argue no problem might arise if a controlled production, supervision and required tests are carried out (29).

In a study conducted by Hammond et al. (1996), a statistical increase in milk productivity and a numeric increase in milk oil were found out as a consequence of an analysis of the impact of soya bean lines on milk production, milk content and rumen parameters in milking cows (29).

In a study conducted by Kan et. al. (2001), it was found out that compared to traditional soybean meal, transgenic soybean meal had a less negative impact on the performance and carcase productivity of broilers, and nutrients of brisket (29).

In a study conducted by Brake and Vlachos in 1998, no difference was observed in terms of weight gain, forage consumption and protein digestibility during a 35 day nutrition study on insecticide resistant corn and broilers (29).

In a study, two species were produced from genetically modified and not genetically modified soya milk in laboratory and these transgenes were determined in different soya milk groups with caution. A cholesterol-rich diet is related to an increased risk of dyslipidaemia development. In the mentioned study, genetically modified and not genetically modified fermented probiotic soya milk was compared in order to assess the reduction in oxidative stress and atherosclerotic plaque. Oxidative stress is a major risk factor in atherosclerosis pathogen. It reduced especially in hamsters fed with not ferment-genetically modified soya milk. Additionally, atherosclerotic plaque development reduced as a consequence of being fed with genetically modified and not genetically modified soya milk. Every genetically modified and not genetically modified soya milk reduced hypercholesterolemia symptoms and atherosclerosis. (30).

Genetically modified plant forage emerged for the very first time in 1996. In 2013, more than 95%, 93% and 90% sugar beet, soya, and cotton and corn were produced in the Usa respectively (USDA National Agricultural Statistics Service, 2013). Consumers make up most of both the global stockbreeding and genetically modified forage plants populations. Independent studies indicated that genetically modified plants equalled new generation plant compositions and no major difference was observed in terms of forage digestibility, performance or health in animals eating genetically modified forage (31). Similarly, no difference was determined in the nutritional profiles of animal products after forage consumption (32). Despite such findings, some states paid attention to the law making it mandatory to label products such as meat, milk and eggs acquired from animals eating genetically modified forage (CAST, 2014). Furthermore, some food companies aimed to make campaigns in order to promote the products acquired from animals eating genetically modified food. Not genetically modified nutrition is less considering that genetically modified nutrition has become common in animal farming. (32).

The USA approved 165 genetically modified products including 19 plant species (clover, canola, corn, cotton, soya bean, sugar beet) in animal feeding (33). Before approval, every genetically modified product is subjected to an extensive risk assessment. Risk analysis of genetically modified organisms is conducted with

international rules developed by Codex Alimentarius Commission. Challenges of forage test are safe and related to every product containing thousands of known bio-active substances acquired from genetically modified plants (34). A meaningful difference can be defined among treatment groups eating all types of foods without creating major differences in compounds. Every toxicologist intends to contribute to safety assessment and determination of slightly negative effects in every genetically modified food (32).

Studies on animals eating genetically modified plants focus less on genetically modified risk assessment, more on nutritional features, and consequences of animal health and performance of animals eating genetically modified plants when fed with isogenic equivalents. Such studies are developed in order to provide definite recommendations in scientific studies (32).

Several studies were conducted on animals used in food production such as sheep, goat, pig, chicken, quail, cattle, buffalo, rabbit and fish eating various genetically modified plant species. Many authors came to the same conclusion despite contradictory data ten years ago. Furthermore, genetically modified forage prevalence was found out to have increased in the last ten years (32).

Animal models such as pigs, cows, quails and fish were examined in long-term nutritional trials (monitoring more than 90 days and 2 years) and studies made in multi-level public research laboratories, among which two major extensive and multi-level studies took place to examine the long-run impacts of genetically modified corn species on food producing animals, especially the milking cow in the German study and the pig in the Irish one (32).

In recent years, anti-nutritional factors which serve as protein resources in fish have decreased and soya use in forage has gradually increased (Krogdhal et al., 2000, 2003). Having compared genetically modified soya and oil acquired from traditional soya in rainbow trout, Chainark et al. (2006) observed that the forage prepared with the oil acquired from genetically modified soya did not lead to any difference in nutritional composition and fish development as a consequence of a twelve week trial. Sanden et

al. (2004) examined the residues of genetically modified and traditional DNAs of soya – a gluten of soya, canola and corn having vegetable protein resources as a good alternative for fish forage – in the digestive system of Atlantic salmon and found out that the genetically modified soya did not leave any different DNA residue in the digestive system of genetically modified soya as a consequence of a six week nutritional trial (29).

Hemre et al. (2005) used genetically modified and traditional soya in Atlantic salmon forage in order to examine the potential health problems as a consequence of genetically modified food use in forage. As a result of a three month trial in which three different applications took place, no major difference was observed in forage assessment, liver and muscle composition and fat acid profile in muscles. On the other hand, only the group in which genetically modified soya was uses had differences as a consequence of kidney, liver, brain and spleen examinations. It was observed that it occurred due to the fact that the erythrocyte cell volume was a little smaller than average. Genetically modified organisms were reported as safe to be used in terms of fish development and health(29).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

In our study, we studied on 20 packaged and 20 unpackaged pure milk samples. No milk or fruit milk containing additives was used to prevent wrong conclusions due to the fact that whether GMO is the result of additives cannot be known. Milk samples were collected from various districts of Istanbul (Ataşehir, Kadıköy, Kartal, Maltepe, Beşiktaş, Şişli, Şile). Unpackaged milk samples were received in sterile sample cups; while packaged ones were received in their own packages. Samples were collected in October and November in 2014. Samples acquired were conveyed to laboratory in cold chain and kept in 2-8 °C until the study day has come.

3.2 Methods

3.2.1 Nucleic Acid Isolation

In our study, MagNA Pure Compact Nucleic Acid Isolation Kit I and MagNA Pure Compact (Roche, Germany) was used in order to do DNA isolations from the samples. 1 litre of milk sample was used as GMO might be rather diluted in milk. And to do that, initially 1 litre milk was received and centrifuged with 50 µl falcon tubes and supernatant was discharged. 500 µl was received from the final sediment and it was isolated as 100 µl elution volume in the automatic DNA device (49).

3.2.2. PCR

All tests were performed with Roche LightCycler 480 II.

3.2.2.1. Nucleic Acid Multiplication Methods

Nucleic acid multiplication and determination methods are examined typically in two groups.

- Nucleic acid probe hybridization methods
- Nucleic acid amplification methods (NAA).

Nucleic acid probe hybridization methods are the oldest and simplest molecular methods. The target nucleic acid sequence in the example is hybridized with a marked probe having a complimentary and diagnosis is made. It is based on the self-matching of DNA.

It is used in order to determine the genomes of microorganisms which cannot be produced or hardly identified with the culture method, and make a diagnosis. It is rarely used due to the fact that there are many required pre-treatments and its area of use is limited.

Nucleic acid amplification methods have been frequently used in recent years. The major one is polymerase chain reaction (PCR), which is based on the self-matching of DNA. It is aimed to multiply the DNA particle using base sequences appropriate to the target region. It is used in many fields such as food and health ones. It is used especially in medical studies.

3.2.2.2. Polymerase Chain Reaction (PCR)

Molecular biology and medicine developed with the invention of PCR by K. Mullis et. Al. in 1985. PCR is an in vitro technique multiplying enzymatically the part which lies between two parts of the DNA chain. While only a small part of a specific gene could be acquired early on, today millions of copies can be obtained from a gene using PCR in a few hours (51). PCR is a sensitive and specific molecular technique used in studies conducted on nucleic acids (50).

PCR enables the selective amplification of the target DNA/RNA, which are:

- Template DNA carrying target directory
- 2 types of oligonucleotide primaries which can be matched to template DNA
- Four types of dNTP (dATP, dCTP, dGTP, dTTP)
- $MgCl_2$

PCR is a three stage method, which are denaturation, annealing and extension following one another.

Denaturation is the transformation of a two-stranded DNA into a one-stranded DNA in a couple of seconds with 94-96°C.

Annealing is for instance that a primary is annealed to the target region through keeping it at 30-60 °C.

Extension is the extension of primaries annealed to one-stranded DNA templates via polymerase enzyme in 5'→3' direction. It usually occurs at 65-72 °C.

These three stages constitute a cycle in PCR (Figure III.10.) and a PCR contains 30-45 on average. PCR occurs in Thermal Cycler devices enabling heat cycles, which are used to set the PCR sample at the programmed temperature and duration.

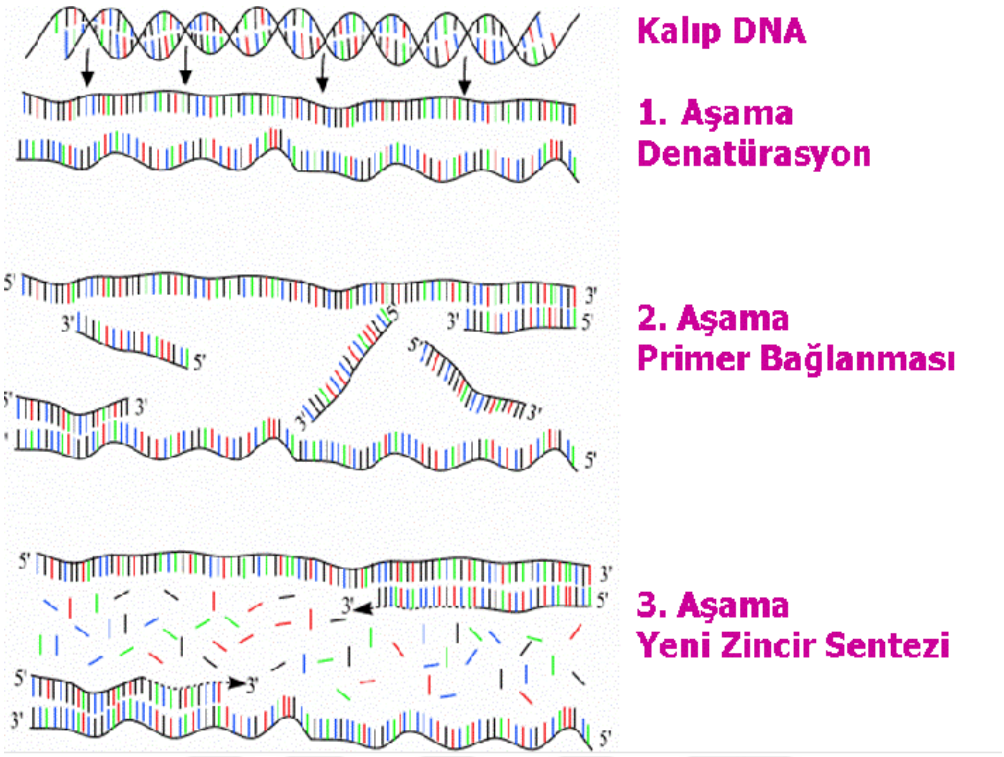

Figure 3.1 Schematic levels of a cycle in a polymerase chain reaction

3.2.2.3. Polymerase Chain Reaction Types

- Inverse PCR
- Homopolymered PCR
- In situ PCR
- Hot start PCR
- Multiplex PCR
- Quantitative PCR
- Nested, semi-nested PCR
- RT (Reverse transcriptase) PCR
- Touch down PCR
- Consensus PCR
- Real time PCR

The most suitable one should be selected among these PCR types. Real time PCR method, which has been frequently used in recent years, is used in our study.

3.2.2.4. Real Tıme PCR

The combination of thermo-cyclers with sensitive measurement devices in order to provide heat cycles in PCR reactions led to the development of a new method named PCR. Products are analysed during reaction in real time PCR. Therefore, there is no need for applications such as agarose gel electrophoresis and DNA bands under ultraviolet light. Flourasence dyes not specific to the sequence or probes specific to the sequence are used in the qualitative and quantitive analyses of real time PCR products.

Real time PCR is a system working according to the adequate fluorescence light coming from the product in each PCR cycle during reaction, monitoring each reaction stage and checking the product until the process is complete. Therefore, it is a system indicating the results simultaneously during working (Figure 3.2).

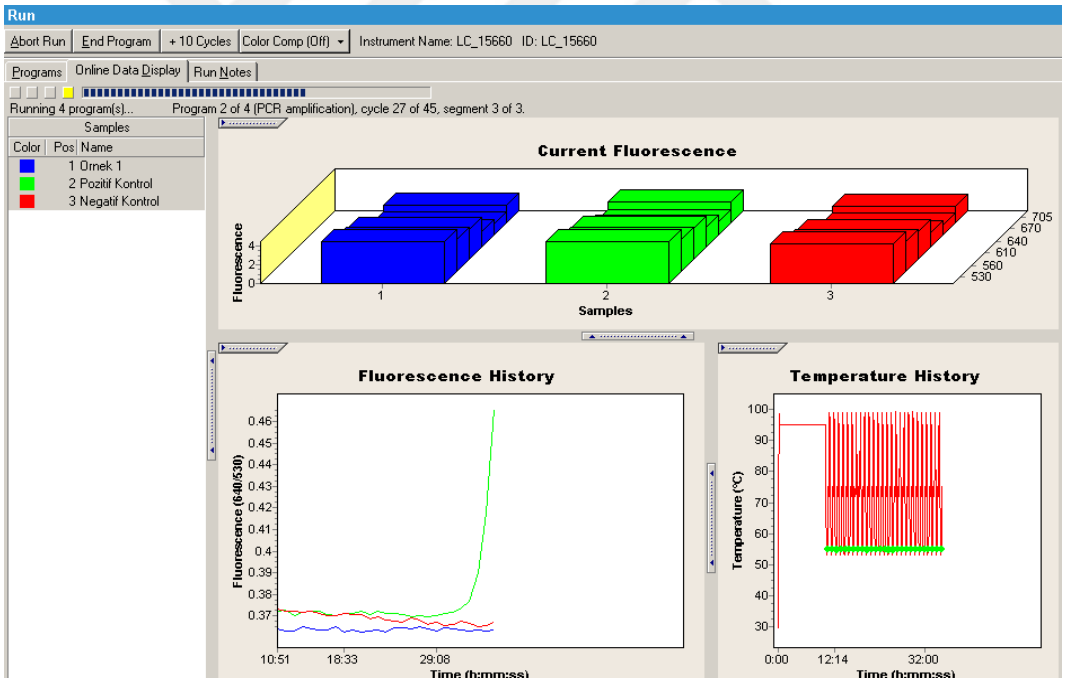

Figure 3.2 Real time PCR image shot during working

DNA and RNA samples can be analysed as qualitative and quantative in a short time thanks to PCR which can work with countless samples and rather low contamination risk. Specific DNA sequences ranging from 25 bp to 10.000 bp can be amplified with

this method. It is rather fast, sensitive and specific. Its sensitivity and specificity increases when probes specific to sequence such as Taqman probes are used.

Various probe systems and dyes are used in real time PC, which are:

Specific fluorescence marked dyes
FRET (Fluorescence Resonance Energy Transfer)
Taqman probe
Scorpion primaries
Hybridization probes
Unspecific fluorescence marked probes
Sybr Green
Ethidium Bromide (47)

3.2.2.5. GMO PCR

In our study, SureFood® GMO SCREEN 4plex 35S/NOS/FMV/IAC kit was used. PCR (Polymerase Chain Reaction) multiplies DNA target directory which is determined through hybridization probe marked with fluorescence in real time. This kit is a GMO real time PCR kit and uses FAM/TAMRA marked TaqMan probes.

This kit can be used for screening of genetically modified organisms (GMOs) in food, feed and seeds. For this purpose, PCR systems for detection of the 35S Cauliflower Mosaic Virus (CaMV) promoter DNA sequence, A. tumefaciens NOS terminator DNA sequence and for detection of the 34S FMV promoter DNA sequence are applied. Additionally the kit contains an internal inhibition control. The real-time PCR assay can be used with established real-time PCR instruments, equipped for detection of four fluorescence emissions at 522 nm, 553 nm, 610 nm and 670 nm (FAM, VIC, ROX and Cy5) at the same time (Rotor-Gene Q, Stratagene MxSeries, BioRad CFX96, Roche LightCycler® 480* etc.). This kit has a limit of detection of $\leq$5 DNA copies (48).

In our study, Roche LightCycler® 480 Instrument II was used. The LightCycler® 480 System is a high-performance, medium- to high-throughput PCR platform (96- or 384-well plates) that provides various methods for gene detection, gene expression analysis, genetic variation analysis, and array data validation. The system features the LightCycler® 480 Instrument, a versatile, plate-based real-time PCR device that supports mono- or multicolour applications, as well as multiplex protocols. The benchtop instrument is easily customizable to meet changing user requirements, and can be integrated into everyday use as a robotically controlled, automated high-throughput solution. The LightCycler® 480 Instrument is designed for general laboratory use and is not intended for use in diagnostic procedures.

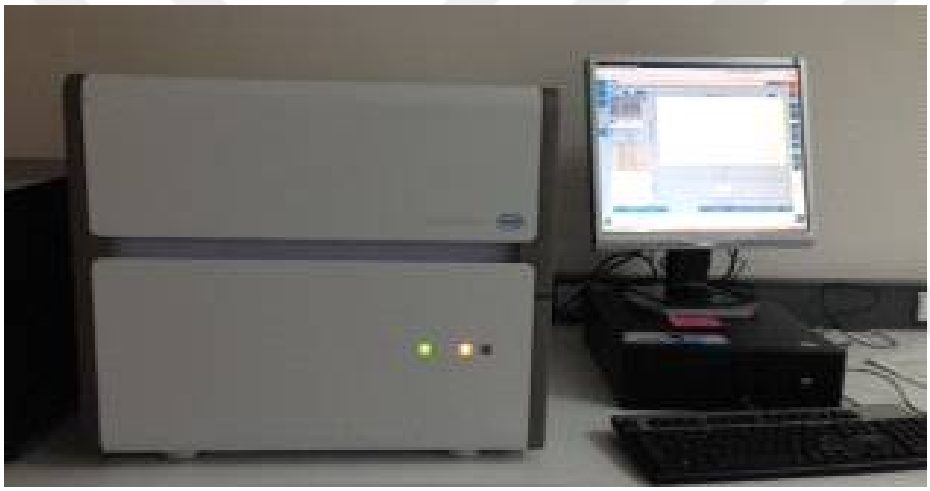

Figure 3.3 Roche The LightCycler® 480 System Machine

PCR Mix Preparation: Positive control was made in each study. During studies, PCR compounds were kept in an ice block, not at room temperature. PCR mixture was prepared according to kit recommendations (Table 3.1). Calculations were made as the number of samples, and distributed to PCR plate as 20 each µl. 5 µl DNA was placed in each well. Sterile distilled water was used for negative control. A final positive control was carried out and a filter film was put on the plaque in order to prevent contamination. Film remained untouched not to affect test performance.

Table 3.1 PCR Mix Preparation

Components for master mix	Amount per reaction
Reaction mix	19.9 µl
Taq polymerase	0.1 µl
Total volume	**20.0 µl**
DNA	**5** µl

PCR Conditions: Centrifuged, the plate was installed to the device and the device was opened in accordance (Table 3.2) with the recommendation of the kit (Figure 3.4).

Table 3.2 PCR Conditions

Analysis Mode	Segment	Cycle	Temperature (°C)	Hold Time (hh:mm:ss)	Ramp Rate (°C/s)	Acquisition Mode
Initial denaturation						
None	1	1	95	5 min.	Maximum	None
Amplification						
Quantification	Denaturation	45	95	15 sec.	Maximum	None
	Annealing/ Extension		60	30 sec.	Maximum	Single
Cooling Step						
None	1	1	40	30 sec.	Maximum	None

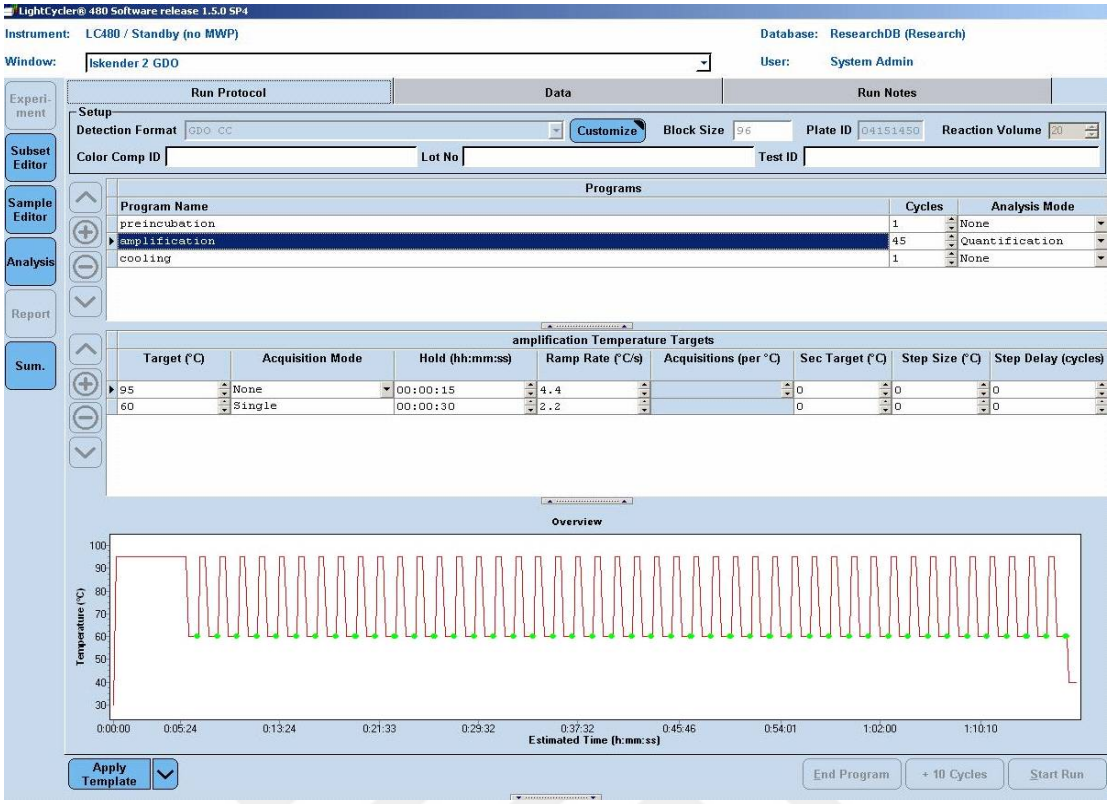

Figure 3.4 Roche The LightCycler® 480 System Screen

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Study Results

Quality Control results: It was checked whether the controls were working in recommended channels (table 4.1) for every region in order to evaluate the performance of the test (figure 4.1-4.3). No contamination was observed in the negative control in the test.

Table 4.1 Interpretation of Results

Region Name	Dye	Detection Channel
35S	FAM	465/510
NOS	Cy5	618/660
FMV	ROX	533/610
Amplification Control	VIC/HEX	533/580

Quality control results were found out to be negative for S gene area (figure 4.1)

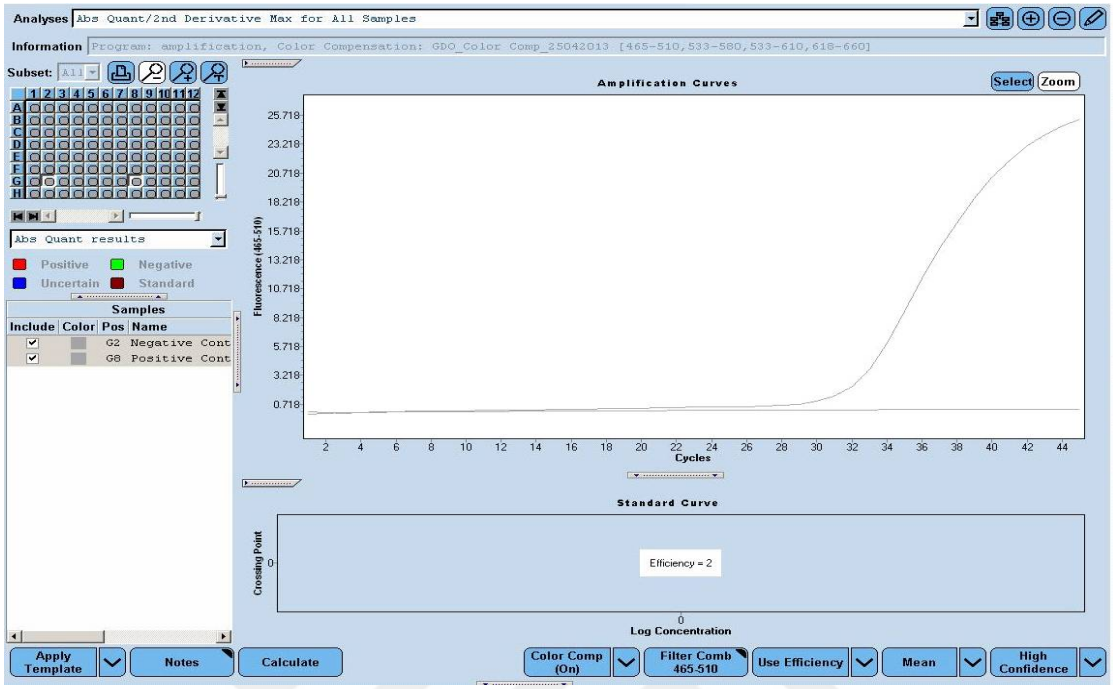

Figure 4.1 Quality control results for 35 S

Quality control results were found out to be negative for NOS gene area (figure 4.2)

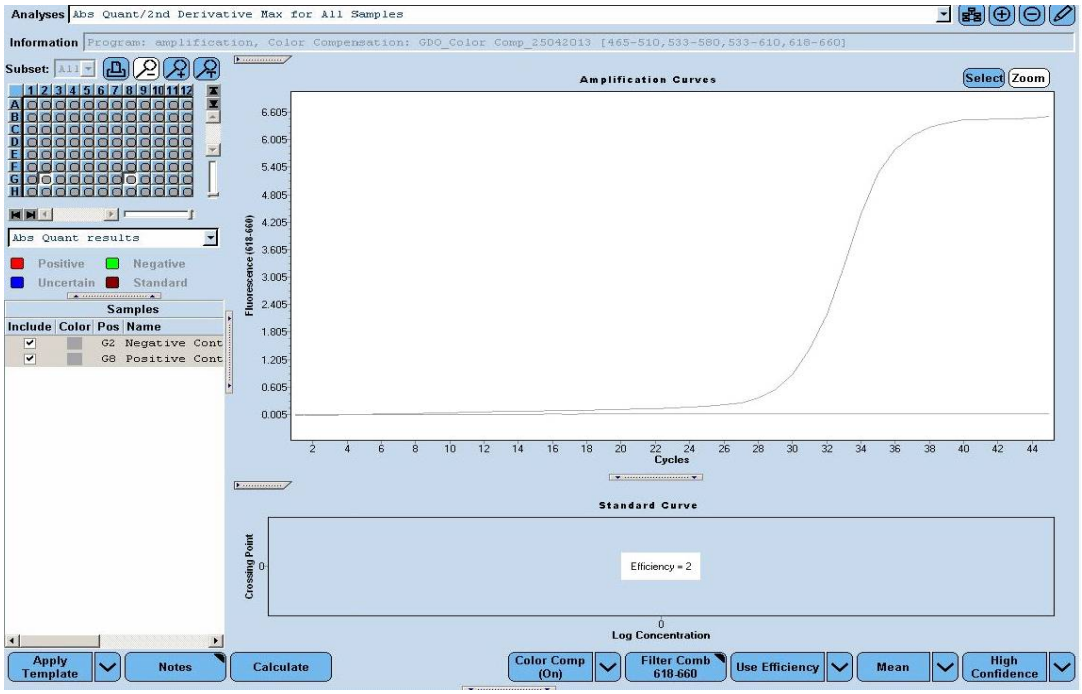

Figure 4.2 Quality control results for NOS

Quality control results were found out to be negative for FMV gene area (figure 4.3)

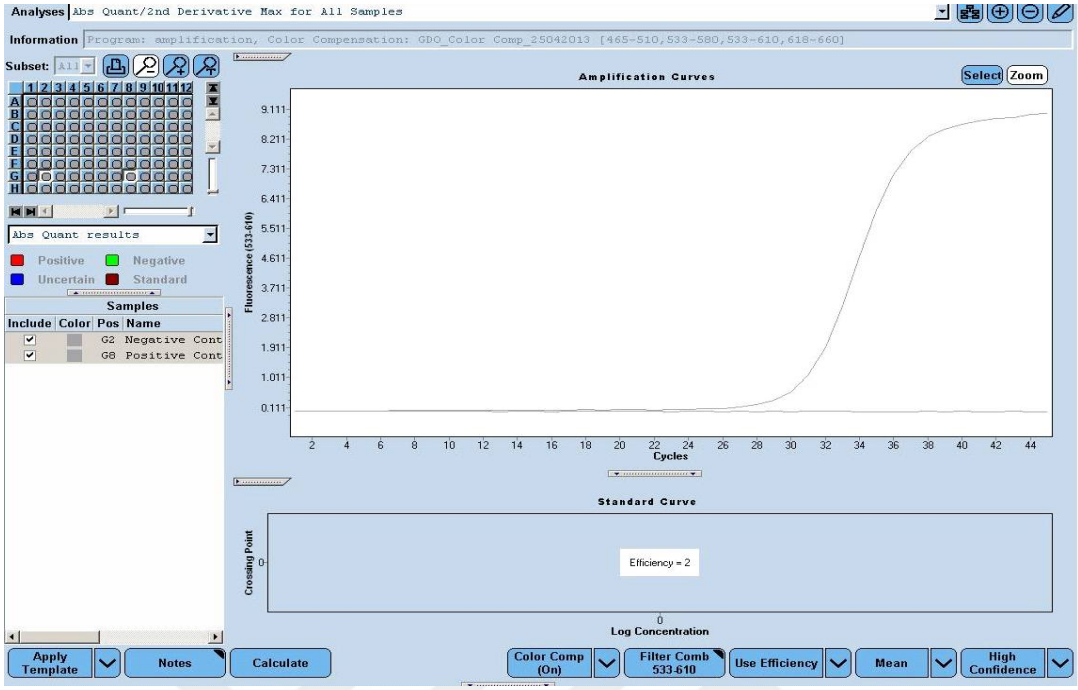

Figure 4.3 Quality control results for FMV

Internal Control Results: Internal Control (IC) PCR is of great importance, especially in terms of our particular study. Because it indicates that PCR is working and whether there is DNA/ RNA in the sample. Every test result was found out to be negative for every area in our study. IC results should be examined in order to understand whether they are truly negative or there is an inhibitor. IC control results were assessed for each sample in 533/580 channel (figure 4.4).

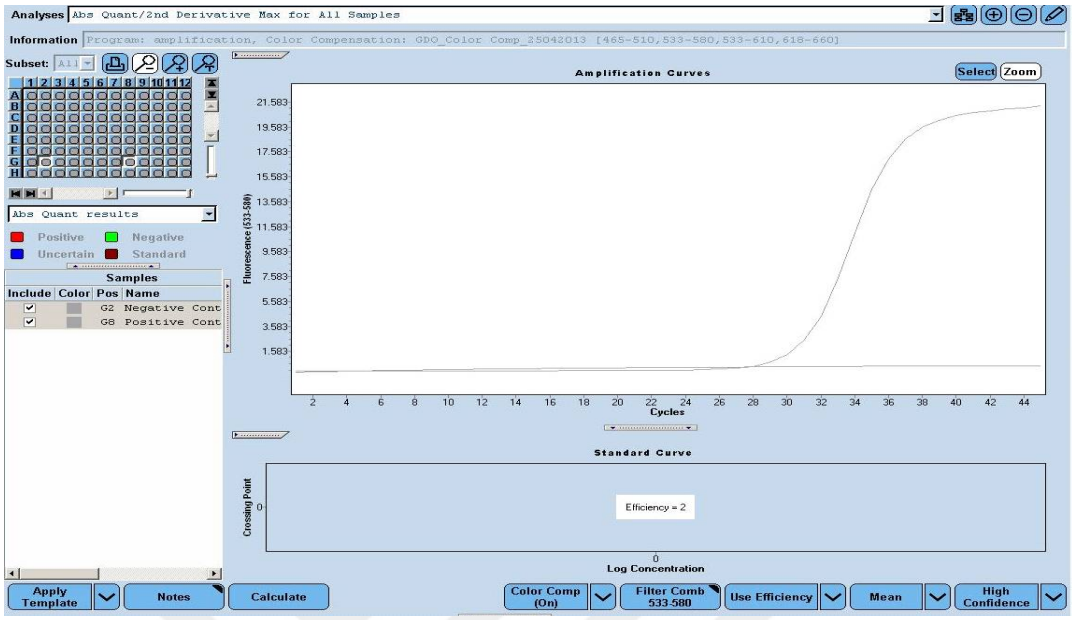

Figure 4.4 Quality control results for internal control

Internal control results for every sample were found out to be positive, which indicates that we should count on the tests.

GMO PCR Results: Results in appropriate channels were observed for each region.

35S Promotor sequence scan results were found out to be positive (figure 4.5)

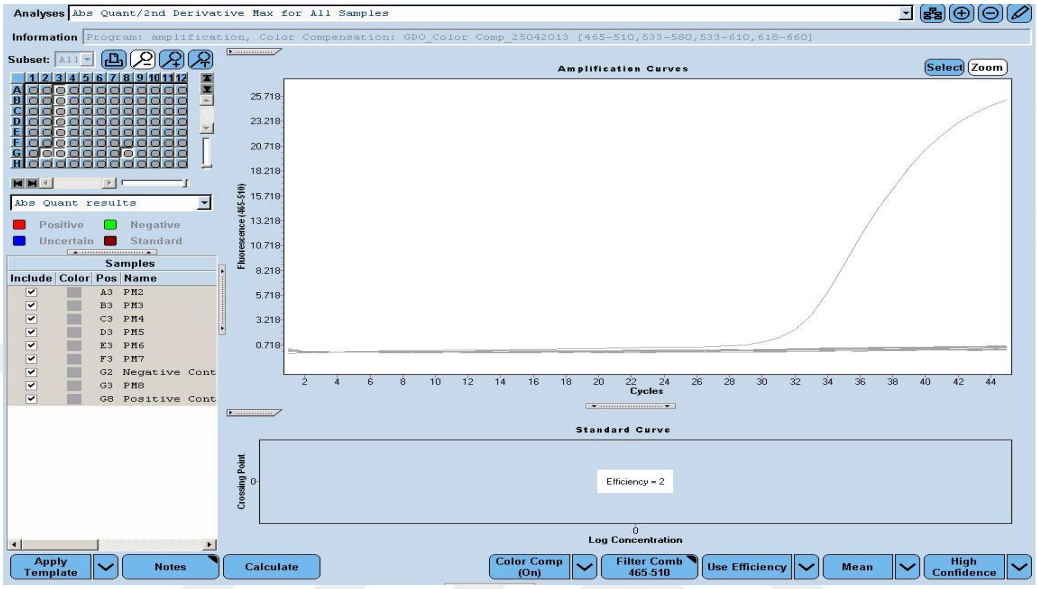

Figure 4.5 35S Promotor sequence scan results

NOS terminator sequence scan results were found out to be positive (figure 4.6)

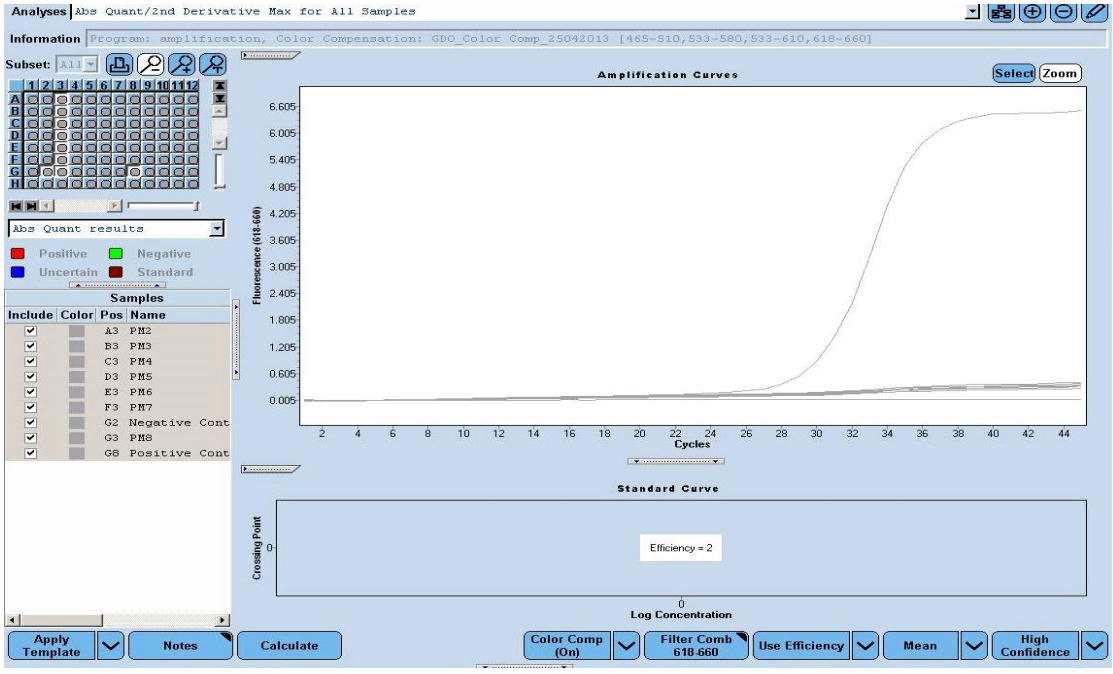

Figure 4.6 NOS terminator sequence scan results

FMV promotor sequence scan results were found out to be positive (figure 4.7)

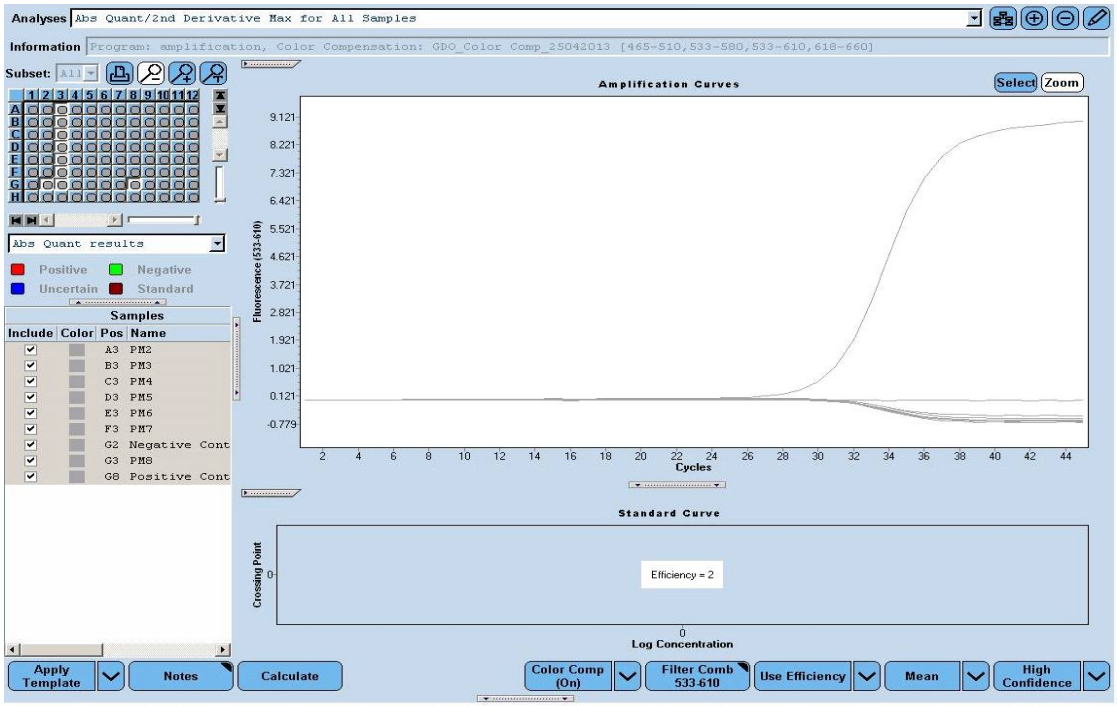

Figure 4.7 FMV promotor sequence scan results

IC results of milk products were found out to be positive (figure 4.8)

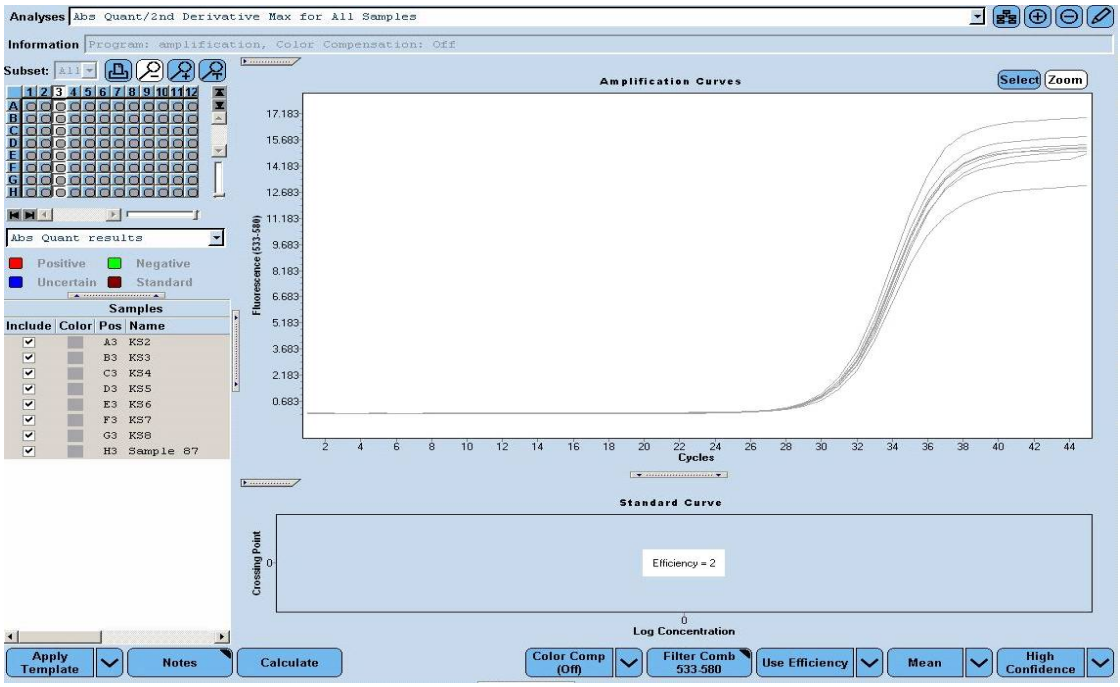

Figure 4.8 IC results of Milk products

No GMO was observed in any packaged or unpackaged milk samples. Test validity was obtained through quality control results (Table 4.2, 4.3)

Table 4.2 Results of unpacked GMO milk samples

Unpacked	35S	NOS	FMV	Amplification Control
Samples	**465/510**	**618/660**	**533/610**	**533/580**
Unpacked Milk 1	Negative	Negative	Negative	Positive
Unpacked Milk 2	Negative	Negative	Negative	Positive
Unpacked Milk 3	Negative	Negative	Negative	Positive
Unpacked Milk 4	Negative	Negative	Negative	Positive
Unpacked Milk 5	Negative	Negative	Negative	Positive
Unpacked Milk 6	Negative	Negative	Negative	Positive
Unpacked Milk 7	Negative	Negative	Negative	Positive
Unpacked Milk 8	Negative	Negative	Negative	Positive
Unpacked Milk 9	Negative	Negative	Negative	Positive
Unpacked Milk 10	Negative	Negative	Negative	Positive
Unpacked Milk 11	Negative	Negative	Negative	Positive
Unpacked Milk 12	Negative	Negative	Negative	Positive
Unpacked Milk 13	Negative	Negative	Negative	Positive
Unpacked Milk 14	Negative	Negative	Negative	Positive
Unpacked Milk 15	Negative	Negative	Negative	Positive
Unpacked Milk 16	Negative	Negative	Negative	Positive
Unpacked Milk 17	Negative	Negative	Negative	Positive
Unpacked Milk 18	Negative	Negative	Negative	Positive
Unpacked Milk 19	Negative	Negative	Negative	Positive
Unpacked Milk 20	Negative	Negative	Negative	Positive

Table 4.3 Results of packed GMO milk samples

Packed	35S	NOS	FMV	Amplification Control
Samples	**465/510**	**618/660**	**533/610**	**533/580**
Packed Milk 1	Negative	Negative	Negative	Positive
Packed Milk 2	Negative	Negative	Negative	Positive
Packed Milk 3	Negative	Negative	Negative	Positive
Packed Milk 4	Negative	Negative	Negative	Positive
Packed Milk 5	Negative	Negative	Negative	Positive
Packed Milk 6	Negative	Negative	Negative	Positive
Packed Milk 7	Negative	Negative	Negative	Positive
Packed Milk 8	Negative	Negative	Negative	Positive
Packed Milk 9	Negative	Negative	Negative	Positive
Packed Milk 10	Negative	Negative	Negative	Positive
Packed Milk 11	Negative	Negative	Negative	Positive
Packed Milk 12	Negative	Negative	Negative	Positive
Packed Milk 13	Negative	Negative	Negative	Positive
Packed Milk 14	Negative	Negative	Negative	Positive
Packed Milk 15	Negative	Negative	Negative	Positive
Packed Milk 16	Negative	Negative	Negative	Positive
Packed Milk 17	Negative	Negative	Negative	Positive
Packed Milk 18	Negative	Negative	Negative	Positive
Packed Milk 19	Negative	Negative	Negative	Positive
Packed Milk 20	Negative	Negative	Negative	Positive

4.2 Discussion

The use of the products acquired from genetically modified plants in foods for human consumption and forages for animal consumption cause some concerns. Especially the use of raw materials such as transgenic corn and soybean in animal forages leads to the questioning of the safety of the products acquired from animals. Potential risks of products acquired from animals eating forage containing genetically modified raw materials on human health are discussed the most. Such suspicions were researched in various animal feeding studies (20).

In the scientific studies conducted on animals, it was observed that biologically active genes and proteins existing in the formation of forages split rapidly with DNA or peptides once received in the body (38). Moreover, no genetically modified plant DNA or protein residue was observed in meat, milk, eggs and other products and tissues acquired from animals eating genetically modified forage (38). No GMO residue was observed in packaged or unpackaged milk samples in our study as well.

Jennings et al. (2003) observed no transgenic DNA residues in various tissues of the broilers, which had eaten forage containing transgenic corn for 42 days, as a consequence of the study (39).

In the study conducted by Kan and Hartnell in 2004, it was observed that GMO soya pulp addition in broiler forage did not change the performance, carcass productivity and the substances of brisket (40). No GMO residue was observed in the milk acquired from cattle consuming GMO forage in our study as well.

As a consequence of a study conducted by Taylor et al. (2004) through canola (Roundup Readyl (Event RT73)) pulp addition, no different effect was observed in carcass performance, carcass fat rate, brisket, wing and leg rates (41)

In another study conducted on broilers in 2008, Mc Naughton et al. gave two different types of forages containing equal amounts of genetically modified soya (DP-305423-1) products and (oil, flour and pulp) conventional soya. As a consequence of the study, it was observed that the two different types of forage did not cause a different impact on the nutritional performance (live weight, death rate and benefiting from forage), liver and kidney weight and carcass features (brisket, wing, leg and abdominal fat rates) (42).

In a study in which Clark and lpharraguerre examined the results of the 23 studies conducted on the subject (2001), stated that the genetically modified corn and soya products have similar effects on living stock like other conventional products. As a consequence of the examination of scientific studies, it was stated that not only genetically modified 1st generation products did not have a different effect on animals' health and nutritional features than conventional products, but also such genetically modified DNA fragments did not pass to products acquired from such animals (30). No GMO was observed in pure milk samples in our study as well. Furthermore, Clark and Ipharraguerre stated that it was early for 2nd generation products to be determined as above, and it had to be indicated through new studies (30).

Seralini et al. observed that the triglyceride amount in the blood of the rats consuming GMO corn increased by 24-40%, their livers grew larger, brains got smaller and kidney parameters disrupted (35). On the other hand, Finamore et al. determined distortion in the immunity system of the rats consuming GMO corn in the study (20-36).

In a 25 month study, 36 cows were divided into two groups which were given genetically modified (BT-MON810) corn and not genetically modified isogene (CON). No change in terms of energy content and chemical compounds was observed in groups consuming CON and BT-MON810 in lactation. The Cry-A1 protein of the cows consuming genetically modified corn was determined to be 6 mg and 6,1 mg in the first and second lactations respectively. Daily lactation amount value was determined to be 18,8 kg and 20,7 kg from each cow during the first and second lactations. No apparent

difference in terms of lactation performance was observed between the cows consuming genetically modified corn and not genetically modified isogene (37).

Malatesta et al. observed no apparent problem in the livers and pancreases of the rats fed with GTS Soya, however determined a deformation in the liver cell nucleus, an increase in the cell nucleus pores and the metabolic speed of the cell thanks to special dyes in the ultrastructural, microscopic and immune-histochemical examinations. Furthermore, an increase in the number and size of the packages containing enzymes in pancreas cells and the intra-cell traffic accelerated in parallel with all the findings. The findings on toxic effects also lead to questionings about the effects of toxic structures on excretion organs (36).

Commercial cattle has been the biggest consumer of genetically modified plants for almost twenty years. No health or physical disadvantage has been observed in such animals as a consequence of comprehensive field studies. Animal agriculture largely depends on such animals and such plants are traded at great levels. Additionally, such plants are traded by countries allowing them. On the other hand, the market of not genetically modified forages is rather expensive and challenging. Those using such plants are have a small scale and have a rather specific market, therefore the costs are pretty much. Today, there is a production under the name of new generation, which seems to have the potential to compete with traditional production if approved. In addition to that, production techniques will lead to big opportunities. In light of such information, two subjects should be emphasized on, one of which is an international draft for genetically modified plants and the other is advanced cultivation techniques to prevent issues in the trade of such plants (31).

In another study, analysis of genetically modified corn is important in terms of security assessment. When compared to not genetically modified corn, the genetically modified one was designed in order to study on composition analysis in the research conducted by Rayan et. Al. Many biochemical compounds' values and genetically modified corn samples were evaluated within not genetically control or literature. In not genetically modified corn samples, a major increase in protein, oil, fibre and oil acids was

observed, which might be a result of the synergic impact of the new features of corn types. Additionally, a great similarity was observed between protein fractions of corn samples studied on in SDS-PAGE analysis. These data indicate that not genetically modified corn equal compositionally to not genetically modified corn and are nutritional (52).

CHAPTER FIVE
CONCLUSION

The distribution of genetically modified animals and foods acquired from them is rapidly increasing. Foods containing oil, flour, starch, glucose syrup, sucrose, fructose produced from corn and soya; biscuits, crackers, puddings, vegetable oils, baby foods, candies, chocolate and waffles, instant soups, cotton and foods acquired from animals such as chicken and the like consuming corn and soya as forage are the leading products having a risk. The short-term and long-term impacts of these products on human health are not sufficiently known and perspectives still vary. Furthermore, there is no return in case such products pose a threat to genetic diversity. No GMO has been determined in the milk samples examined in our study, which seems to be promising. Therefore, having potential negative and positive impacts, GMO products should be available for consumption once adequate scientific studies have been made and always be legally controlled.

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