UNIVERSITY OF GAZİANTEP
GRADUATE SCHOOL OF
NATURAL & APPLIED SCIENCES

EFFECTS OF MODIFIED ATMOSPHERE ON *LISTERIA MONOCYTOGENES* and *SALMONELLA* TYPHIMURIUM DURING STORAGE OF MEAT, SUCUK AND WHITE CHEESE

M. Sc. THESIS
IN
FOOD ENGINEERING

BY
AYKUT ÖNDER BARAZİ
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Effects of Modified Atmosphere on *Listeria monocytogenes* and *Salmonella* Typhimurium during storage of meat, sucuk and white cheese

M.Sc. Thesis
in
Food Engineering
University of Gaziantep

Supervisor
Prof. Dr. Osman ERKMEN

by
Aykut Önder BARAZİ
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DEDICATED TO;

My Mother,

Grandmother, and

Specially my beloved Wife

ABSTRACT

EFFECTS OF MODIFIED ATMOSPHERE ON *LISTERIA MONOCYTOGENES* and *SALMONELLA* TYPHIMURIUM DURING STORAGE OF MEAT, SUCUK and WHITE CHEESE

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In this research effect of modified atmosphere (MA) on *Listeria monocytogenes* in meat, sucuk and white cheese stored at 4°C, and *Salmonella* Typhimurium in meat and sucuk stored both 4 and 12°C have been studied. These two pathogens were studied at separate time periods. During *S.* Typhimurium and *L. monocytogenes* counts at manufacturing and storage periods, Aerobic bacteria (AB), Lactic acid bacteria (LAB) and mold and yeasts (MY) were also counted. Head space gas analysis, pH and a_w values of sucuk dough, meat and cheese have been also recorded during storage period. Numbers of *S.* Typhimurium and AB increased at all packages in both of meat and sucuk stored at 12°C. Numbers of *S.* Typhimurium decreased in sucuk and meat packaged by vacuum and 100 % CO_2 at 4°C. Numbers of AB were increased in meats stored at both 4 and 12°C, and the increase was higher at 12°C than 4°C. AB numbers in sucuks increased in sucuks stored at 12°C, but decreased in sucuks stored at 4°C. In meats packaged with CO_2 at 4°C *L. monocytogenes* survived without increasing the growth, but in the presence of low CO_2 (30% CO_2) the number of counts of *L. monocytogenes* have been increased slightly. AB counts have been increased at 4°C in meat packaged by vacuum and 100 % CO_2 at 4°C by 3 and 2 logs, respectively. In sucuks, numbers of *L. monocytogenes* and AB were decreased but this decrease differed according to the atmospheric conditions of the packages. In packages containing CO_2, inhibition was observed more. LAB counts of sucuks have been increased during the fermentation period, and become constant at further storage. Similar microbiological results have been seen at cheese samples, both AB and *L. monocytogenes* numbers decreased during 22 days storage and the highest decrease was observed in 100% CO_2 packages by approximately 3 logs. Sucuks were also produced without *S.* Typhimurium and *L. monocytogenes* and sensory analyzed for quality parameters like color, odor, taste, cutting (texture) and general acceptance quality (GAQ) by panelists. And all parameters except cutting property gave scores as 7/10. Color analyses of meats packaged with MA in this research were made during storage, meats packaged with 100% CO_2 darkened during storage and contaminated meats have lost their acceptable appearance because of microbial spoilage. Head space gas analysis was observed in sucuk and CO_2 amounts in 100 and 60% CO_2 packages decreased due to the dissolution of CO_2. Amount of O_2 in air packages was decreased due to the consumption of O_2 by microorganisms.

Presence of CO_2 in package atmosphere has an inhibition effect on bacteria (both pathogens). The results of this research show that; meat, sucuk and cheese packed with CO_2, pathogen bacteria can be inhibited. The shelf life of these products (meat, sucuk and cheese) can be increased by using MA packaging.

Keywords: Modified atmosphere packaging, vacuum packaging, *Listeria monocytogenes*, *Salmonella* Typhimurium, meat, sucuk, cheese

ÖZET

MODİFİYE ATMOSFER PAKETLEMENİN ET, SUCUK ve BEYAZ PEYNİRİN SAKLANMASI SIRASINDA *LISTERIA MONOCYTOGENES* ve *SALMONELLA* TYPHIMURIUM ÜZERİNE ETKİLERİ

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Bu araştırmada modifiye atmosfer (MA) paketlemenin 4°C'de saklanan et, sucuk ve beyaz peynirde *Listeria monocytogenes*, 4 ve 12°C'de saklanan et ve sucukta *Salmonella* Typhimurium üzerine etkileri çalışılmıştır. Bu iki patojen farklı zamanlarda çalışılmış ve sonuçları farklı zamanlarda kaydedilmiştir. Üretim ve saklama zamanlarında, *S.* Typhimurium ve *L. monocytogenes* sayımlarının yanında, Aerobik bakteri (AB), Laktik asit bakterileri (LAB) ve Küf ve maya (MY) sayımları da yapılmıştır. Ayrıca kuşbaşı et, sucuk ve beyaz peynirin pH, paket gaz analizi ve su aktivitesi saklama süresince kaydedilmiştir. 12°C'de saklanan sucukta ve kuşbaşı ette, tüm paket atmosferlerinde *S.* Typhimurium sayısında artış gözlenmiştir. Et ve sucukta *S.* Typhimurium sayısında azalma en fazla 4°C'de vakum ve %100 CO_2 ile paketlendiğinde görülmüştür. AB sayısında her iki saklama sıcaklığında artış tespit edilmiş fakat bu artış 12°C daha fazla olmuştur. Kuşbaşı etlerde 4°C'de CO_2 varlığında *L. monocytogenes* çoğalmadan canlılığını koruduğu fakat düşük CO_2 varlığında (%30 CO_2) az da olsa sayısında artış olduğu belirlenmiştir. 4°C'de AB sayısında artış gözlenmiş, bu artış vakum ve %100 CO_2 ile paketlenmiş etlerde sırasıyla 2 ve 3 log kadar olmuştur. *L. monocytogenes* sayısında 4°C'de %100 CO_2 ile paketlendiğinde azalma olmuş ve bu azalma yaklaşık 1 log kadar gerçekleşmiştir. Sucuklarda *L. monocytogenes* ve AB sayıları sucukların saklama süresince azalmış fakat bu azalma ortamın atmosfer yapısına göre değişmiştir. CO_2 içeren atmosferlerde inhibisyon daha fazla gerçekleşmiştir. LAB sayısı sucukların fermantasyonu süresince artmış daha sonraki saklama süresince sabit kalmıştır. Benzer mikrobiyolojik sonuçlar farklı atmosferlerde saklanan peynirlerde de gözlenmiştir. AB ve *L. monocytogenes* sayıları 22 günlük saklama süresince düşmüş, en fazla düşüş %100 CO_2 paketlerinde 3 log kadar gözlenmiştir. Araştırmada Salmonella ve Listeria eklenmemiş sucukların renk, koku, tat ve kesin özelliği gibi kalite parametreleri panelistler yardımıyla tespit edilmiş, bunun sonucunda kesim özelliği hariç sucuklar ortalama 7/10 puan üzerinde skor almışlardır. Araştırmada farklı modifiye atmosferlerle paketlenmiş kuşbaşı etlerin saklama süresince renk tayinleri de yapılmış ancak mikrobiyal bozulma sebebiyle etler kabul edilebilir görünüm kalitesini kaybetmiştir. Sucuklarda saklama süresince ayrıca paket gaz atmosferi de ölçülmüş ve %100 ve %60 CO_2 içeren paketlerde CO_2 miktarı çözünmeden dolayı azalmıştır. O_2 miktarları da saklama süresince azalmıştır.

Ortam atmosferinde CO_2 olması bakteriler (her iki patojen) üzerinde daha fazla inhibisyon etki yaratmıştır. Araştırmamız sonuçları et, peynir ve sucukların CO_2 varlığında paketlenerek 4°C'de saklandığında patojen mikroorganizmaların inhibe edilebileceğini göstermiştir. Bu da MA ile paketlemenin et, sucuk ve beyaz peynirin raf ömrünü artırabileceğini göstermektedir.

Anahtar kelimeler: Modifiye atmosfer paketleme, vakum paketleme, *L. monocytogenes*, *Salmonella* Typhimurium, et, sucuk, peynir

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LIST OF SYMBOLS / ABREVIATIONS

AB	Aerobic Bacteria
Ar	Argon
a_w	Water Activity
BHIA	Brain Heart Infusion Agar
BHIB	Brain Heart Infusion Broth
CA	Controlled Atmosphere
CAP	Controlled Atmosphere Packaging
CAS	Controlled Atmosphere Storage
cfu	Colony Forming Unit
CO	Carbon monoxide
CO_2	Carbon dioxide
DFD	Dark Firm Dehydrated
EMA	Equilibrium Modified Atmosphere
EU	Europe
EVA	Ethylene Vinyl Acetate co-polymer
EVOH	Ethylene vinyl alcohol copolymer
GAQ	General acceptance quality
HFFS	Horizontal Form Fill Seal
kPa	Kilo Pascal (pressure)
LAB	Lactic Acid Bacteria
Log	Logarithmic
MA	Modified Atmosphere
MAP	Modified Atmosphere Packaging
MPa	Mega Pascal (pressure)
MRSA	De Man Rogosa Sharp Agar
MY	Mold and Yeast
N_2	Nitrogen gas
O_2	Oxygen gas
OPP	Oriented polypropylene
PA	Poly Amide (nylon)
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
PE	Polyethylene
PET	Polythene terephthalate (Polyester)
PVC	Polyvinyl Chloride
RH	Relative Humidity
ROP	Reduced oxygen packaging
spp.	Species
UK	United Kingdom
US – USA	United States of America
VP	Vacuum Packaging

CHAPTER 1

INTRODUCTION

Modified atmosphere packaging (MAP) is the term which is very widely used for the packages that include other gases than air at specific known amounts. Replacement of gas atmosphere of packages of food materials with other gases rather than air may also alter the environmental gaseous conditions of food materials, and may keep freshness and high quality of foods for longer times. Besides these protective properties of MAP, it may also help to keep microbiological conditions in package atmosphere safe by using antimicrobial effects of gas compositions in modified atmosphere (MA). Gas compositions of MAs include various gases like carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2). Some pathogens can be kept at low numbers by using these gas mixtures in MA packaged food materials. If food material includes pathogens in high numbers, various foodborne illnesses and disorders may occur after the consumption of these high microbial loaded foods.

Because of increasing numbers of food poisoning caused by two pathogens *Listeria monocytogenes* and *Salmonella* Typhimurium recently and high need of keeping food products at high quality and fresh, MAP seen as an alternative method in order to solve these problems. These two pathogens are mainly responsible from the food poisoning caused by the consumption of meat, meat products and milk products like cheese. Foodborne illnesses like salmonellosis and listeriosis are the names that given these microorganisms based food poisonings. Salmonellosis and listeriosis are reported to cause illnesses thousands in USA and in whole EU annually. Preservative additives and other chemicals are mostly used to protect food products from these pathogens and prevent their growth during storage period. But these additives may also have side effects on human health and increase added cost to food products. MA may help to decrease using of these preservatives and keep the freshness of food products.

Meat and meat products are most susceptible media for growth of these two pathogenic microorganisms. Meat and sucuk are mostly vacuum packaged. Vacuum packaging uses vacuum pressure to evacuate all the atmospheric air around the food product and keep the product free of any atmospheric gases. This condition mostly helps to prevent growth of aerobic microorganisms and help to prolong the shelf life of food products. Sucuk is a very popular example to vacuum packaged products. It can be defined as Turkish style sausage made from fermented ground meat mixed with some spices. Sucuk can be made from a mixture of sheep and beef meat. It has a ripening period in order to make a successful fermentation by using starter cultures. It is totally open to microbiological threads during this period. Spoilage microorganisms cause off flavors and odors in sucuk. In order to prevent spoilage, atmospheric gas mixtures in MA packages may help to prevent the growth of these microorganisms and help to prolong the shelf life.

White cheese is a very popular dairy product and it is also the most common carrier of the illness named as listeriosis caused by the bacterium *L. monocytogenes*. Microbiological conditions may be very dangerous and not visible until spoilage of cheese. If raw milk or processing conditions are heavily contaminated with spoilage and pathogenic bacteria, white cheese may be a potential risk to human health. MAP may help to prevent both pathogenic and spoilage microorganisms on the surface of white cheese and prolong the shelf life.

Besides microbiological spoilage, chemical deteriorations and changes may cause the spoilage in food product during storage. Atmospheric gases around the food products may favor these changes. For example lipid oxidation may be accelerated because of high oxygen concentration in package atmosphere. Lipid oxidation has been accepted as one of the major quality deterioration in meat and dairy products.

MAP has a very wide usage area in food industry. Different food products may be packaged with different gas mixtures. It needs a serious pre-study in order to determine a suitable package atmosphere for a specific food product. According to microbiological aspect, mold and yeast growth, growth of possible pathogenic microorganisms should be prevented mainly.

In this thesis study; first *S.* Typhimurium added meat and sucuk samples were monitored during storage at 4 and 12°C in MA packages with different gas

compositions. Aerobic bacteria (AB), mold and yeast (MY), and *S.* Typhimurium numbers were counted during storage period. And then *L. monocytogenes* added meat, sucuk and white cheese were stored at 4°C and AB, lactic acid bacteria (LAB), and *L. monocytogenes* numbers were monitored during manufacturing and storage. Besides microbiological analyses, chemical analysis like pH (meat, sucuk and cheese), a_w (sucuk), head space gas analysis, color analysis (meat) and sensory analysis (sucuk) were observed.

These two pathogens were studied at different times and the effects of MAP on these pathogens were examined in order to determine whether MAP is an acceptable way of preserving food products and preventing growth of pathogenic microorganisms in food products or not.

Therefore, aims of this master thesis were to;

1. Determine fate of two common pathogens, *S.* Typhimurium and *L. monocytogenes* in sucuk and meat packaged with MAP.
2. Detect the general microbiological conditions during the storage of meat, sucuk and white cheese packaged with different gas atmospheres.
3. Detect both effects of storage temperatures and storage times in MA packaged foods.
4. Find which gas mixture is most suitable and effective against pathogenic microorganisms in MA storage of meat, sucuk and white cheese.
5. Use of MA storage of foods in food safety.

CHAPTER 2

LITERATURE REVIEW

2.1. Modification of storage atmosphere of foods

The food industry is moving away from preservative methods that physically or chemically alter foodstuffs in favor of gentler techniques that protect the inherent quality of the food and leave the product unchanged. There are lots of preservation methods of foods. In recent years, modification of the storage environment to preserve foods has become one of the most used methods. Storage of many convenient and ready-to-eat foods is preserved with this technology without uses of any chemical additives and by less processing treatments. Modification of atmosphere provides the food industry with a "preservative-free" method of extending the shelf life of a range of fresh and processed foods. The shelf life of food can be enhanced by modifying the gas composition of air, eg, increasing or decreasing the oxygen (O_2) amount and/or increasing the level of carbon dioxide (CO_2). The presence of O_2 is one of the major factors of spoilage of foods and causes: (i) Oxidation reactions, damaging vitamins, fatty substances, pigment formation, formation of flavoring substances that are often catalyzed by enzymes; and (ii) growth and activity of aerobic microorganisms (aerobic bacteria, yeast and molds). In practice four different techniques are used to modify the atmosphere to increase acceptable quality of foods; controlled atmosphere (CAP), modified atmosphere packaging (MAP), vacuum packaging (VP) and active packaging (AP) (Erkmen and Bozoglu, 2008).

2.1.1. Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) is the replacement of air in a pack by a different mixture of gases, where the proportion of each component is fixed when mixture is introduced, but no further control is exercised during storage (Davies and Gibbs, 1994). In MAP, a food is enclosed in a high gas-barrier packaging material, the air is removed from the package, which is then flushed and filled with a

particular gas or combination of gases, and the packaging is hermetically sealed (Erkmen and Bozoglu, 2008).

In the 1920s the Low Temperature Research Station showed that the shelf life of apples could be increased by storing them in atmospheres containing low levels of O_2 and increased CO_2, and in the 1930s beef carcasses were transported in atmospheres containing CO_2, which approximately doubled the storage life previously obtained. It was Marks & Spencer in 1979 who paved the way for Britain's pre-eminence today in the world market place for modified atmosphere (MA) products with its test launch of MAP meat (Parry, 1993). In the 70's MA packages reached the stores when bacon and fish were sold in retail packs in the UK. Since then the development has been stable and the interest into MAP has grown due to increasing consumer demand for fresh and chilled convenience foods containing fewer preservatives.

The principal factors in a successful MAP operation are the selection of (Erkmen and Bozoglu, 2008):

1- A gas or gas mixture to minimize the selected deteriorative reactions in the food to insure the required shelf-life;

2- A suitable packaging material to maintain the desired gas composition; and

3- An appropriate packaging machine.

In order to provide stated shelf-life by using MAP, control of the cold-chain is a critical factor to insure that no temperature abuse (temperatures outside the specified range for a particular product) occurs.

The potential advantages and disadvantages of MAP have been given as below (Modified from Farber, 1991);

Advantages:

- Shelf-life increase of 50-400% possible. Extended shelf life is achieved without addition of preservatives which means foods can be served to consumer as "preservative-free".
- Economic losses reduced (longer shelf-life to spoilage).

- Products can be distributed over longer distances and with fewer deliveries, leading to decreased distribution costs.
- Antimicrobial effect: obtained by reduction O_2 and presence of CO_2 inhibit the growth of aerobic microorganisms.
- Provides a high quality product.
- Increase shelf-life quality in the distribution chain by days or even weeks, which increases the availability of fresh food to consumers.
- Reduce the return of spoiled foodstuffs.
- Easier separation of slices.
- Improved product visibility.

Disadvantages:

- Visible added cost.
- Temperature control necessary. Chilled storage is necessary for the premium quality and microbiological safety.
- Different gas formulations for each product type.
- Special equipment and training required.
- Product safety to be established.
- Increased requirement for display space.

Storage of food under modified atmosphere (MA), eg, an atmosphere where the ratios between the air components have been changed MAP, can be achieved in several ways, usually the term MA implies the use of some form of package based on a laminated plastic film with a substantial head space, at least at the moment of packaging. However, a “vacuum package” (VP) also provides a MA; air is removed and the head space is decreased. In this limited space the product and/or the metabolic activities of contaminating microorganisms change the proportions of O_2 and CO_2. To distinguish between a vacuum package where the atmospheric modification is passively created and the type of package where gas has been

deliberately flushed into the package, the term “gas package” has been used. However, the term is mostly avoided in the industry because of the possible negative implications (Lund et al., 2000).

2.1.1.1. Machines used at MA

a) Vacuum chambers

As seen in Figure 2.1, these machines use preformed bags and utilize the compensated vacuum technique to replace air. Preformed high-barrier bags are manually placed within the chamber before evacuation, back-flushing with the desired gas mixture, and heat sealing. These machines can be used for small scale production of vacuum or gas flushed catering packs.

The product to be packaged is put into a film pouch and placed in the vacuum chamber. When the lid has been closed, the programmed level of vacuum is produced in both the vacuum chamber and the pouch. The pouch is then either sealed in a vacuum (vacuum package) or the chamber (and thus the pouch as well) is filled with a MAP gas before the sealing operation (modified atmosphere package).

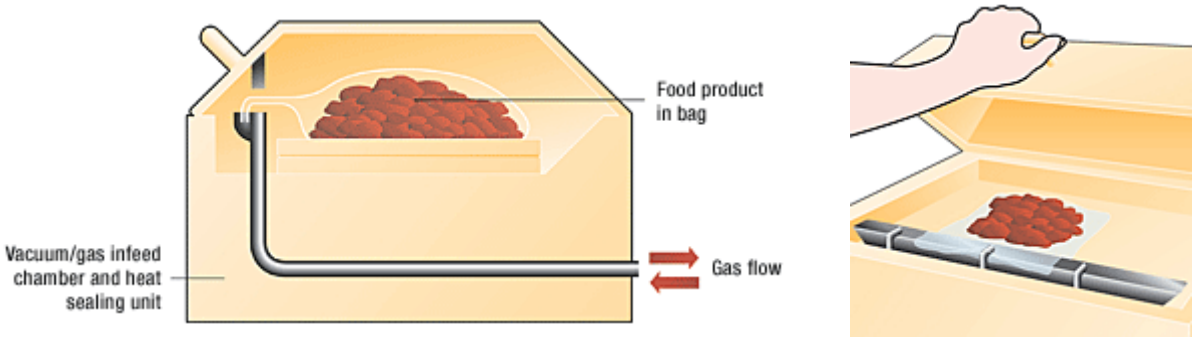

Figure 2.1 Vacuum chamber illustrations.

b) Snorkel type

These machines use the compensated vacuum technique to produce bulk MA catering bag-in-box packs (Figure 2.2). Alternatively, they can gas flush conventionally packaged retail products, such as overwrapped packs of red meat, into large master packs. In these machines, preformed plastic bags are positioned on a

heat seal mandrel and retractable snorkels pull a vacuum and then back-flush with a desired gas mixture before heat sealing.

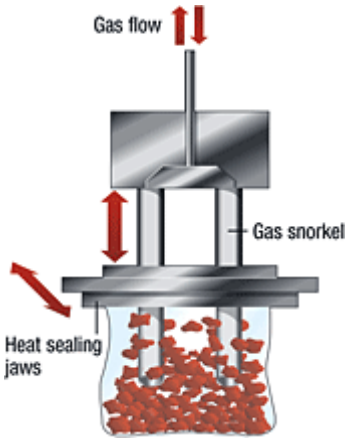

Figure 2.2 Illustration of snorkel type.

c) Tray lidding

A traysealer uses ready-made trays that are sealed in much the same way as a thermoformer. The top web of packaging material (lid film) covers the filled pockets/trays (Figure 2.3). The air is evacuated from the sealing die and protective gas is added. Then the pack is sealed by the application of heat and pressure. Tray lidding machines are available from tabletop (manual) for the small producer, to fully automatic inline versions for larger processors.

d) Horizontal form-fill-seal

These so-called flow-pack machines (Figure 2.3) are capable of making flexible pillow-pack pouches from only one reel of film. Horizontal form-fill-seal machines can also overwrap a pre-filled tray of product. The air from the package is removed by a pulse of gas or continuous gas flushing, but gas mixtures containing levels of O_2 > 21% cannot be used due to the use of hot sealing jaws at the end of the machine. For certain very porous products (e.g. some bakery goods), gas flushing is not capable of reducing the residual O_2 within the package to low levels. In some cases, a gas injection station can be fitted to the machine infeed so that the product itself is purged with gas immediately prior to packaging.

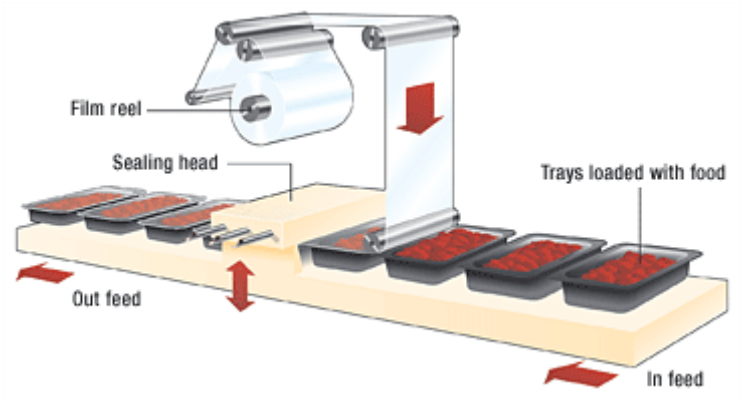

Figure 2.3 Illustration of tray lidding horizontal form.

e) Vertical form-fill-seal (VFFS)

A vertical machine forms a tube, it then fills with product (in most cases dropped from an overhead multi-weigher), purges with gas and then seals (Figure 2.4). At the same time film is transported vertically downwards. VFFS machines are predominantly used for packaging foods in powder, granular, shredded and dried form.

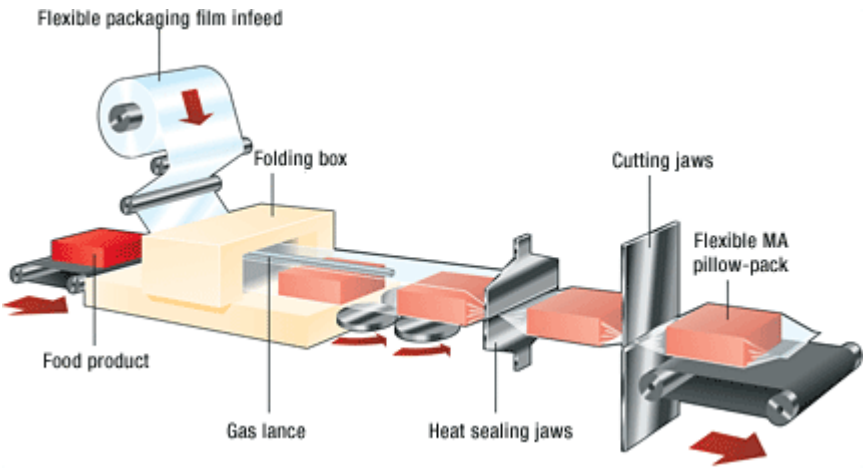

Figure 2.4 Illustration of vertical form.

f) Thermoform-fill-seal

Packaging material for the base web (thermoformable film) is unwound from the reel (Figure 2.5). It is heated in the forming die and formed into pockets/trays. The formed pockets are loaded manually or automatically. The top web of packaging material (lid film) covers the filled pockets/trays. The air is evacuated from the sealing die and protective gas is added. Then the pack is sealed by the application of heat and pressure. The web of packs is cut across the machine direction initially.

Production of the individual packs has been completed after the longitudinal cutting operation.

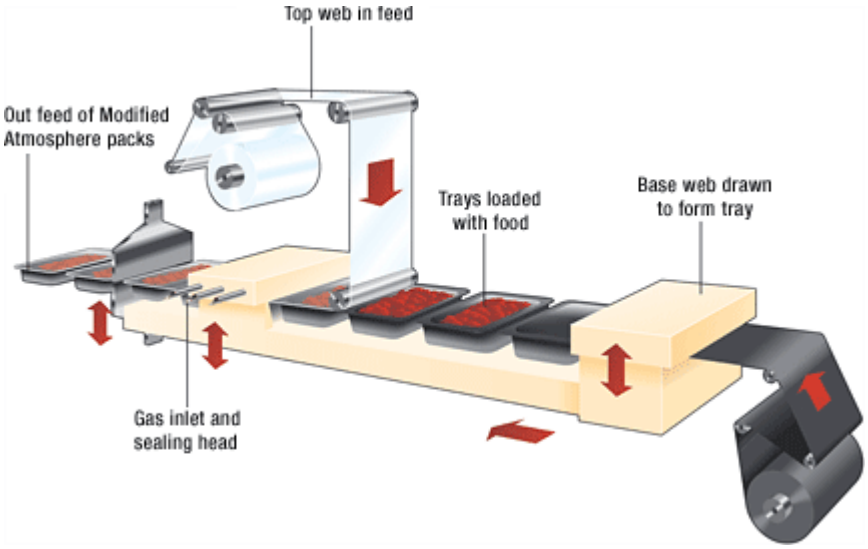

Figure 2.5 Illustration of Thermoform.

g) Gas mixer

For food companies packing a varied range of products and may be a complicated arrangement of packing machines with frequent product changes, it may be beneficial to use an adjustable gas mixer (Figure 2.5). These are available in flow rates to suit the common MAP gas requirements in gas combinations.

Figure 2.6 An equipment of Witt gas mixer.

The relative benefits of this type of installation are:

- Flexible supply of food grade mixed gas
- More portable arrangement of equipment
- The mixtures can be adjustable to meet the demands of production

Some gas mixers incorporate alarms for gas supply failure and an electrical supply would be required. In some cases gas mixers will require a receiver, the size dependent upon the type and flow rate of the packaging machine.

2.1.1.2. Gases used at MA

The basic concept of the MAP of fresh foods is the replacement of the air surrounding the food in the package with a mixture of atmospheric gases different in proportion from that of air.

Table 2.1 Gaseous composition of dry air at sea level (Parry, 1993)

Gas	Percentage
Nitrogen (N_2)	78.03
Oxygen (O_2)	20.99
Argon (Ar)	0.94
Carbon dioxide (CO_2)	0.03
Hydrogen (H_2)	0.01

The gas atmosphere must be carefully adapted to the individual foodstuff and its properties. In the case of low-fat products with a high moisture content, MAP focuses on inhibiting the growth of microorganisms in particular. Oxidation protection is the primary objective, however, in the case of products with a high fat content and low a_w (Linde Gas, 2005).

The three major gases used commercially in MAP are oxygen (O_2), nitrogen (N_2) and carbon dioxide (CO_2), although several other gases have been investigated e.g. carbon monoxide (CO), sulphur dioxide (SO_2), nitrous oxide (N_2O), ozone (O_3) and chlorine (Cl). However, the use of these has been limited by safety concerns,

legislation and negative effects on organoleptic properties and cost (Davies and Gibbs, 1994).

The altered ratio of these gases (N_2, O_2, and CO_2) makes a difference in the prolongation of shelf life. By reducing the O_2-level and increasing the CO_2-level, ripening of fruits and vegetables can be delayed, respiration and ethylene production rates can be reduced, softening can be retarded and various compositional changes associated with ripening can be slowed down.

In food industry, atmospheres in packages are adjusted according to required quality of products and microbial load of foods that will be produced or processed. This makes the standardization of gas atmospheres for each food product difficult, and also additional pre-experiments of these gas atmospheres are required strictly to produce good quality food products. For some types of foods recommended gas mixtures are given in Table 2.2.

Table 2.2 Recommended gas mixtures of MAP (Parry, 1993)

Product	**% O_2**	**% CO_2**	**% N_2**
Red meat	60-85	15-40	-
Cooked/cured meats	20-35	65-80	-
Poultry	-	25	75
Fish (white)	30	40	30
Fish (oily)	-	60	40
Salmon	20	60	20
Hard cheese	-	100	-
Soft cheese	-	30	70
Bread	-	60-70	30-40
Non-dairy cakes	-	60	40
Dairy cakes	-	-	100
Pasta (fresh)	-	-	100
Fruits and vegetables	3-5	3-5	85-95
Dried/roasted foods	-	-	100

2.1.1.2.1. Oxygen

Food deteriorates due to physical, chemical and microbiological factors. Oxygen is probably the most important gas in this context being used metabolically by both aerobic spoilage microorganisms and plant tissues and taking part in some enzymic reactions in food including the compounds such as vitamins and flavors. For these reasons, in MAP, O_2 is either excluded or the levels set as low as possible. The exceptions occur where O_2 is needed for fruit and vegetable respiration, color retention as in the case of red meat or to avoid anaerobic conditions in white fish (Parry, 1993).

In MAP, O_2 levels are normally set as low as possible to reduce oxidative deterioration of foods. O_2 will generally stimulate the growth of aerobic bacteria and can inhibit the growth of strictly anaerobic bacteria, although there is a very wide variation in the sensitivity of anaerobes to O_2. One of the major functions of O_2 in MAP meats is to maintain myoglobin in its oxygenated form, oxymyoglobin. This is the form responsible for the bright red color, which most consumers associate with fresh red meat (Farber, 1991).

Oxygen is toxic to all life provided the partial pressure is high enough. Explanations of its toxicity include the following: (1) inactivation of certain enzymes, (2) increasing intracellular generation of hydrogen peroxide, (3) oxidation of membrane lipids, and (4) *in vivo* production of the superoxide radical. These effects may coincide but formation of superoxide radicals is probably of major importance. For example, O_2 resistance of cells can be correlated with their superoxide dismutase activity (Lund et al., 2000).

Oxygen will generally stimulate the growth of aerobic bacteria and can inhibit the growth of the strictly anaerobic bacteria although there is a very wide variation in the sensitivity of anaerobes to O_2. O_2 is very important in fresh meats to maintain myoglobin in its oxygenated form (oxymyoglobin), which gives fresh meat its bright red color. Its presence may cause problems with oxidative rancidity or color in some products (a.g. fatty fish and cured meats, respectively) (Davies and Gibbs, 1994).

Oxygen is essential when packaging fresh fruits and vegetables as they continue to respire after harvesting. The absence of O_2 can lead to anaerobic respiration in the

package which accelerates senescence and spoilage. Too high levels of O_2 do not retard respiration significantly and it is around 12% of O_2 where the respiration rate starts to decrease. So O_2 is used in low levels (3-5 %) for positive effect.

Oxygen is perhaps the major factor determining the shelf-life of meat products. It is the essential gas being used metabolically by aerobic spoilage organisms. It takes part in some enzyme-catalyzed reactions in foods including the oxygenation of myoglobin in meat and the oxidation of fat. As the red color of meat is an important criterion for its acceptability and marketability, O_2 is used in fresh-meat packaging to maintain the red color. On the other hand, it can reduce the shelf-life of meat due to oxidative rancidity in certain O_2-sensitive products and the growth of aerobic spoilage microflora. A low level of O_2 may favor rapid metmyoglobin formation and cause browning of uncured meat products or greening in undercooked meat products. Some researchers recommended the incorporation of O_2 to help reduce the risk of growth of anaerobic pathogens and toxin production (Rao and Sachindra, 2002).

2.1.1.2.2. Nitrogen

Nitrogen (N_2) is an inert tasteless gas with low solubility in both water and lipid. It is used to displace O_2 in packs so as to delay oxidative rancidity and inhibit the growth of aerobic micro-organisms. Because of its low solubility it is used as a filler gas to prevent pack collapse which may occur in high CO_2-containing atmospheres. (Davies and Gibbs, 1994)

Nitrogen reduces the vacuum effect and is also a natural component of the air. “Pack Collapse,” which is common in MAP packs due to absorption of CO_2 into meat tissue, can be prevented by using N_2 in combination with CO_2 as inert filler. (Rao and Sachindra, 2002).

If there is not any microbiological concern during storage, N_2 may be used alone for preventing pack collapse but if the package atmosphere wanted as an antimicrobial factor for prolonging shelf life of product, so some other gases like CO_2 should be included into the package besides N_2. Mostly N_2 is used as a filler gas for packaging of snack foods for example.

2.1.1.2.3. Carbon dioxide

Carbon dioxide extends the shelf-life of perishable foods by retarding bacterial growth. CO_2 increases both the lag phase and the generation time of spoilage organisms. CO_2 is effective due to its ability to penetrate the bacterial membrane causing intra-cellular pH changes of greater magnitude than those resulting from similar external acidification that can be effectively buffered by the organism. The state of knowledge regarding the inhibition of bacterial growth in the presence of CO_2 can be summarized as follows: (a) The replacement of O_2 by CO_2 may slightly contribute to the preservative effect, by slowing down the growth rate of aerobic organisms; (b) the penetration of CO_2 into the cells may facilitate its chemical effects on internal metabolic processes; (c) CO_2 is able to produce rapid acidification of the cells thus influencing metabolic activities; (d) CO_2 appears to have an effect on certain enzyme systems (Rao and Sachindra, 2002).

Carbon dioxide (CO_2) is both water-and lipid- soluble and is mainly responsible for the antimicrobial effect seen on microorganisms in modified atmospheres. This antimicrobial effect is influenced by the concentration of CO_2, the age of and load of the initial bacteria population, storage temperature and type of product to be packaged. Although this antimicrobial effect of CO_2 has been known for many years, the precise mechanism of its action is still not clearly understood. Farber summarized the theories regarding the influence of CO_2 on the bacterial cell as (Farber, 1991):

1. Alteration of cell membrane function including effects on nutrient uptake and absorption.
2. Direct inhibition of enzymes or decreases in the rate of enzyme reactions.
3. Penetration of bacterial membranes, leading to intracellular pH changes.
4. Direct changes to the physicochemical properties of proteins.

With high moisture high fat foods such as meat, poultry and seafood, excessive absorption of CO_2 can lead to the phenomenon known as ‘pack collapse’. Increased in-pack drip is also caused by dissolution of the gas into the surface of fresh muscle foods which reduces their pH sufficiently to weaken the water holding capacity of the proteins (Davies and Gibbs, 1994).

CO_2 has an important regulatory influence in the living cell. All cells require CO_2 for growth, but if the partial pressure of CO_2 increases over a certain critical level, metabolic activity will be retarded. (Lund et al., 2000)

Several hypotheses to explain the growth-inhibitory effects of CO_2 have been put forward. Some examples are that carbon dioxide should: (1) cause a decrease of the intracellular pH, (2) inhibit (or stimulate) enzymatically catalyzed reactions and enzyme synthesis and (3) interact with the cell membrane. There are probably two major effects involved: (i) effects of both aqueous CO_2 and bicarbonate (HCO_3^-) on the state of the cell membranes and/or the function of the membrane proteins, and (ii) effects on cytoplasmic enzymes. The former effects are probably the most important ones in those organisms that are relatively resistant to CO_2, e.g. where a relatively high level of CO_2 is required for growth inhibition. However, for more sensitive organisms that are affected by small increases in the partial pressure of CO_2, interference with enzymes and their activities is probably more important. It has also been concluded that "most probably, a major factor in the efficacy of CO_2 lies in its ability to penetrate the bacterial membrane, causing intracellular pH changes." Generally speaking, CO_2 inhibition of microbial growth cannot be attributed to a single target in the cell. Inhibition by CO_2 seems to be the overall result of a complex interaction with several key physiological reactions (Lund et al., 2000).

The retarding effect of CO_2 on bacterial growth increases along with increasing concentration. CO_2 resistance is different for different types of microorganisms. Examples of CO_2-sensitive organisms are frequently found in the genus *Pseudomonas* and among many of the molds that are associated with food. Their growth is often significantly retarded at a partial pressure as low as 10 kPa (0.1 atm or 10% [v/v] CO_2 in the gas phase at atmospheric pressure) (Lund et al., 2000).

When packaging meat and fish, the high CO_2-levels are effective bacterial and fungal growth inhibitors. In the case of vegetables and fruits, CO_2 is not a major factor since CO_2-levels above 10% are needed to suppress fungal growth significantly. Unfortunately higher levels than 10% of CO_2 are working phytotoxic for fresh produce. CO_2 has a more limited impact on the growth of anaerobic microorganisms.

CO_2 inhibits the increase of most aerobic bacteria. In general one can say the higher the CO_2 concentration the longer the durability of the perishable food. However fat

and water absorb CO_2 gases very easily and excessive CO_2 concentrations cause quality failures regarding taste, loss of humidity and the concentration of the packaging (so called vacuum effect). It should therefore be considered carefully! As to how long the product has to be durable and how acceptable are the reductions caused by CO_2. If CO_2 is intended to regulate the growth of bacteria, a concentration of at least 20% is recommended. CO_2 is a natural gas, which is found in small concentrations in the air.

The antimicrobial effect is influenced by the concentration of CO_2, the partial pressure of CO_2, volume of headspace gas, the type of microorganism, the age and load of the initial bacterial population, the microbial growth phase, the growth medium used, the storage temperature, acidity, water activity, and the type of the product being packaged (Church, 1994; Farber, 1991; Phillips, 1996; Church and Parsons, 1995). Yeasts which produce CO_2 during growth are stimulated by high levels of CO_2 and thus for some products where they are potentially a major cause of spoilage, MAP may not be an advisable option. Also the food-associated pathogens *Clostridium perfringens* and *Clostridium botulinum* are not affected by the presence of CO_2 and their growth is encouraged by anaerobic conditions. In general CO_2 is most effective in foods where the normal spoilage organisms consist of aerobic, Gram-negative psychrotropic bacteria (Hotchkiss, 1998; Phillips, 1996).

For maximum antimicrobial effect, the storage temperature of MAP product should be kept as low as possible, because the solubility of CO_2 decreases dramatically with increasing temperature. Thus, improper temperature control will usually eliminate the beneficial effects of elevated CO_2. The absorption of CO_2 is highly dependent on the moisture and fat content of the product. If product absorbs excess CO_2, the total volume inside the package will be reduced, giving a vacuum package look known as “pack collapse”. Excess CO_2 absorption in combination with “pack collapse” can also reduce water holding capacity of meats, resulting in unsightly drip. Some dairy products (e.g. cream) are very sensitive to CO_2 concentrations and will be tainted if packed in MA with high CO_2 levels. Fruits and vegetables can suffer physiological damage due to high CO_2 levels. For practical purposes, in most foods, gaseous CO_2 is applied to a biological tissue would exist in the liquid phase of the tissue primarily dissolved CO_2 gas forming and carbonic acid (about 2%). At $pH < 6.0$, carbonic acid will dissociate to form bicarbonate and hydrogen ions, the latter of which likely

causes pH drop (< 0.1 pH unit or more) often observed in muscle tissue packaged in a CO_2 atmosphere (Daniels et al. 1985).

2.1.1.2.4. Other Gases

a) Carbon monoxide

Carbon monoxide (CO) is an odorless, tasteless, and colorless gas, but it is highly reactive and flammable. Although its solubility is low in water, it can be soluble in some organic solvents. Using CO in meat and meat products are under research and it can prevent browning in lettuce. Because of high risk of its toxicity and explosion when mixed with air its use has been restricted and limited.

This has been found to be very effective in maintaining the red color in fresh meat due to the formation of carboxymyoglobin. It has not been used commercially for this purpose however since CO; a highly toxic gas is not approved by the regulatory authorities owing to the possible health hazard to packaging machine operatives (above 30%). Its use has, however, been sanctioned in the United States to prevent browning in packed lettuce. CO has little inhibitory effect on micro organisms.

b) Noble gases

They are gases that have not ability to react with anything. They can be used as an alternative for N_2 and as a filler gas for food products. Some of these gases are; helium (He), argon (Ar), xenon (Xe), and neon (Ne). Among these noble gases Ar has advantages compared to N_2: solubility of Ar is higher than N_2, it can be used instead of N_2 in some packages that contain O_2. Ar is 2.5 times more soluble than N_2 in water and 2 times more in lipids which is similar as solubility of O_2.

2.1.1.3. Packaging materials used at MAP

There is a wide range of packaging materials for Modified Atmosphere Packaging. When choosing materials, the following consideration must be taken into account:

Packaging format

- Pouch; which is more commonly a plastic bag with a modified atmosphere.
- Flowrap (Horizontal or Vertical); form-fill machines are used and then gases are filled and bags were wrapped with films.

- Tray Lid; Trays including food materials are filled with modified atmosphere and then lid was closed with films.
- Thermoforming; packaging materials are heated in the forming die and formed into pockets/trays. After that, modified atmosphere was filled into trays and lid was closed with films.

Typical film structures used at MAP and other applications are given in Table 2.3.

The wide range of materials used for MAP is:

a) Glass and Metal Containers; ex: jars, or metal containers

b) Semi-rigid and Plastic Containers; ex: plastic bags, plastic based packaging materials

c) Flexible Packaging Materials; ex: paper, paperboard, aluminum foil are used

Table 2.3 Typical film structures and their uses

Structure	**Applications**				
	MAP	Hot Filling	Chilled food	Frozen Food	Pausterization
APET/PE	•		•		
APET/EVOH/PE	•		•		
PS/EVOH/PE	•	•	•	•	•
PP/PE	•	•	•		•
PP/EVOH/PE	•	•	•		•
PP/EVOH/PP	•	•	•		•
EPP/EVOH/PE	•	•	•	•	•
PE/PA	•	•	•	•	•
PP/PA/PP	•	•	•		•
PETBlend/PE	•		•	•	
PETBlend/EVOH/PE	•		•	•	
EPETBlend/PE	•		•	•	
EPETBlend/EVOH/PE	•		•	•	
PVC/PE	•	•	•	•	
PVC/EVOH/PE	•	•	•	•	

The different types of packaging materials used for MAP. In general, packaging films with O_2-permeability of 100 $cm^3/m^2/atm/day$ at 25 C are used for vacuum and MAP. O_2-permeability of films used for VP has significantly greater effects on the sensory characteristics of products than the permeability of films used for gas-packed products. O_2-permeability in vacuum packs results in immediate contact of O_2 with the surface of the product and can promote rapid growth of aerobes at the interface between the product and the film. Although it does not produce a complete anaerobic environment, VP of meat in films with low permeability impedes the diffusion of O_2 to meat (Rao and Sachindra, 2002).

The O_2-permeability of the film is likely to affect not only the developing microflora, but also product color and flavor. Discoloration and development of off-odor in vacuum-packaged meat is inversely related to film permeability. To maintain the red color of meat, it is necessary to use gas-impermeable films. The development of rancid odor and flavor due to rapid oxidation in packaged poultry was found to be retarded when the O_2 level in the pack is reduced below 2% and low permeability films are used (Rao and Sachindra, 2002).

As a preservative, packaging will only be effective while an appropriate in-pack atmosphere is maintained, and thus a packaging must necessarily be composed of a material that has limited capabilities of transmitting gases. Moreover, the pack must be sealed, rather than closed by clipping or crimping, to avoid any direct exchange of gases between the pack atmosphere and air. A wide range of equipment, designed for various commercial functions, is available for the preservative packaging of meat (Gill, 1995).

Packaging materials should have various certificates that prove it has not any risk of health when contact with food products.

2.1.1.3.1. Permeability of packaging materials

The permeability of the packaging material determines the atmospheric conditions in the headspace and ultimately the shelf-life of the product. If an atmosphere higher in CO_2 and / or lower in O_2 is required, the material should be impermeable to the gases. Vegetables and fruits require a certain amount of O_2 in the headspace for maintenance of quality; therefore, packaging material for these products should be

quite permeable to the oxygen, to allow atmospheric O_2 to replenish the gas in the package. Transparency of packaging material to light is also important.

The choice of packaging material is an extremely important part of the MAP operation. The materials must be cost effective, have low water vapor transmission rate, high gas barrier, and mechanical strength to withstand machine handling and subsequent storage and distribution of the finished pack as well as have the capability of giving high integrity seals to ensure retention of gas within the pack until opened by consumer. Also, once a gas atmosphere is applied, the level and proportion of headspace gas is controlled only by judicious selection of packaging material with specified permeability characteristics.

When selecting packaging films for Equilibrium modified atmosphere packaging (EMAP) of fruits and vegetables the main characteristics to consider are; gas permeability, water vapor transmission rate, mechanical properties, transparency, type of package and sealing reliability. Traditionally used packaging films like LDPE (low density polyethylene), PVC (polyvinyl chloride), EVA (ethylene vinyl acetate co-polymer) and OPP (oriented polypropylene) are not permeable enough for highly respiring products like fresh-cut produces, mushrooms and broccoli. As fruits and vegetables are respiring products, there is a need to transmit gases from and to the package. Films designed with these properties are called permeable films.

These materials provide a range of permeability to gases and water vapor together with the necessary package integrity needed for MAP (Table 2.4).

Table 2.4 Permeability of Plastic Films to Gases (at 30°C) and Water Vapor (at 25°C, 90% R.H)

	Permeability of gases and vapor (mlm^{-2} MPa^{-1} per day)			
Material	**N_2**	**O_2**	**CO_2**	**H_2O**
PE (0.922)	120	300	2300	5300
PE (0.954 - 0.960)	18	71	230	860
PP (0.910)	-	150	610	4500
PVC	2.7	8.0	67	10000
PVdC	0.07	0.35	1.90	94
PS	19	73	590	80000
PA (Nylon 6)	0.67	2.50	10	47000
PET (MylarM)	0.33	1.47	10	8700

The major factors to be taken into account while selecting the packaging materials are:

• The type of package (i.e. rigid or semi-rigid lidded tray or flexible pouch),

• The barrier properties needed (i.e. permeabilities of individual gases and gas ratios when more than one gas is used),

• The physical properties of machinability and strength,

• Integrity of closure (heat sealing), fogging of the film as a result of product respiration,

• Printability

The correct atmosphere at the start will last long only if the permeability of the barrier material does not allow any rapid changes to occur. The transmission rate of the films used should be proportional to the surface area of the package and inversely proportional to the film thickness.

Many of the films used in MAP, singly do not offer all the properties required for a MA pack. To provide packaging films with a wide range of physical properties, many of these individual films are combined through processes like lamination and co-extrusion.

It is very important to analyze food products' requirements for O_2 and vapor permeability. Foods that high in fat should be protected from oxidative rancidity and in order to prolong shelf-life this type of product should be packaged with high O_2 barrier films.

2.1.2. Controlled Atmosphere Packaging

Controlled atmosphere packaging (CAP) is packaging in an atmosphere where the composition of gases is continuously controlled throughout storage. This technique is used primarily for the bulk storage of products and requires constant monitoring and control of the gas composition (Davies and Gibbs, 1994).

Mostly this technique is used for transportation of food products or storage for long times in same storage place.

CAP, in which the atmosphere that is first established remains unaltered in composition throughout the life of the package. The MA and controlled atmosphere CA packagings are variously appropriate for different types of meat and for differing commercial circumstances. Their proper usages can therefore be appreciated only with understanding of the critical differences in deteriorative processes between various fresh meats, the preservative capabilities of the various packaging systems, and the various commercial circumstances within which preservative packagings may be required to function (Gill, 1995).

A truly CA is obtained when product is sealed in a gas-permeable pouch filled with an oxygen-free atmosphere. If CO_2 is a major, or the sole component of the input atmosphere, then the quantity of added gas must be adjusted to assure that the intended atmosphere persists after dissolution of the gas in the product (Gill, 1995).

High-pH beef of average microbiological quality will have a shelf life of about 6 weeks at 5°C or about 20 weeks at -1.5°C, when stored in CAP under CO_2 (Gill, 1995).

CAP with CO_2 atmosphere is increasingly being used for sea freighting lamb primal, and whole lamb carcasses, to distant overseas markets. With that type of packaging, and optimum storage temperatures, microbial spoilage can be delayed beyond 20 weeks when the product is prepared to a high hygienic standard. Moreover, the high concentration of CO_2 inhibits the textural deterioration that would otherwise ruin the eating qualities of the product after that time in storage (Gill, 1995).

2.1.3 Vacuum packaging

Vacuum packaging (VP) is defined as "the packaging of a product in a high barrier package from which air is removed to prevent growth of aerobic spoilage organisms, shrinkage, oxidation, and color deterioration." VP could also be considered as a type of MAP because the removal of air from the environment itself is a modification of the atmosphere and the consumption of residual O_2 in the packs by microorganisms results in the production of CO_2 within the packages (Rao and Sachindra, 2002).

The vacuum package is the most frequently used package type for meat where a MA is applied. The meat is put into a pouch made of suitable plastic film, the air is evacuated, and the pouch is sealed. Other technical procedures are also used, e.g., a

hot web can be molded to the meat on a stiff lower web by establishing a vacuum above the upper web is released (vacuum skin package) (Lund et al., 2000).

However it is produced, a vacuum packed product is encircled by a plastic film that ideally should be gas impermeable. There is no vacuum inside these packages. The air space is strongly restricted, and the proportions of O_2 and CO_2 are altered by the scavenging of O_2 by the meat and the microflora, and by the production of CO_2. These changes affect the metabolism of the microflora (growth and end-product formation), and the product's shelf life will be prolonged. (Lund et al., 2000).

The evolving CO_2 in a meat package can originate from the following: (1) diffusion from a preformed pool in the meat, (2) aerobic energy metabolism of the meat cells, (3) other biochemical reactions of the meat cells, and (4) microbial activity. It is not clear which of these are the most important. However, the presence of O_2 seems to be of minor importance for the initial CO_2 production, when about $2x10^{-3}$ ml CO_2 cm^{-2} of meat surface per hour is produced (Lund et al., 2000). Because CO_2 is highly soluble in water, even after a considerable of the package amount has been produced, the appearance of the package can remain largely unaffected. In vacuum packaged fresh meat that has been chill stored for some days, the concentration of CO_2 will be expected to be least 20% to 40% (v/v), and frequently it is around 90% (v/v), measured in the tiny head space of the package, and the O_2 concentration will be below 1% (v/v). The concentrations vary and are dependent on several factors, eg, the quality of the package film, head space, storage temperature, meat quality, and the composition and activity of the microflora. From the bacteriological point of view, this is the major drawback with the VP of a meat, ,such as, even if a satisfactory shelf life is achieved in a majority of the packages, there will always be a certain number with an unsuspected short shelf life, due to leakage and/or unsatisfactory CO_2 generation. As a consequence, the safety margin between maximum obtainable shelf life and the storage time must be kept wide (Lund, et al., 2000).

In general, VP of refrigerated meat can extend the shelf life three to five times compared with a high pH, for example dark firm dehydrated (DFD) beef or poultry, the extension of shelf life is smaller, and is only about twice that obtained in air (Lund et al., 2000).

The gaseous atmosphere of the VP is likely to change during storage (from metabolism of the product or microorganisms) and therefore the atmosphere becomes modified indirectly (Davies and Gibbs, 1994).

2.1.4 Active Packaging

Active packaging (AP) employs a packaging material that interacts with the internal gas environment to extend shelf life of a food (Erkmen and Bozoglu, 2008). Packaging may be termed active when it performs some role other than providing an inert barrier to external conditions. AP has developed as a series of responses to unrelated problems in maintenance of the quality and safety of foods. Accordingly a range of types of active packaging has been developed. A simple example of this situation is when a plastics package has adequate moisture barrier but an inadequate O_2 barrier. AP solutions could be the inclusion of an O_2 scavenger, or an antimicrobial agent if microbial growth is the quality-limiting variable (Rooney, 1995).

The atmosphere in vacuum packages or modified atmosphere packages can be controlled by the use of O_2 absorbers or carbon dioxide emitters. These techniques can also be used to introduce or withdraw other components in the package, such as, ethylene, which affects the ripening of fruits and vegetables, or ethanol, which suppresses mold growth and retards the staling of bread. The industrial use of O_2 scavengers and ethylene absorbers is increasing, especially in Japan and the United States (US) (Lund et al., 2000).

AP can be seen in one sense as a means of maintaining the optimum conditions to which a food was exposed at the immediately preceding step in its handling or processing. Passive packaging has been used in an effort to minimize the deleterious effects of a limited number of external variables such as O_2, water, light, dust microorganisms, rodents and to some extent, heat. Hence, AP has the potential to continue some aspects of the processing operation or to maintain chosen variables at particular levels. This aspect of AP is a unifying theme and crosses the border between foods such as plant produce, and processed foods, including those thermally processed. A second aspect of AP is that it can be involved in the preparation of the food for consumption. This includes aspects of temperature modification either for

organoleptic or food safety purposes. These properties therefore include heating, cooling and foaming (Rooney, 1995).

2.1.5. Equilibrium modified atmosphere packaging

Equilibrium modified atmosphere packaging (EMAP) is a MAP technique used primarily for the vegetable and fruits; either the pack is flushed with the required gas mix or the produce is sealed within the pack with no modification to the atmosphere. Subsequent respiration of the produce and the gas permeability of the packaging allow an equilibrium-modified atmosphere (EMA) to be reached (Davies and Gibbs, 1994).

When packaging vegetables and fruits the gas atmosphere of package is not air (21% O_2, 0.01% CO_2, 78% N_2) but consists usually of a lowered level of O_2 and a heightened level of CO_2. This kind of package slows down the normal respiration of the product and so prolongs the shelf-life of the product. Of course there are other factors like the size of the product, severity of preparation, maturity of the product and type of tissue that have an effect to the shelf-life of an EMA packaged produce.

2.1.6. High level O_2 MAP

This type of modified atmosphere packaging contains atmospheres that have high levels of O_2 in their composition. This situation has several reasons. Depending on product quality and microbiological safety this conditions may vary. Especially at meat packaging using MAP may need more O_2 in their atmospheres because of keeping color of meat during storage of packages.

Displayed red meats are usually rendered unsalable by discoloration of the muscle tissue surfaces, rather than by microbial spoilage. High concentrations of O_2 will retard that deterioration, by increasing the fraction of oxidation-resistant oxymyoglobin in the pigment at the tissue surface. Moreover, the increased oxymyoglobin concentration will intensify the desirable red color of the meat. Although a high O_2 concentration will delay color deterioration, it will have no adverse effect on the rates of growth of the aerobic spoilage bacteria. The growth of the aerobic bacteria can, however, be slowed by moderate concentrations of CO_2. When the CO_2 content of the atmosphere exceeds 20%, the rate of growth of the population of aerobic bacteria is approximately halved. The rate of growth increases

with decreasing concentration below that value, but higher concentrations cause little further decrease of the growth rate. Thus, an atmosphere of 80% O_2: 20% CO_2 would be optimal for stabilizing the color and microbiological condition of red meat, but in practice such an atmosphere cannot readily be maintained within a display pack. The difficulty arises because O_2 is converted to CO_2 by both the meat and the bacteria; and because CO_2 is highly soluble in meat, and lost more rapidly than O_2 through the films used for lidding display trays. Consequently, the initial concentration of CO_2 must be greater than 20% to allow for the dissolution of that gas in the meat. As storage progresses, the O_2 partial pressure will not be critically important, but excessive reduction of the atmosphere volume will lead to pack collapse. Collapsed retail packs will be essentially unsalable. Although high O_2 MAP master packaging is in use for distribution of centrally prepared retail packs, the system has limited application, because product color will deteriorate, albeit at a reduced rate, while the product is in store, and the preservative atmosphere is lost once the master pack is opened for display of the somewhat compromised product (Gill, 1995).

2.1.7. Low level O_2 MAP

When microbiological condition of a food product is required as good quality, package atmosphere of food products like meat should contain low O_2 in their concentration. But this situation is not suitable for preserving meat color.

Low-O_2 MAP is aimed at exploiting inhibitory effects of CO_2 on the spoilage bacteria without any particular regard for the preservation of meat color. Low-O_2 MAP is typically used for bulk-packed product, with a non-hooded snorkel machine being used to establish the pack atmosphere. It is used to draw only a low vacuum in the pouch, so substantial and variable volumes of air persist when the pouch is gassed. Although pouches may be gassed with CO_2 alone, the dissolution of that gas in the product will require the pouch to be initially over-inflated if the pouch is not to collapse tightly around the product. Therefore, N_2 is often included in the input gas. There is a widespread belief that O_2 in a pack atmosphere will preclude the growth of *Clostridium botulinum*. In fact, anaerobic niches where these bacteria can grow if any are present and if other factors of the environment are favorable, will occur in any package of raw meat irrespective of the surrounding atmosphere. None the less, some O_2 is often included in the gas mixture used for establishing Low-O_2 MAP. As a result of those various factors, the atmosphere initially established in Low-O_2 MAP

can vary widely, with CO_2 from 50 to 90%, N_2 from 10 to 40 %, and O_2 from 1 to 10%. During storage, O_2 concentrations are likely to decrease, so conditions within a pack of initially low O_2 content may finally preclude the growth of strictly anaerobic organisms. In the other packs, the O_2 content may remain sufficient for the growth of strict aerobes to be uninhibited, except by the effects of CO_2. Consequently, the delaying of microbial spoilage by Low-O_2 MAP can range from the modest time obtained with High-O_2 MAP to approaching the very long times attainable with CAP. Obviously Low-O_2 MAP cannot be used when the meat color will be unacceptably degraded by exposure of the product to low concentrations of O_2 (Gill, 1995).

Reduced oxygen packaging (ROP) is another terminology used for Low-O_2 MAP for prolonging the storage life of food products.

2.2 Food products used with MA

2.2.1. Meat

The earliest applications of MAP were on the meat and meat products. During 1920s meat was transported by using MAP in UK. After that studies of prolonging shelf life and keeping freshness of meat by using MAP were accelerated.

The storage life of fresh meats can be extended by sealing products into packages that contain an atmosphere differing from air in the concentrations of N_2, O_2 and CO_2. During the storage, the atmosphere first established in the pack may alter, through interactions between the product and the atmosphere, and/or by the exchange of gases across the plastic film used for forming the pack (Gill, 1995).

2.2.1.1. Spoilage

The initial microflora of meat, for example, beef, lamb, pork, and poultry, always represents a complex mixture originating from skin and mucous membranes, and from environmental sources such as soil and water. Typical groups present are *Acinetobacter*, *Pseudomonas*, *Flavobacterium*, *Moraxella*, *Enterobacteriaceae*, *Staphylococcus*, *Salmonella*, *Listeria*, and *Micrococcus*. But even if the microbial variety is huge, the total numbers of organisms are usually relatively low. The environment then enforces a selection pressure on the microflora, and the types of organisms most fitted for the specific environment will outgrow the others, become

dominant, and reach high numbers and spoil the meat. The microbial spoilage point of a food can be defined as "the time at which the activities of microbes present in a product become offensively evident to the senses." (Lund et al., 2000).

The typical spoilage flora of refrigerated meat stored at 8°C or lower in air is composed mainly of *Pseudomonas* spp.

The interior of fresh meat from healthy animals is almost free of bacteria and other microorganisms. The surface is where that the microorganisms multiply and reach numbers high enough to cause spoilage. As a general rule, meat acquires an offensive odor when the bacterial flora reaches about 10^7 colony forming unit (cfu) cm^{-2} of meat surface, and when the numbers have reached about 10^8 cfu cm^{-2} the meat surface becomes slimy. At this point, the easily available nutrients on the surface become depleted; the microbial flora starts to become proteolytic and penetrates into the meat. Before the bacteria attack the protein, they utilize more easily available carbon and energy sources; proteins must be hydrolyzed to amino acids before they can easily be utilized. Easily utilized compounds in meat, other than free amino acids (about 0.4% w/w) are lactic acid (0.9%) and glucose (0.01%). In spite of these relatively low concentrations, the spoilage flora can be well supported up to around 10^7 cfu cm^{-2} due to diffusion of nutrients from the interior of the meat (Lund et al., 2000).

Off-odors result primarily from the production of ethyl and methyl esters, short chain fatty acids, and sulfur-containing compounds. From the bacteriological point of view, no general differences exist among the spoilage of meat from cows, pigs, lambs, and poultry. However, the shelf-life can differ because of differences in contamination levels, the pH of the meat and the ratio between meat surface and volume, which is often high for poultry (Lund et al., 2000).

2.2.1.2. Vacuum package

VP is the most frequently used package type of meat where a modified atmosphere is applied. The meat is put into a pouch made of a suitable plastic film, the air is evacuated, the pouch collapses around the product, and the pouch is sealed. The vacuum packed product is encircled by a plastic film that ideally should be gas impermeable. There is no vacuum inside these packages. The air space is strongly

restricted, and the proportions of O_2 and CO_2 are altered by the scavenging of O_2 by the meat and the microflora, and by the production of CO_2. These changes effect the metabolism of the microflora (growth-and end-product formation), and the product's shelf life will be prolonged. The evolving CO_2 in a meat package can originate from the following: (1) diffusion from a preformed pool in the meat, (2) aerobic energy metabolism of meat cells, (3) other biochemical reactions of the meat cells and (4) microbial activity. It is not clear which of these are the most important. However, the presence of O_2 seems to be of minor importance for the initial CO_2 production, when about $2x10^{-3}$ ml CO_2 cm^{-2} of meat surface per hour is produced. Because CO_2 is highly soluble in water, even after a considerable amount has been produced, the appearance of the package can remain largely unaffected. The concentrations vary and are dependent on several factors, such as, the quality of the package film, head space, storage temperature, meat quality, and the composition and activity of the microflora. From the bacteriological point of view, this is the major drawback with the vacuum packaging of meat, in other words; even if a satisfactory shelf life is achieved in a majority of the packages, there will always be a certain number with an unsuspected short shelf life, due to leakage and/or unsatisfactory CO_2 generation.

As a consequence the safety margin between maximum obtainable shelf life and the storage time must be kept wide. In general VP of refrigerated meat can extend the shelf life three to five times compared with that obtained with air. However, for meat with a high pH, for example DFD beef (dark firm dehydrated beef) or poultry, the extension of shelf life is smaller, and is only about twice that obtained in air (Lund et al., 2000).

2.2.1.3. Modified atmosphere packing with a head space of meat

MAP of meat can be used for both display package and bulk packaging. The optimal gas mixture can be selected either (1) to obtain a limited shelf-life of meat and retain a desirable color or (2) for a maximal prolongation of shelf life without any major considerations about the meat color. The former mostly used for display packaging and the latter for bulk packaging (Lund, et al., 2000).

The color of raw meat is to a high degree dependent on the oxidation state of myoglobin. The biological role of myoglobin is to carry O_2, and the oxygenated myoglobin (oxymyoglobin) is bright red. This is the color most commonly associated

with freshness because the consumer is accustomed to the appearance of meat exposed to the O_2 of the air. The oxygenation is rapidly reversible with changes in the partial pressure of O_2; at low partial pressures the meat color darkens due to the purple color of the deoxygenated myoglobin. The time taken for myoglobin to turn to oxymyoglobin when exposed to air is 0.5 to 1 hour (Lund et al., 2000).

If the color is important, as in a display package the meat can be packed in an atmosphere of 70 to 80% (v/v) O_2 (to ensure a fresh red color) and 20 to 30% CO_2 in order to obtain a reasonable shelf life. This type of package gives a shorter shelf life than VP, but provides a shelf life about twice that of storage in air (Lund et al., 2000).

Raw meat and prepared meat in Turkish codex has microbiological limitations these are given in Tables 2.5-2.6 respectively.

Table 2.5 Microbiological criteria for raw meat (Turkish Codex of Raw Meat 2006/31)

	n	**c**	**m**	**M**
Aerobic mesophilic bacteria	5	2	$5.0x10^5$	$5.0x10^6$
Escherichia coli O157:H7	5	0	Should be zero in 25 g	
Staphylococcus aureus	5	2	$5.0x10^2$	$5.0x10^3$
Pseudomonas	5	2	5.0×10^4	5.0×10^5
Salmonella spp.	5	0	Should be zero in 25 g	

Table 2.6 Microbiological criteria for prepared meat (Turkish Food Codex of prepared meat 2006/31)

	n	**c**	**m**	**M**
Aerobic mesophilic bacteria	5	2	$5.0x10^5$	$5.0x10^6$
Escherichia coli O157:H7	5	0	Should be zero in 25 g	
Staphylococcus aureus	5	2	$5.0x10^2$	$5.0x10^3$
Bacillus spp.	5	2	$1.0x10^4$	$1.0x10^5$
Clostridium perfringens	5	2	$2.0x10^1$	1.0×10^2
Pseudomonas	5	2	5.0×10^4	5.0×10^5
Total mold and yeast	5	2	$1.0x10^3$	$1.0x10^4$
Salmonella spp.	5	0	Should be zero in 25 g	

n : Sample number for analysis
c : Sample number that can be taken into analysis which has microorganism numbers between m and M
m : The highest acceptable number of microorganism that can be found in 1 g of sample which are taken as (n – c) number
M : The highest acceptable number of microorganism that can be found in 1 g of sample which are taken as c number.

2.2.2. Meat products

Meat and meat products includes a wide variety of different products, not always within stringent borderlines. The group includes traditional cured meats such as bacons, beef, sausages (like sucuk), and hams and also convenience food products such as premade hamburgers and meatballs. Especially important factors to consider are whether the product has been heat treated, the concentration of NaCl, and the water activity.

2.2.2.1. Sucuk

Sucuk is a fermented meat product which can be also called as "Turkish style dry fermented sausage". Sucuks are sausages of beef meat, mixed with fat and other ingredients, stuffed into sheep or beef casings and exposed to drying, including sometimes sun drying. They are often annular shaped (Erkmen, 2008). Composition of meat skeletal muscle (lean) used in the production of sucuk are given in Table 2.7.

Table 2.7. Approximate composition of sucuk meat (% wt). (Erkmen and Bozoglu, 2008)

Component	Composition (%)
Water	75.0 (65-80)
Protein	18.5 (16-22)
Lipids	3.0
Non-protein nitrogenous substances	1.5
Free amino acids	0.3
Carbohydrates	1.0
Inorganic compounds	1.0
pH	5.5-5.9

The sucuk has pinkish color, cherry rotten, and marble appearance, not soft or hard with finger pressure, and no pieces on the cutting knife. Manufacturing procedures of sucuk involves the following basic steps: (i) reducing the particle size of high-quality raw meat; (ii) incorporation of salt, nitrate/nitrite, glucose, spices, sausages, and a desired specific inoculum of LAB; (iii) uniformly blending all ingredients and further reducing particle size; (iv) vacuum stuffing into a semipermeable casing to minimize the presence of O_2; (v) incubation (ripening) at or near the temperature optimum of the starter culture until a specific pH end-point; (vi) heating (product dependent) the product to inactivate the inoculum and ensure pathogen destruction; and (vii) drying

(aging) the product to the required moisture/protein endpoint (Erkmen and Bozoglu, 2008).

Formulation of sucuk (Erkmen and Bozoglu, 2008): Basic ingredients for 100 kg: 90 kg fresh beef meat (with 18% fat) (or 60 kg beef meat, 30 kg mutton or lamb meat), 10 kg tail fat and seasoning. Seasoning formula per 1 kg: 5.5 g cumin, 1.1 g cinnamon, 11.4 g allspice, 0.5 g clove, 5.5 g red pepper, 11 g black pepper, 20.6 g garlic, 0.4 g ginger, 2.0 g sugar, 18 g NaCl, 0.3 g $NaNO_3$, 0.05 g $NaNO_2$ and 2.1 g olive oil. Starter culture is added into sucuk dough with 10^{6-7} cfu/g.

Turkish codex for meat products (TFC 2000/4) includes sucuk as a fermented meat product. And limitations are given below:

Chemical criteria for fermented sucuk

- the fat content should be 40% maximum,
- the moisture content should be 40% max,
- Salt content must be 3.30% max,
- Protein content must be 20% max,
- pH must be 5.4 max,
- No coloring matter present, or added,
- During sucuk production starch must not be used.

Organoleptic criteria for fermented sucuk

- Flavor; must be in its original flavor,
- Taste; must be no rancid, no sour, no bitter,
- Appearance; must be in appropriate color and texture

Microbiological criteria for sucuk

Sucuk as a fermented meat product has high microbial activity in it. So it should be checked in detail for keeping public health safe. According to Turkish food codex microbiological criteria of meat products are given below. Microbiological criteria of sucuks are given in Table 2.8.

Table 2.8. Microbiological criteria of meat products in Turkish food codex (2000/4)

	n	c	m	M
Escherichia coli (cfu/g)	5	1	5×10^1	1×10^2
Escherichia coli (cfu/g)*	5	0	Should be zero	
E. coli O157: H7(cfu/g)	5	0	Should be zero	
Staphylococcus aureus (cfu/g)	5	1	5×10^2	5×10^3
Clostridium perfringens (cfu/g)	5	2	1×10^1	1×10^2
Salmonella (cfu)	5	0	Should be zero in 25 g	
Listeria monocytogenes (cfu)	5	0	Should be zero in 25 g	
Mold and yeast (cfu/g)	5	2	1×10^1	1×10^2

* Heat treated products
n : Sample number for analysis
c : Sample number that can be taken into analysis which has microorganism numbers between m and M
m : The highest acceptable number of microorganism that can be found in 1 g of sample which are taken as (n – c) number
M : The highest acceptable number of microorganism that can be found in 1 g of sample which are taken as c number.

2.2.3. Dairy products

Raw milk is mainly spoiled by *Pseudomonas fluorescens* biovar1, *Pseudomonas fragi*, *Pseudomonas lundensis*, and *Pseudomonas fluorescens* biovar 3. the bacteriological shelf life of refrigerated milk can be prolonged by applying an increased partial pressure of CO_2 (Lund et al, 2000).

2.2.3.1. Cheese

Defects in cheeses may be caused several types of microorganisms; (i) Mold and yeats, (ii) lactic acid bacteria (LAB), (iii) coliform bacteria, (iv) spore forming bacteria (Erkmen and Bozoglu, 2008). In cheese major microbiological problem is mold and yeasts on the surface. They can be suppressed by a modified atmosphere containing low O_2 levels and increased CO_2 concentrations. VP occasionally fails to protect against molds (Lund et al., 2000). The most common molds associating on cheeses are, *Penicillium* spp., with others, including *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Hormodendrum*, and *Mucor*. If the initial contamination level is limited, growth of fungi can be inhibited with further preservation methods. These include packaging to reduce O_2 (or increase CO_2), cold storage, and use of antimicrobial chemicals such as, sorbate, propionate, and

natamycin (pimaracin). Liquid smoke addition is also a potent mold inhibitor. None of these control measures is completely effective alone. Some of the molds are resistant to sorbate. *Penicillium* spp. are sorbate-resistant molds and also they can degrade it by decarboxylation to 1,3-pentadiene (Erkmen and Bozoglu, 2008).

Among the pathogenic microorganisms, especially *Listeria monocytogenes* (pathogen interested in this thesis) can easily grow and may become a threat in cheeses.

2.3. Effect of MA on microorganisms in meat, sucuk and cheese

As all known, microbial activity in foods occur in two major ways as spoilage and illness or pathogenic, such as, the microorganisms in foods may spoil the food or may become illness in human body. Both pathogenic and spoilage microorganisms can be inhibited by MAP. First of all initial microbial load of foods should be controlled and besides the natural microbial flora and dominating taxa there should not be any other foodborne pathogen and spoilage microorganisms. Natural microbial flora in foods such as meat, sucuk and cheese may spoil the foods during long storage time, these can be stopped by using suitable atmospheric conditions for these types of foods but pathogens may still grow and cause foodborne illnesses to human body. In this research as pathogenic microorganisms; *Salmonella* Typhimurium and *Listeria monocytogenes* were studied with MAP meat, sucuk, and cheese. Other microorganisms; aerobic bacteria (AB), mold and yeasts (MY), and also lactic acid bacteria (LAB) were counted and results were recorded.

2.3.1. Spoilage of Modified atmosphere packaged meat, sucuk and cheese

2.3.1.1. Spoilage of modified atmosphere packaged meat

The predominant microorganisms in MAP meats are lactobacilli and *B. thermosphacta*. The vacuum and gas packaged products can be spoiled by psychrotrophic *Lactobacillus* (including homofermentative *Lb. curvatus*, *Lb. sake* and heterofermentative *L. viridescens*) and *Leuconostoc* (*Leu. carnosum*, *Leu. gelidium*, *Leu. mesenteroides*). They cause;

- Cloudy appearance,
- Accumulation of CO_2 and liquid

- Slime formation due to bacterial cells
- Dextrane production by *Leuconostoc* spp. In products containing sucrose.

Serratia spp. Produce ammonia-like flavor due to amino acid breakdown. Some refrigerated foods may have brown to yellow spots with concentration of nitrites is present in vacuum-packaged meat products, they inhibit *B. thermosphacta* and psychrotrophic *Enterobacteriaceae,* so lactic acid bacteria (LAB) can dominate. Refrigerated meat in MAP, such as in a mixture of CO_2 and O_2 will favor the microaerophilic *B. thermosphacta*, especially in meat with pH 6.0 or higher.

Lactobacillus spp. metabolize glucose to produce lactic acid, and the amino acids, leucine and valine to isovaleric and isobutyric acids (cheesy odor, at level over 10^8 cells/cm^2) when they metabolize cysteine and produce H_2S, the product will have undesirable odor and color. *Shewanella putrefaciens* can metabolize amino acids under both aerobic and anaerobic conditions to produce methysulfides and H_2S in large quatities. H_2S oxidizes myoglobin to metmyoglobin and causing greening of the meat (Erkmen and Bozoglu, 2008).

2.3.1.2. Spoilage of modified atmosphere packaged sucuk

Main spoilage factors in sucuks were;

- Lipid oxidation (malonaldehyde formation); one of the most important sucuk deterioration causes.Color, flavor, texture and nutritional value.
- Color formation (nitrosomyoglobin conversion); when nitrite is first added to meat the color is changed from the purple read color of myoglobin to the brown of metmyoglobin. With time and reducing conditions, the color is converted to dark red nitrosomyoglobin (red cured color).
- Formation of biogenic amines; depends on the ripening and storage conditions of product, presence of amine positive microorganisms, free amino acids, quality of raw materials and poor process conditions.

Microorganisms responsible from spoilage of VP and MA packaged meat and meat products are listed in Table 2.9.

Table 2.9 Types of microbial spoilage in VP and MA packaged meat and meat products (Modified from Erkmen and Bozoglu, 2008)

Meat and Meat product	Alteration	Microorganisms
Vacuum packaged meats	Putrefaction and gas production	*Clostridium, Alcaligenes*
	Souring, off odor	*Lb. carnosus, Lb. gelidium, Leu. mesenteroides, Alcaligenes, Pediococcus*
Vacuum packaged cooked meats	Souring off odor	*Leuconostoc, Lactobacillus, Carnobacterium*
MAP meat in 100% CO_2	Souring	*B. thermosphacta*
MAP cured meat	Cheesy odor, souring rancid	*Micrococcus, Lactobacillus, Alcaligenes, Bacillus, Clostridium*

2.3.1.3. Spoilage of modified atmosphere packaged white cheese

Cheeses undergo spoilage with bacteria, yeasts and molds (Table 2.10). The most common bacterial spoilage is slimy curd caused by *Acinetobacter*, *Alcaligenes*, *Enterobacter*, *Proteus*, and *Pseudomonas*. *Clostridium* spp. are responsible from gassiness of cheese. Fungi cause stale, musty, moldy, and yeasty defect. Molds generally causing spoilage on cheese are *Alternia*, *Geotrichum*, *Mucor* and *Penicillium* (Erkmen and Bozoglu, 2008).

2.3.2 Foodborne Illness

Foodborne illnesses were caused by various types of foods and microorganisms. The number of different microorganisms recognized to cause foodborne illness has increased from around 5 identified by the beginning of the 20th century, to 20 by the early 1950s to more than 40 by the early 1990s. New foodborne agents continue to be identified and in the last 5 years at least six new ones have been reported. Some of the years in which evidence of the association of a microorganism with Foodborne illness was first reported are listed in Table 2.11.

Table 2.10 Types of microbial spoilage in cheese (Erkmen and Bozoglu, 2008).

Defect	**Microorganisms**	**Products**
Early gas	Coliforms, yeasts, *Lactobacillus*	CO_2, hydrogen
Fruity	*Lactococcus*, Pseudomonas, yeasts	Ethanol
Late gas	*Clostridium* spp.	CO_2, hydrogen
Off-flavor	Heterofermentative lactobacilli	CO_2
Phenolic discoloration	*Lb. casei* subsp. *alactosus*, *Lb. casei* subsp. *rhamnosus*	Phenolic compounds
Pink discoloration	*Lb. bulgaricus*, *Fusarium culmorum*, *Propionibacterium*, yeasts	High redox potential
Rancid	Psychrotrophic bacteria	Free fatty acid
Slime curd	*Acinetobacter*, *Enterococcus*, *Alcaligenes*, *Pseudomonas*	Polymeric products
Soft defect	*Lb.* casei subsp. *casei*	Organic acids
White crystallines	Heter. *Lactobacillus* spp.	Excessive D-lactate

Table 2.11. Some dates of first reported evidence of foodborne or waterborne transmission of the following pathogenic agents (Lund et al., 2000)

Year	**Pathogenic Agent**
1884	*Vibrio cholera*
1888	*Salmonella*
1896	*Shigella*
1897	*Clostridium botulinum*
1914	*Staphylococcus aureus*
1939	*Yersinia enterocolitica*
1945	*Clostridium perfingens*
1949	*Listeria monocytogenes*
1950	*Bacillus cereus*
1951	*Vibrio parahemolyticus*
1976	*Cryptosporidium*
1977	*Campylobacter jejuni*
1978	Small round structured viruses
1982	Verocytotoxigenic *Escherichia coli*
1986	Cyclospora
1997	Bovine spongiform encephalopathy agents

Foodborne illnesses cause financial lost besides public health lost. For example, in 1994 there was an incident in the US involving *Salmonella enteritidis* contamination of a pasteurized ice cream mix that resulted in an estimated 224,000 persons becoming ill. Examples of estimated financial costs of food poisoning incidents resulting from commercially produced foods in the UK are given in Table 2.12.

Table 2.12. Examples of Costs of Foodborne Illness in the UK Caused by Commercially Produced Foods (Lund et al., 2000)

Year	Illness	Food Involved	Cost Estimate*
1964	Typhoid	Canned corned beef[1]	£30 million
1978	Botulism	Canned salmon[1]	£4.0 million
1979	Staphylococcal food poisoning	Canned corned beef[1]	£2.0 million
1987	Salmonellosis	Infant dried milk	£3.0 million
1989	Salmonellosis	Salami sticks[1]	£5.0 million
1989	Botulism	Hazelnut yogurt	£6.5 million

*Approximate costs (inflated to 1997 costs) based on available information on costs of investigations, losses of earnings of victims, medical costs, and industry costs (recall, destruction of stock, loss of business).
[1]Imported Product

It was reported that the total burden of foodborne illness per year caused approximately 76 million illnesses, 323 000 hospitalizations and 5000 deaths in the USA (Table 2.13). Three pathogens (*Salmonella*, *Listeria* and *Toxoplasma*) were responsible for 1500 deaths per year which is more than 75% of those caused by known pathogens. Unknown agents caused 62 million illnesses, 265 000 hospitalizations and 3200 deaths. Using a population size of 270 299 000 (USA Census Bureau 1998) this equates to 28% of the population suffering from food poisoning each year and 0.1% being hospitalized due to food poisoning (Forsythe, 2000).

2.3.2.1. *Salmonella*:

Salmonella is a genus of the Enterobacteriaceae family. They are Gram-negative, facultative anaerobic, non-sporeforming short (1-2 μm) rods. The majority of species are motile with peritrichous flagella. Salmonella ferment glucose with the production of acid and gas but are unable to metabolize lactose and sucrose.

Table 2.13. Estimated illnesses, hospitalizations, and deaths caused by known foodborne pathogens in the USA (modified from Forsythe, 2000)

Disease or agent	**Illnesses**				**Hospitalization**		**Deaths**	
Bacterial	**Total**	**Foodborne**	**% Foodborne transmission**	**% of total foodborne**	**Foodborne**	**% of total foodborne**	**Foodborne**	**% of total foodborne**
Bacillus cereus	27 360	27 360	100	0.2	8	0.0	0	0.0
Botulism, foodborne	58	58	100	0.0	46	0.1	4	0.2
Brucella spp.	1 554	777	50	0.0	61	0.1	6	0.3
Campylobacter spp.	2 453 926	1 963 141	80	14.2	10.539	17.3	99	5.5
Clostridium perfingens	248 520	248 520	100	1.8	41	0.1	7	0.4
Listeria monocytogenes	2 518	2 493	99	0.0	2 298	3.8	499	27.6
Salmonella typhi	824	659	80	0.0	494	0.8	3	0.1
Salmonella, non-typhoidal	1 412 498	1 341 873	95	9.7	15 608	25.6	553	30.6
Staphylococcus aureus	185 060	185 060	100	1.3	1 753	2.9	2	0.1
Streptococcus, foodborne	50 920	50 920	100	0.4	358	0.6	0	0.0
Vibrio chlorae,toxigenic	54	49	90	0.0	17	0.0	0	0.0
Yersinia enterocolitica	96 368	86 731	90	0.6	1 105	1.8	2	0.1

Their optimum growth temperature is about 38°C and their minimum is about 5°C. Since they are non-sporeformers, they are relatively heat sensitive, being killed at 60°C in 15-20 minutes.

Characteristic symptoms of salmonella food poisoning included (Erkmen and Bozoglu, 2008):

- Diarrhea
- Nausea
- Abdominal pain
- Mild fever and chills
- Sometimes vomiting, headache and malaise

The incubation period before the illness is between 16 and 72 hours. The illness is usually self-limiting, lasting 2-7 days. The infected person will be shedding large numbers of salmonellae in the feces during the period of illness. A wide range of foods is associated with Salmonella food poisoning, including raw meats, meat products, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces and salad dressings, cake mixes, cream-filled desserts and toppings, dried gelatin, peanut butter, cocoa and chocolate.

Contamination of the food is through poor temperature control or handling practices, or cross-contamination of processed foods from raw ingredients. The organism multiplies an infectious dose. *S. typhi* and *S. paratyphi* A, B and C produce typhoid and typhoid-like fever in humans. Typhoid fever is a life threatening illness.

Typical symptoms of typhoid fever are:

- Sustained fever as high as 39-40°C
- Lethargy
- Abdominal cramps
- Headache
- Loss of appetite
- A rash of flat, rose-colored spots may appear

The fatality rate of typhoid fever is 10% compared to less than 1% for most forms of salmonellosis (Forsythe, 2000).

2.3.2.2. *Listeria monocytogenes*:

Listeria are Gram-positive, non-sporeforming bacteria. They are motile by means of flagella and grow between 0 and 42°C.they are less sensitive to heat compared with *Salmonella* and hence pasteurization is sufficient to kill the organism. The genus is divided into eight species of which *L. monocytogenes* is the species of primary concern with regard to food poisoning. It is resistant to diverse environmental conditions and can grow at temperatures as low as 3°C. The foods include *L. monocytogenes* are pasteurized milk and cheese, meat (including poultry), and meat products, raw vegetables, fermented raw-meat sausages as well as sea food and fish products. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying. Its ability to grow at temperatures as low as 3°C, permits multiplication in refrigerated foods. *L. monocytogenes* is responsible for opportunistic infections, preferentially affecting individuals, whose immune system is perturbed, including pregnant women, newborns and the elderly.

Symptoms of listeriosis are:

- Meningitis, encephalitis or septicemia
- It can lead to abortion, stillbirth or premature birth when pregnant women are infected in the second and third trimesters
- The incubation period is extremely wide at 1-90 days.
- *L. monocytogenes* may invade the gastrointestinal epithelium.

Listeriosis has a very high mortality rate. When listeric meningitis occurs overall mortality may be as high as 70%. Cases of septicemia have a 50% fatality rate whereas in perinatal-neonatal infections the rate is greater than 80%. In infections during pregnancy the mother usually survives. Infection can be symptomless, resulting in fecal execrators of infectious *Listeria*.

2.3.2.3 Mold and Yeasts

Molds and yeasts are most popular groups of microorganisms responsible from spoilage. Especially with the presence of oxygen in the gas atmosphere they can spoil the food products easily. Molds are predominate spoilage microorganisms in the meat cuts when the surface is too dry for bacterial growth or when beef can be

treated with antibiotics such as with tetracylines. Molds do not grow on meats when bacteria grow freely. This would be due to higher growth rate of bacteria and consumption of free oxygen.

Most important genera of molds spoiling fresh meats are *Thamnidium*, *Mucor* and *Rhizopus* to produce "whiskers"; white growth by *Mucor mucedo*, *M. lusitanicus*, *M. racemosus*, *Thamnidium chaetocladioides*, *T. elegans*, *Sporotrichum carnis*, *Rhizopus*, and others; black spot by *Cladosporium herbarum*; and green patches by *Penicillium expansum*, *P. asperulum* and *P. oxalicum*. Molds do not grow on meats if the storage temperature is below -5°C. Genera of yeasts spoiling refrigerated meats are *Candida* (*C. lipolytica*, *C. zeylanoides*), *Torulapsis* and *Rhodotorula* (Erkmen and Bozoglu, 2008).

Fermented products like sucuk has a microbial flora containing lactic acid bacteria and mold and yeasts cannot suppress this strong microflora mostly. But minced meat (as raw material of sucuk) more rapidly spoiled than meat cuts because of increasing surface area which are suitable for growth of spoilage microorganisms.

2.3.2.4. Starter cultures

These cultures are added to fermented foods during fermentation and responsible from an appropriate fermentation in foods. During sucuk preparation, starter cultures are added to decrease pH and prepare a medium for fermentation. Bacterial starter cultures have a variety of functions including: (i) Boosting acidity (decreasing pH), (ii) Intensify the curing color (acid environment catalyses curing reaction), (iii) Counteract rancidity of fats (due to enzymatic impacts), (iv) Development of flavor and taste, and (v) Texture improvement of ripened products (by supporting formation of protein gel in sucuk mixes). Through their physiological activity, the starter cultures ensure the rapid decrease of the pH, guarantee the safety of the product and the uniformity of its flavor, color and texture and a shortening of the production cycles.

Starter cultures used for the production of sucuk can be isolated from naturally fermented meat or commercially obtained. The most commonly isolated LAB from dry-fermented sucuk and used for sucuk manufacturing are *Lb. sake*, *Lb. curvatus*, *Lb. plantarum*, *Ped. pentosaceus*, and *Ped. acidilactici* (Erkmen and Bozoglu, 2008).

Currently, there are bacterial starter cultures which belong to the *Lactobacillus*, *Pediococcus, Micrococcus* and *Staphylococcus* genus and which are commercialized for meats as singular cultures or as mixtures of 2 or 3 strands. The bacterial starter cultures are available frozen, freeze-dried or as liquids stabilized at low temperatures.

2.4. Other modified atmosphere packaged food products

2.4.1. Bread and bakery products

The main spoilage factors for bakery products are mold growth and chemical breakdown. Fermentation may also cause problems in pastries or breads that have been filled. Since the water activity of bakery products is low, the growth of microorganisms other than mold is seldom a problem. Mold is an aerobic microorganism which can be effectively controlled by packaging the produce in a MA of CO_2. This extends shelf-life by many valuable days. MAP is especially suitable for rye bread, sweet bakery products and pies (Linde Gas, 2005).

Bread can be packed in vacuum or modified atmosphere in order to delay staling or to hinder the growth of molds, such as, 70 to 80% (v/v) CO_2 and 20 to 30% N_2 (Lund et al., 2000).

2.4.2. Seafood

Fresh fish and seafood rapidly lose their original quality. This is due to their high water activity, neutral pH (where microorganisms thrive) and the presence of enzymes, which rapidly undermine both taste and smell. The breakdown of proteins by microorganisms gives rise to unpleasant odors.

The oxidation of unsaturated fats in high-fat fish such as salmon, herring and mackerel also results in unappetizing taste and smell. Fish such as herring and trout can turn rancid even before microbial deterioration is detectable (Linde Gas, 2005).

2.4.3. Fruits and vegetables

MAP can help to preserve the quality and extend the shelf life of fresh fruit and vegetables. MAP is only suitable for produce of the highest original quality. All fruit and vegetables intended for MAP must be handled hygienically and gently during harvesting, storage, preparation, packaging and distribution and have been maintained at the correct chilled temperature (Linde Gas, 2005).

The rate of respiration of fruits and vegetables can be reduced by raising the partial pressure of CO_2 and decreasing the partial pressure of O_2. However, a too high level of CO_2 will result in tissue damage, similar to that caused by too low temperatures. Several processes in vegetables and fruits other than respiration are influenced by the gas atmosphere, such as, ripening, production of ethylene, breakdown of chlorophyll, and structural changes due to the breakdown of pectin. These processes are different for different products, for example, between apples and pears. Thus, the design of controlled or modified atmospheres is different for different types of fruits and vegetables.

Apples can be stored in a mixture of 5 to 10% (v/v) CO_2 and about 3% O_2 and N_2 as the filler; tomatoes can be kept in an atmosphere of 3 to 9% CO_2, 3 to 9% O_2 and N_2; suitable atmosphere for strawberries is 10 to 20% CO_2, with low O_2 concentration and N_2 as a filler gas (Lund et al., 2000).

2.4.4. Fats and oils

Oils and fats, and foods with a high oil or fat content, are susceptible to deterioration through oxidation. The objective here is to preserve quality by avoiding oxidation. Oxidation refers to a chemical reaction where atmospheric O_2 attacks the fat acid chains of triglyceride molecules. The O_2 attack can take place at ambient temperatures or below (i.e. during normal storage of oils or finished food products). It can also occur at elevated temperatures, such as during processing or deep-fat frying. In order to protect the oil and improve oil stability, O_2 should never come in contact with the product at any stage of the production process. In line with the worldwide trend towards the replacement of harsh physical/chemical preservative methods with less severe alternatives, the food industry is increasingly relying on N_2 to resolve the oxidation challenge. Because N_2 is an inert gas, it does not interact directly with the product. It is used to displace atmospheric air (and thus O_2) and water vapor to avoid oxidation. There are two main processes for replacing O_2 with N_2: sparging to remove the dissolved O_2; and blanketing to assure the absence of O_2 in the headspaces. Sparging involves injecting a gas into a liquid. The process used will obviously depend on the type of product and the processing stage. For example, N_2 blanketing is beneficial for the holding tanks for intermediate oils and the storage tanks for crude oils and finished oils. Sparging is applied to remove O_2 from a

product. There is a third process called fluffling. This is used widely in the fats and mayonnaise industry. The main objective of this technique is to change the texture of the product. It is mainly applied to fats for bakery products to assure the desired density and avoid the presence of air (Linde Gas, 2005).

2.4.5. Prepared catered foods

With prepared foods, the rate of deterioration depends on the ingredients and varies considerably from one product to another. Take meat-based ravioli or lasagna, for example. The meat spoils at a different rate than the pasta. One of the major challenges associated with prepared foods is how to avoid introducing microbial contamination during the manufacturing process. To achieve this, manufacturers must ensure the highest standards of hygiene and the highest quality raw materials (Linde Gas, 2005).

CHAPTER 3

MATERIAL AND METHODS

3.1. *Listeria monocytogenes* contaminated Meat, Sucuk, and White Cheese

3.1.1. Preparation of Media and Solutions

3.1.1.1. Preparation of media

For culturing of microorganisms and microbiological analysis different media were prepared. In order to make total count, BHIA (Difco) was used. BHIA was prepared and poured on petri plates and kept for further use(Erkmen 2007). For culturing, BHIB was prepared.

For *L. monocytogenes* count, Listeria selective agar (LSA, Oxoid) were prepared. For preparation of LSA, Listeria selective supplement (Oxoid) was added.

For mold and yeast count, potato dextrose agar (PDA, Difco) was prepared and pH of the medium was adjusted with addition of tartaric acid after sterilization of medium.

For lactic acid bacteria (LAB) count, MRSA was prepared.

All agar media were sterilized in autoclave at 121°C for 15-20 min. And then they were poured into sterile petri dishes.

3.1.1.2. Preparation of solutions

a) Preparation of peptone water

Peptone water is used for sample and dilution waters. Peptone was readily powdered for mixing with distilled water and it was weighed on sensitive balance. 0.1 % peptone water was used during preparation of dilution waters, culture solution and preparation of sample waters. Simply 1 g of peptone powder was mixed with 1000

ml of distilled water in a clean balloon, then distributed to bottles and test tubes in required amount and sterilized at 121ºC for 20 min in autoclave.

b) Preparation of dilution water

Dilution water is used for diluting the sample of food products that is expected to include microorganisms in it. In order to prepare sterile dilution water, first 9 ml of 0.1% peptone water was put into test tubes in aseptic conditions and mouths of tubes were closed by the lids. After preparing 9 ml 0.1 % peptone water including test tubes, they were sterilized in autoclave at 121°C for 20 min. The test tubes were stored at refrigerator for further use. For homogenization of solid food samples 225 ml 0.1 % peptone water was prepared in 250 ml flasks. 225 ml of 0.1 % peptone water was poured into 250 ml flask, a magnetic stirrer rod was put into the flask too and the mouths of flasks were closed by cotton and aluminum foil and tied. After closing flasks, they were sterilized in autoclave at 121°C for 20 min. The flasks were stored at refrigerator for further use.

3.1.2. Preparation of culture

3.1.2.1. *Listeria monocytogenes*

Listeria monocytogenes serotype 4a and ATCC 13932 were obtained from University of İstanbul, Faculty of Medicine, Microorganism's Culture Collection Research and Applied Center, İstanbul, TURKEY and LGC Standards GmbH Mercatorstr, 51 46485 Wesel Germany, respectively. Bacteria on slant agar medium was subcultered twice in brain heard infusion broth (BHIB, Difco) at 35^{o}C for 48 h. Brain heard infusion agar (BHIA, Difco) slant of stock cultures were incubated at 35°C for 18 h and stored at refrigerator during the usage period of experiments. It was activated in BHIB to prepare subculture when it was desired to use.

For addition into foods, subcultered *L. monocytogenes* was inoculated twice into BHIB and incubated at 35^{o}C for 18 h. Two 250 ml flasks of BHIB were inoculated by *L. monocytogenes* and incubated at 35°C for 18 h. Cells of *L. monocytogenes* were harvested by centrifugation at 5000 g for 20 min, washed twice with sterile saline and resuspended in saline solution (0.85 % NaCl) and used as working culture. One loop of the stock culture was then streaked onto sterile listeria selective agar (LSA,

Difco) to ensure for the confirmation of pure *L. monocytogenes* and incubated at 35°C for 48 h. The working culture was added into food samples.

3.1.2.2. Lactic acid bacteria

A commercial dry mixed starter culture (*Lactobacillus plantarum*, *Pediococcus acidilactici* and *Staphylococcus carnosus*) (Bactoferm, Chr.Hansen, Pohlheim, Germany) was used as lactic acid bacteria (LAB) and suspended in sterile saline solution (0.85 % NaCl) to obtain a cell density of approximately 10^8 cfu ml^{-1}. This suspension was added into sucuk dough to obtain a cell density of approx. 10^5 cfu g^{-1}. The number of LAB in saline solution and sucuk dough were determined by serial dilution in a diluent containing 0.1 % sterile peptone water and then cultured on de Man-Rogosa-Sharp agar (MRSA, Difco) followed by incubation at 28°C for up to 48 h.

3.1.3. Preparation of Packaging Film

Packaging film was taken from Polinas Plastik Sanayii ve Ticaret A.S. Manisa, Turkey. It (Y10C1, 90 μm) was made from Polyethylene/Polyamide (PE/PA) mixture. It has one side sealing, clear surface with barrier films. Oxygen transmission rate (OTR) is 160 $cm^3m^{-2}day^{-1}$ (oxygen permeability), and water vapor transmission rate (WVTR) is 8.5 g $m^{-2}day^{-1}$ (water vapor permeability) at 25°C.

From the whole roll of packaging film, 20x12 cm (for meats) and 20x30 cm (for sucuks and cheeses) pouches were prepared. Packaging films were cut with a clean knife and three side of pouch was closed with hot sealing. Upper side was kept open in order to close after the sample was put in.

3.1.4. Meat

3.1.4.1. Preparation of meat

Approximately 6.0 kg meat (small cuts like 0.5cm x 0.5cm) was purchased from local butcher at the day of experiments; analyses were started at the same day. *L. monocytogenes* cultured twice in BHIB at 35°C for 18 h was added to 1 liter bottle including 0.1 % sterile peptone water. Approximately 2500 g of meat dishes was added into the bottle containing *L. monocytogenes* culture (2x1000 ml) solution in 5L jar. Contamination of *L. monocytogenes* was obtained by mixing meat dishes in

bacterium solution for 5 min. Meat containing solution was filtered through cheese cloth (boiled for 10 min in boiling water). Meat dishes from filtrate were exposed to air to remove excess moisture and water by spreading on a dust free surface on the filter paper from dishes in a sterile closed container. They were used for packaging with modified atmosphere.

3.1.4.2. Packaging of meat

About 40 g of meat dishes (*L. monocytogenes* contaminated and not contaminated) were placed in PE/PA film packages (20x12 cm) under aseptic condition. According to the manufacturer's data, PE/PA film has permeability at 25°C; oxygen: 160 $cm^3m^{-2}day^{-1}$ and water vapor: 8.5 g $m^{-2}day^{-1}$. The packages containing the meat cuts were packed with double heat sealing in vacuum packaging machine (La Minerra, D.V.P. Vacuum Technology, s.r.l., Italy) with air, vacuum and different modified atmospheres (MAs). The CO_2, O_2 and N_2 were mixed in the various combinations using Witt-Gas mixer (GmbH and Co Kg, Deutschland) and the gases were flushed into vacuum machine during packaging. Meats contaminated with *L. monocytogenes* were packed with the following atmospheres: (1) with air, (2) with vacuum, (3) with 100%CO_2, (4) with 60%CO_2+40%N_2, (5) with 20%CO_2+30%O_2+50%N_2, (6) 40%CO_2+30%O_2+30%N_2 and (7) 40%CO_2+60%N_2 (The seal was visually inspected to ensure appropriate closure before storage). These meats packaged with different MAs were stored immediately at 4±1°C in a cold room (< 60 % RH) equipped with a temperature controller until the sampling day. Four packages were prepared for each atmosphere type, and for each storage condition 28 packages were prepared, so totally 56 packages were prepared. Control meat (without *L. monocytogenes* count) was packaged with only air.

3.1.4.3. Sampling and preparation of meats for analyses

One meat package was taken at the each following sampling time for microbiological and chemical analysis: before and after addition of *L. monocytogenes* (0 day), 1, 3, 6, 12, 18 and 24 days. For microbiological and pH analysis; 25 g of meat sample was added into 225 ml 0.1 % sterile peptone water containing Warring blender (Torrington, CT, US) and the mixture was homogenized for one minute at aseptic conditions. After homogenization the mixture settled for 2 min and the dilution amount was recorded as

10^{-1}. Further decimal dilutions were prepared with 9 ml sterile 0.1 % peptone water (Erkmen, 2007).

3.1.5. Sucuk

3.1.5.1 Preparation of sucuk

Sucuk was prepared from basic formula per kg: 900 g beef meat (containing 18 % fat), 100 g tail fat and spices; 5.5 g cumin, 1.1 g cinnamon, 11.4 g allspice, 0.5 g clove, 5.5 g red pepper, 11 g black pepper, 20.6 g garlic, 0.4 g ginger, 2.0 g sugar, 18.0 g NaCl, 0.3 g $NaNO_3$, 0.05 g $NaNO_2$ and 2.1 g olive oil. Starter culture is added into sucuk dough with a level of approximately 10^5 cfu g^{-1}. *L. monocytogenes* cells obtained from centrifugation was added into sucuk dough and final number of *L. monocytogenes* cells was 6.06 log cfu/g of sucuk dough. The olive oil was added to soften the mixture and to help in peeling the casing from the sucuk.

The control sucuk dough was also prepared without starter culture and *L. monocytogenes* addition.

The general outline processing of sucuk was given in Figure 3.1. The meat was minced in a meat grinder (Tefal Prep'Line 1600, France) to about 1.3-2.5 mm, and spices were added and mixed with the minced meat in the cutter. Dough was held for 24 h at 4°C (conditioning) and then refrigerated tail fat was added, mixed and minced to about 3 mm in the meat grinder. Sucuk dough was used to prepare two different batches: (i) dough to which the starter culture and *L. monocytogenes* was added, (ii) dough to which the starter culture and *L. monocytogenes* was not added (control).

After that, each dough was filled (about 100 g) into natural casings (25 mm diameter) under aseptic conditions using a filling machine (Tefal, Prep'Line 1600, France). During filling, air bubbles formed on the surface of casings were removed by using finger moves out of the casing. The filled casings were allowed to remaining for 2-6 h at 22°C (conditioning) with RH <60 %.

After conditioning, sucuk dough was fermented in air conditioning equipment at 22°C for 1st and 2nd days with 90 % RH and for 3rd days at 20°C with 85 % RH.

After 3 days fermentation, the sucuks were ripened with a step wise RH and temperature reduction: Sucuk is dried at 18°C for 2 days at 85 % RH, at 17°C for 2 days at 75% RH and at 17°C for 1 day at 70% RH. After 5 days of ripening and drying, sucuks are packaged at different atmospheres.

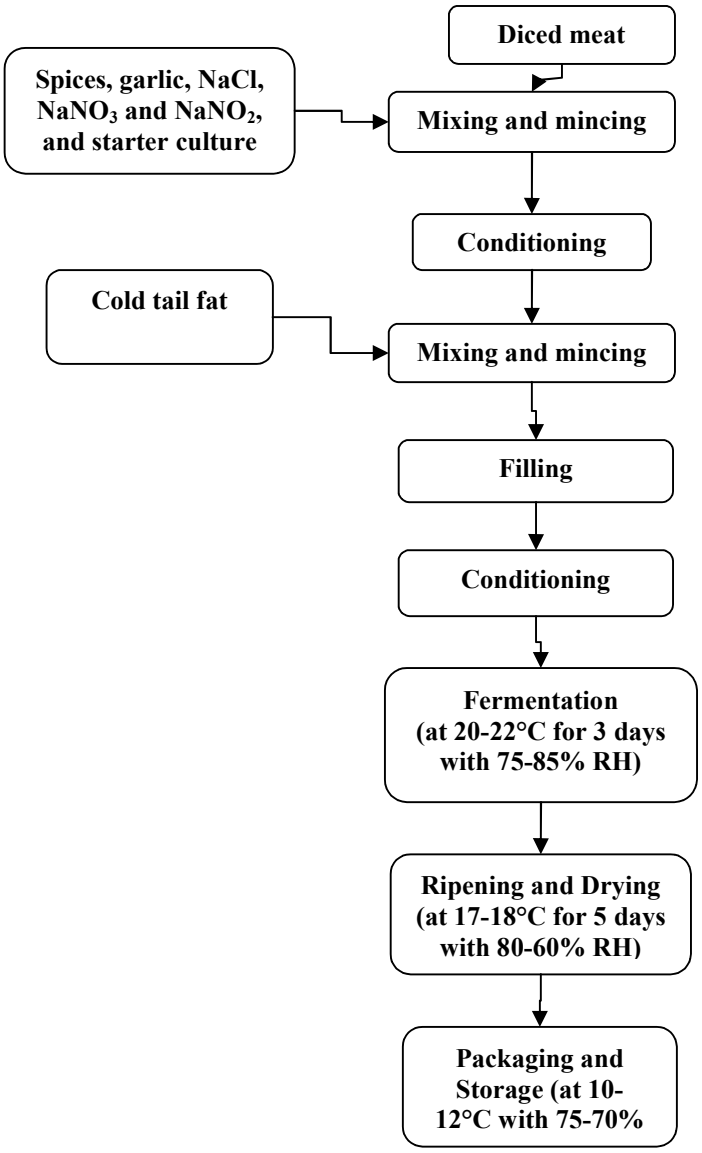

Figure 3.1 General outline processing of sucuk (Erkmen and Bozoglu, 2008)

3.1.5.2. Packaging and storage of sucuk

Two pack of sucuk in natural casing (about 100 g) was placed in PE/PA film packages (30x20 cm) under aseptic condition. According to the manufacturer's data, PE/PA film has a permeability at 25°C; oxygen: 160 $cm^3m^{-2}day^{-1}$ and water vapor: 8.5 g $m^{-2}day^{-1}$. The packages containing the sucuk were packaged and double sealed

with heat sealing in vacuum packing machine in one of five different atmospheres: (1) with air, (2) with vacuum, (3) with 100%CO_2, (4) with 77%CO_2+11%O_2+12%N_2 and (5) with 30%CO_2+40%O_2+30%N_2. The CO_2, O_2 and N_2 were mixed in the various combinations using Witt-Gas mixer and the gases were flushed into vacuum machine during packaging. After packaging, sucuks were stored at 4±1^oC in a cold room (< 60 % RH) equipped with a temperature controller until the sampling day. The seal was visually inspected to ensure appropriate closure before storage.

3.1.5.3. Sampling and preparation of sucuk for analyses

One sucuk package was taken at the each following sampling time for microbiological and chemical analysis: before and after addition of *L. monocytogenes* (0 day), 3, 6, 12, 18, 24 and 30 days. For a_w analysis 30 g of sample was separated and then for microbiological and pH analysis; 25 g of sucuk sample was added into 225 ml 0.1% sterile peptone water containing Warring blender (Torrington, CT, US) and the mixture was homogenized for one minute at aseptic conditions. After homogenization the mixture settled for 2 min and the dilution amount was recorded as 10^{-1}. Further decimal dilutions were prepared with 9 ml sterile 0.1 % peptone water (Erkmen, 2007).

3.1.6. White cheese

3.1.6.1 Contamination of cheese

Approximately 3.0 kg of low fat white cheese was purchased from market at the day of experiments done and analyses were started at the same day. *L. monocytogenes* culture solution (in 0.1 % peptone water) was added in 2 liter bottle including 0.1% sterile peptone water to prepare a stock culture. Cheeses were cut (as rectangle) with a sterile knife and added into 1L of *L. monocytogenes* culture solution in a bottle. Contamination of *L. monocytogenes* was obtained by mixing cheese with stock culture for 10 min. Liquid was removed by filtering through cheese cloth (boiled for 10 min in boiling water), and then cheese pieces taken from filtrate were allowed to remain for removing excess water.

3.1.6.2 Packaging of cheese

After drainage of cheese on a dust-free table, about 80 g of cheese was placed in PE/PA film packages (20x30 cm) under aseptic condition. According to the

manufacturer's data, PE/PA film has a permeability at 25°C; oxygen: 160 $cm^3m^{-2}day^{-1}$ and water vapor: 8.5 g $m^{-2}day^{-1}$. The packages containing cheese were packed and double sealed with heat sealing in vacuum packaging machine with air and different modified atmospheres. The CO_2, O_2 and N_2 were mixed in the various combinations using Witt-Gas mixer and the gases were flushed into vacuum machine during packaging. Cheese contaminated with *L. monocytogenes* was packaged with the following atmospheres: (1) air, (2) vacuum, (3) 100%CO_2, (4) 30%CO_2+70%N_2 and, (5) 100%N_2. Addition to these, control packages containing cheese that were not contaminated with *L. monocytogenes* were prepared also. Six packages were prepared for each atmosphere type, and so totally 60 packages were prepared. After packaging, cheeses were stored at 4±1°C in a cold room (< 60 % RH) equipped with a temperature controller until the sampling day. The seal was visually inspected to ensure appropriate closure before storage.

3.1.6.3. Sampling and preparation of cheese for analyses

One cheese package was taken at the each sampling time for microbiological and chemical analysis: before and after addition of *L. monocytogenes* (0 day), 2, 7, 15, 22 and 30 days. Cheese from packages was used in the following analysis: 30 g of cheese sample was used for RH analysis and 25 g for microbiological and pH analysis. Twenty five grams of cheese sample was added into 225 ml 0.1% sterile peptone water containing sterile Warring blender (Torrington, CT, US) and the mixture was homogenized for one minute at aseptic conditions. After homogenization the mixture settled for 2 min and the dilution amount was recorded as 10^{-1}. Further decimal dilutions were prepared with 9 ml sterile 0.1 % peptone water (Erkmen, 2007).

3.1.7. Analyses

3.1.7.2 Microbiological analyses

L. monocytogenes counts were done at homogenized and diluted cheese, meat and sucuk samples by spread plate technique using two Listeria selective agar (LSA; Difco) for each dilution of samples (Erkmen, 2007). After drying of surface of the inoculated plates, they were incubated at 35°C for 24 and 48 h. After incubation, characteristic colonies of *L. monocytogenes* on LSA were counted from plates containing colonies between 30 and 300.

Aerobic bacteria (AB) counts were done at homogenized and diluted meat, sucuk and cheese samples by spread plate technique by using brain heard infusion agar (BHIA; Difco). After drying of surface of the inoculated plates, they were incubated at 35°C for 24 and 48 h in an incubator. After incubation, formed colonies on BHIA were counted from plates containing colonies between 30 and 300 (Erkmen, 2007).

LAB counts were done at homogenized and diluted meat and sucuk samples by spread plate technique using De Man-Rogosa Sharp agar (MRSA; Difco). After drying of surface of the inoculated plates, they were incubated at 30°C for 24 and 48 h in anaerobic jars (Oxoid, England). After incubation, formed colonies on MRSA were counted from plates containing colonies between 30 and 300 (Erkmen, 2007).

Mold and Yeast (MY) counts were done by spread plate technique using potato dextrose agar (PDA; Difco). After drying of surface of the inoculated plates, they were incubated at 25°C for 72 h in incubator. After incubation, formed colonies on PDA were counted from plates containing colonies between 30 and 300 (Erkmen, 2007).

Averages of AB and *L. monocytogenes*, LAB, and MY counts were calculated for each sample time. Calculated bacteria counts were recorded as colony forming unit (cfu) per gram and logarithms were taken. Then, plots were made by using "SigmaPlot" (SigmaPlot 10 by Systat Sofware, Inc, 2006).

3.1.7.3. Chemical analysis for meat, sucuk and cheese

1) Head space gas analysis for sucuk

Head space gas analyses were made for only sucuk packages. Gas analysis of sucuk packages that were taken at each sampling time were done by using a gas measurement device OXYBABY (OXYBABY-V; Cambridge Sensotec Ltd, 31 Elizabeth Court, St, Ives, CAMBS, PE27 5BQ, England) that measures CO_2 and O_2 gases. Packages containing nitrogen in their gas atmospheres were determined by subtracting total CO_2 and/or O_2 amounts from 100.

2) Relative humidity analysis of sucuk and cheese

Approximately 30 g of sucuk, chopped into small pieces, were put into sample cup of Rotronic hygolab and then sample cup holder was placed into the probe (AW-DIO

Probe) of Rotronic hygrolab device (Rotronik HygroLab 3 set; 160 E. main street, Huntington, NY 11743, USA) to measure RH. After RH equilibrium was remained constant, RH amount was read from device and it was recorded as a_w of sucuk.

3) Measurement of pH of meat, sucuk and cheese

After microbiological analysis, 50 ml of homogenized sample (cheese, meat and sucuk) was used for pH measurement and pH of samples was measured by a pH-meter (WTW pH/mV/Temperature Meters, Models 720; 3150 Commercial Ave Northbrook, IL 60062, USA) equipped with an electrode (WTW-pH electrode Sen Tix 92).

3.1.7.4. Organoleptic (sensory) properties of sucuks

Sucuks were exposed to sensory analysis according to organoleptic properties (odor, taste, cutting property, color, and general acceptance) by 20 panelists at the end of the 8 days of storage time. Panelists were chosen as local citizens who consume sucuk produced by factory (commercial) and also local butcher (traditional) have an advanced experience in sucuks. Both of sucuk prepared in this research and factory (commercial) were analyzed. Panelists were not informed whether sucuks were made of starter culture or packaged with MAP or not. Also, they were exposed to this analysis without explaining that this research was about prolonging of shelf life of MAP sucuk. Each panelist was wanted to test both control and sucuks that we prepared according to five organoleptic properties (Turkish Food Codex 2000/4). Then they gave scores for each sucuk samples from 1 (worst) to 10 (best). *Cutting property:* was described to panelists as "cutting sucuks without any traces on knife easily". *Taste:* Sucuks that were cooked at seperate pans were eaten by panelists and they gave scores according taste of sucuks. *Color property:* Color was defined to panelists as "Turkish sucuk appearance property includes purple-brown in Turkish Food Codex" and they gave scores to color. *Odor property:* Odor property of sucuk was described to panelists as "Turkish sucuk is a meat product mixed with various spices". They gave scores to this property. *General acceptance quality:* Panelists were asked to give scores to this property by deciding at what degree they see these sucuks acceptable for consumption.

3.1.7.5 Color measurement of meat

Effects of modified atmosphere on meat color were observed in this research. Color measurements (L* = lightness, a* = redness, and b* = yellowness) of meat samples were made by using a Hunter Lab ColorFlex (A60-1010-615 Model Colorimeter, Hunter Lab, Reston, VA) according to CIE Lab scale. The results are averages of two measurements. Then averages for both samples were calculated and recorded.

3.1.7.6 Statistical analysis

The results reported here were the mean of two repeated experiments and duplicate samples of each experiment. $P<0.05$ values were considered to be signification. The significant differences among sucuks were compared statistically by a computer program, Sigmaplot 10.0 (Systat., Inc, 2006).

3.2. *Salmonella* Typhimurium in meat and sucuk

3.2.1. Preparation of Media and Solutions

3.2.1.1 Preparation of Media

For culturing of microorganisms and microbiological analysis different media were prepared. In order to make total count BHIA (Difco) was used.

BHIB was used for culturing of *S.* Typhimurium.

For *S.* Typhimurium count Bismuth sulfid agar (BSA, Merck, Darmstadt, Germany) were prepared.

For MY count Potato Dextrose Agar (PDA, Difco) was prepared and pH of the medium was adjusted with addition of tartaric acid.

For Lactic acid bacteria count De Man Rogosa Sharp Agar (MRSA, Difco) was prepared.

All media except BSA (not autoclaved) were sterilized in autoclave at 121°C for 20 min and then poured into sterile petri dishes.

3.2.1.2. Preparation of solutions

a) Preparation of peptone water

Peptone water is used for sample and dilution waters. Peptone was readily powdered for mixing with distilled water and it was weighed on sensitive balance. 0.1 % peptone water was used during preparation of dilution waters, culture solution and preparation of sample waters. Simply 1 g of peptone powder was mixed with 1000 ml of distilled water in a clean balloon, then distributed to bottles and test tubes in required amount and sterilized at 121ºC for 20 min in autoclave.

b) Preparation of dilution water

Dilution water is used for diluting the sample of food products that is expected to include microorganisms in it. In order to prepare sterile dilution water, first 9 ml of 0.1% peptone water was put into test tubes in aseptic conditions and mouths of tubes were closed by the lids. After preparing 9 ml 0.1 % peptone water including test tubes, they were sterilized in autoclave at 121°C for 20 min. The test tubes were stored at refrigerator for further use. For homogenization of solid food samples 225 ml 0.1 % peptone water was prepared in 250 ml flasks. 225 ml of 0.1 % peptone water was poured into 250 ml flask, a magnetic stirrer rod was put into the flask too and the mouths of flasks were closed by cotton and aluminum foil and tied. After closing flasks, they were sterilized in autoclave at 121°C for 20 min. The flasks were stored at refrigerator for further use.

3.2.2. Preparation of culture

3.2.2.1. *Salmonella* Typhimurium

S. Typhimurium KUEN 1357 was obtained from University of İstanbul, Faculty of Medicine, Microorganism's Culture Collection Research and Applied Center, İstanbul, TURKEY. It was subcultered twice in BHIB, incubated at 37^{o}C for 24 h. Cells of *S.* Typhimurium was harvested by centrifugation at 5000 g for 20 min, washed twice, and resuspended in saline solution (0.85 % NaCl) to obtain a cell density of about 10^{9} cfu ml^{-1}, which was determined by serial dilution in a diluent containing 0.1 % sterile peptone water and then cultured on BHIA followed by incubation at 35^{o}C for 24 h. In order to confirm, *Salmonella* culture was inoculated by streak plate

technique on bismuth sulfide agar (BSA, Difco). Working culture was added into foods to contaminate the foods (meat and sucuk).

3.2.3. Preparation of Packaging Material

Packaging material was taken from Polinas Plastik Sanayii ve Ticaret A.S. Manisa, Turkey. This packaging film (Y10C1, 90 μm) was made from Polyethylene/Polyamide (PE/PA) mixture. It has one side sealing, clear surface with barrier films. Oxygen transmission rate is 160 $cm^3m^{-2}day^{-1}$ (oxygen permeability), and water vapor transmission rate is 8.5 g $m^{-2}day^{-1}$ (water vapor permeability) at 25°C.

From the whole roll of packaging film, 20x12 cm (for meats) and 20x30 cm (for sucuks and cheeses) pouches were prepared. Packaging films were cut with a clean knife and three side of pouch was closed with hot sealing. Upper side was kept open in order to close after the sample was put in.

3.2.4. Meat

3.2.4.1. Preparation of meat

An amount of resuspended *S.* Typhimurium culture (in 0.85% NaCl) was added into 0.1 % sterile peptone water (pH=5.78) in a 2 liter bottle and mixed. Meat (its postmortem age was about 24 h) was purchased from a butcher, transported to laboratory within 15 min, and contaminated with *S.* Typhimurium. Meat with 14 % fat was cut to small dishes (about 0.5x0.5 cm) on a clean cutting board under aseptic condition. Meat dishes were added into bottle containing peptone water contaminated with *S.* Typhimurium, thoroughly mixed for 2 min and then the contents of bottle were filtered through a sterile cheese cloth under aseptic condition and allowed for 10 min to drain. The meat dishes placed to a dust free area for 10 min at room temperature to reduce excess water and moisture from meat. Control meat batch was also prepared without contamination of *S.* Typhimurium. *S.* Typhimurium contaminated meat and control meat (without *S.* Typhimurium) batches were packaged.

3.2.4.2. Preparation of sucuk

Sucuk was prepared from basic formula for 1 kg: 900 g beef meat (containing 18 % fat), 100 g tail fat and spices; 5.5 g cumin, 1.1 g cinnamon, 11.4 g allspice, 0.5 g clove, 5.5 g red pepper, 11 g black pepper, 20.6 g garlic, 0.4 g ginger, 2.0 g sugar, 18 g NaCl, 0.3 g $NaNO_3$, 0.05 g $NaNO_2$ and 2.1 g olive oil. The meat was minced in a meat grinder (Tefal Prep'Line 1600, France), and spices were added and mixed with the minced meat in the cutter. Spice-meat mixture was held for 24 h at 4°C (conditioning) and then refrigerated (4°C) tail fat was added, mixed and minced to about 3 mm in the meat grinder. After that, each dough was filled (about 100 g) into natural casings (25 mm diameter) under aseptic conditions using a filling machine (Tefal, Prep'Line 1600, France). During filling, air bubbles formed on the surface of casings were removed by using finger moves out of the casing. The filled casings were allowed to remaining for 2-6 h at 22°C (conditioning) with RH <60 %.

Sucuk dough was used to prepare following hatches: (i) dough were prepared without LAB and *S.* Typhimurium were added (control), and (ii) dough to which the starter culture and *S.* Typhimurium were added.

After that, each dough was filled (about 100 g) into natural casings (25 mm diameter) under aseptic conditions using a filling machine at 2°C. The filled casings were allowed to remaining for 2-6 h at 22°C (conditioning) with RH <60 %.

After conditioning, sucuk dough was fermented at 22°C for 1^{st} and 2^{nd} days with 90 % RH and for 3^{rd} days at 20°C with 85 % RH.

After 3 days fermentation, the sucuks were ripened and dried with a step wise RH and temperature reduction: Sucuk is ripened at 18°C for 2 days at 85 % RH, at 17°C for 2 days at 75% RH and at 17°C for 1 day at 70% RH. After 5 days of ripening and drying, sucuks are packaged at different atmospheres. The general outline processing of sucuk is given in Figure 2.1.

3.2.4.3. Packaging of sucuk and meat

About 30 g of meat dishes or a pack of sucuk was placed in polyethylene/polyamide film (PE/PE; Polinas Plastik Sanayii ve Ticareti A.S., Manisa, Turkey) packages (20x12 cm) under aseptic condition. According to the manufacturer's data, PE/PA

film has a permeability at 25°C; oxygen: 160 $cm^3m^{-2}day^{-1}$ and water vapor: 8.5 g $m^{-2}day^{-1}$. The packages containing the meat dishes were packed and double sealed with heat sealing in vacuum packaging machine (La Minerra, D.V.P. Vacuum Technology, s.r.l., Italy) with vacuum packaging (VP) and gas mixtures. The CO_2, O_2 and N_2 were mixed in the various combinations using Witt-Gas mixer (GmbH and Co Kg, Deutschland) and the gases were flushed into vacuum machine during packaging. Sucuks and meats contaminated with *S.* Typhimurium were packed with the following atmospheres: (1) with air, (2) with vacuum, (3) with 100%CO_2, (4) with 60%CO_2+40%N_2 and (5) with 20%CO_2+30%O_2+50%N_2. Sucuk made without LAB *S.* Typhimurium was also packed with only air. Twelve packages were prepared for each packaging type. After packaging, sucuks were stored at 12°C in a refrigerator and at 4±1°C in a cold room (< 60 % RH) equipped with a temperature controller until the sampling day. The seal was visually inspected to ensure appropriate closure before storage.

3.2.5. Sampling

One sucuk sample for microbiological analysis and pH determination was taken at following sampling time: before and after LAB and *S.* Typhimurium addition (0 day), during fermentation (after 1 and 3 days), after ripening (after 5 days), and during storage (after 7, 13 and 22 days).

One meat package for microbiological analysis was taken at following sampling time: before *S.* Typhimurium addition (0 day), 2, 5, 8, 10 and 12 days of storage.

A sample from the raw meat and ingredients of sucuk was taken for preparation. *S.* Typhimurium, aerobic bacteria (AB) and LAB counts, and pH analysis were performed in duplicates. Sucuk samples were cut into small pieces under aseptic condition.

Homogenization:

Twenty-five gram portions of sucuk and respective meat sample were homogenized in a sterile Warring blender (Torrington, CT, US) containing 225 ml of 0.1 % sterile peptone water. Homogenized meat or sucuk samples were serially diluted using 0.1 % sterile peptone water (Erkmen, 2007).

3.2.6. Microbiological analyses

a) *Salmonella* Typhimurium

S. Typhimurium count was done by using Bismuth sulfide agar (BSA). One ml of homogenized and diluted sample were spread plated on BSA for Salmonella count, after drying the surface of inoculated petri plates they have incubated at 35^{o}C for 24 - 48 h in incubator (Erkmen, 2007). The whole experimental procedure was performed twice. After incubation *Salmonella* specific colonies between 30 and 300 on BSA were counted. The average value of microbial counts was recorded for each time point. According to dilution and inoculation numbers of *S.* Typhimurium were calculated. The number of survivors was expressed as cfu and logarithms were taken and recorded as log cfu g^{-1} of sucuk or meat.

b) Aerobic bacteria

One ml of homogenized and diluted sample were spread plated on BHIA for aerobic bacteria (AB), and incubated at 35^{o}C for 24 h. The average number of colonies from the duplicate samples was then recorded for each sample. The average number of microbial counts was recorded for each time point. The number of survivors was expressed as log cfu g^{-1} of sucuk or meat.

c) Lactic acid bacteria

One ml of homogenized and diluted sample were spread plated on MRSA for LAB, and incubated at 28^{o}C for 48 h. The average number of colonies from the duplicate samples was then recorded for each sample. The whole experimental procedure was performed twice. The average value of LAB was recorded for each point of time. The number of survivors was expressed as log cfu g^{-1} of sucuk.

d) Mold and Yeast

One ml of homogenized and diluted sample were spread plated on PDA for MY count, and incubated at 25^{o}C for 3-5 days. The average number of mold and yeasts from the duplicate samples were then recorded for each sample. The whole experimental procedure was performed twice. The average value of microbial counts was recorded for each time point. The number of survivors was expressed as log cfu g^{-1} of sucuk or meat.

3.2.7. Statistical Analysis

The results reported here were the mean of two repeated experiments and duplicate samples of each experiment. $P<0.05$ values were considered to be signification. The differences among sucuks packaged with different atmospheres and with and without LAB culture were compared statistically by a computer program, SigmaPlot 10.0 (Systat., Inc. 2006).

CHAPTER 4

RESULTS AND DISCUSSION

During this research, survivals of two pathogenic microorganisms in foods were studied depending on different modification of storage atmospheres. In this research, meat, sucuk and cheese were contaminated with *L. monocytogenes* stored in packages with different atmospheres at 4°C, and meat and sucuk were contaminated with *S.* Typhimurium packaged with different atmospheres and then stored at 4 and 12ºC. Pathogenic bacteria were inoculated into foods and stored at low temperature. Different microbiological and chemical analyses were made during preparation and storage periods.

4.1. Survival of *Listeria monocytogenes* in meat, sucuk and cheese

4.1.1. Meat Packaged with Different Modified Atmosphere

Aerobic bacteria (AB), *L. monocytogenes*, color measurement and pH analyses were done at meat samples during storage period.

4.1.1.1. Changes in pH of meat during storage period

Changes of pH during storage time in meats packaged with six different gas atmospheres were shown in Table 4.1. A 0.5 pH unit reduction was observed from the initial meat pH from 5.6 to 5.10. The pH of meat was reduced at all storage atmospheres except packages without CO_2. The reduction in pH would be due to growth of LAB naturally present in meats. The decrease in pH was higher in meat packaged with 100% CO_2 than 60% O_2/40% N_2 atmosphere. CO_2 dissolved on the surface of meats might cause a reduction in pH of meats. During 12 day of storage period, pH of meats packaged with 100% CO_2 atmosphere decreased from 5.60 to 5.10, on the other hand, meat packaged with 60%O_2/40% N_2 atmosphere had a reduction from 5.60 to 5.46. Also due to lack of CO_2 in package atmosphere pH increased from 5.6 to 5.76 at the end of 2nd day. This would be due to growth of proteolytic microorganisms at the beginning of storage. CO_2 can induce growth of

LAB and this would be resulted with higher reduction of pH in meat packages containing CO_2.

Table 4.1 Changes of pH in meats packaged with different modified atmospheres during storage at 4°C

	pH of meats during storage				
	Days of storage				
Package atmospheres	**0**	**2**	**5**	**8**	**12**
1) Air	5.60	5.54	5.42	5.30	5.36
2) Vacuum	5.60	5.65	5.31	5.20	5.18
3) 100% CO_2	5.60	5.56	5.22	5.15	5.10
4) 60% CO_2 + 40% N_2	5.60	5.83	5.36	5.25	5.20
5) 30% CO_2 + 30% O_2 + 40% N_2	5.60	5.82	5.45	5.36	5.30
6) 60% O_2 + 40% N_2	5.60	5.76	5.54	5.53	5.46

4.1.1.2. Changes in color of meat

The changes in color of meats packaged with different MAs during storage at 4°C are given in Table 4.2. L* values in CO_2 containing packages increased during storage which means meats became darker. On the other side, a* values of CO_2 containing packages decreased. Especially the highest decrease occurred in 100% CO_2 from 10,775 to 5,125 which explain that meats lost redness. a* values in O_2 containing packages (60% O_2) also decreased but this decrease was lower than the others. Due to the contamination with *L. monocytogenes* color results of meat samples have changed immediately during storage and abnormal color changes occurred because of growth of microorganisms on the surface of meats. Greenish spoilage colors were increased during storage. And blurry appearance was observed at all meats.

Table 4.2 Changes in color of meats packaged with different modified atmospheres during storage at 4°C

		Days of storage			
Package atmospheres		**2**	**5**	**8**	**12**
1) Air	**L***	35.90	34.63	37.77	39.10
	a*	10.77	8.26	9.97	5.12
	b*	13.88	13.24	13.28	10.27
2) Vacuum	**L***	35.89	32.42	44.43	45.62
	a*	10.65	11.29	8.92	5.24
	b*	12.76	13.08	14.20	15.47
3) 100%CO_2	**L***	32.83	42.81	42.64	49.29
	a*	8.55	10.86	6.89	6.10
	b*	10.63	13.26	10.32	13.29
4) 60%CO_2 + 40%N_2	**L***	37.50	30.95	42.69	40.90
	a*	9.84	10.79	8.94	6.83
	b*	12.33	12.81	13.95	14.94
5) 30%CO_2 + 30%O_2 + 40%N_2	**L***	37.22	39.03	37.04	42.12
	a*	9.73	9.30	10.00	6.37
	b*	12.95	13.18	14.78	12.91
6) 60%O_2 + 40% N_2	**L***	34.51	34.70	40.47	37.04
	a*	10.96	9.73	5.93	4.85
	b*	11.56	13.50	12.78	11.92

4.1.1.3 Microbiological changes of meat

Effects of MA on *L. monocytogenes* and AB at 4°C storage in meat packaged with different gas compositions were studied. During the storage period AB, LAB and *L. monocytogenes* counts were determined. Gas atmospheres were; (i) air, (ii) vacuum, (iii) 100% CO_2, (iv) 60%CO_2 + 40% N_2, (v) 30%CO_2 + 30% O_2 + 40% N_2, and (vi) 60% O_2 + 40% N_2.

4.1.1.3.1 Survival of aerobic bacteria

The effect of different gas compositions on AB in meat samples stored at 4°C are shown in Figure 4.1. The numbers of AB were increased during storage period at all storage atmospheric conditions. The growth of AB was significantly ($p<0.05$) lower at package atmospheres containing high amount of CO_2 than the other atmospheres. Growth of bacteria was increased at first five days of storage, but during the further storage period (up to 12^{th} day), growth was slowed down. This slowing in the growth of AB would be due to the consumption of O_2 in packages. Until 5 days of storage

period, the increase in the AB number was 0.73 log in packages containing 100% CO_2 but between 5 and 12 days this increase was 1.31 log. On the other hand, O_2 containing packages (60%O_2 + 40%N_2) had a high increase during first 5 days as 3 log but after 5 days (due to the consumption of O_2 in the package) growth was slowed and at the end of the 12 day of storage AB numbers in 60%O_2 packages were increased by 4.4 log. Counts of AB in packages of meat with different atmospheres showed significant differences ($p<0.05$). Bodnaruk and Draughon (1998) packaged the meat with different gas atmospheres and they determined increase at AB counts in meat packages at different gas atmospheres stored at 4°C, but this increase was higher at air and O_2 containing packages than 100%CO_2 containing packages as obtained in this research. Rutherford et al. (2007) found a 4.5 log cfu/g increase in the aerobic psychrophilic bacteria counts in meat packaged with MA and stored at 3, 7 and 12°C for 15 day storage time. Again in this research, increase of this microorganism was higher at packages containing higher amount of O_2 and air than the packages containing 100%CO_2.

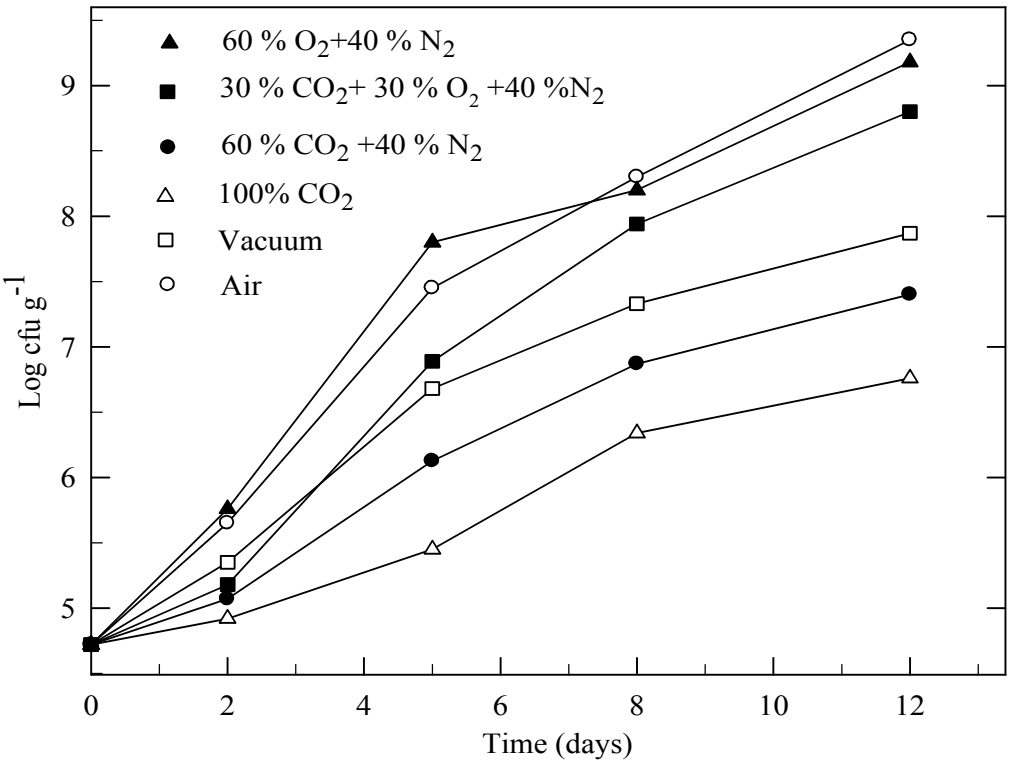

Figure 4.1 Growth of aerobic bacteria in meat packaged with different gas atmospheres and stored at 4°C

Sorheim et al. (1999) recorded that aerobic counts showed an increase of 4.0 log cfu g^{-1} in meat packaged with higher amounts of CO_2 ($60\%CO_2 + 40\%N_2$) stored at 4 and 8°C.

4.1.1.3.2. Lactic acid bacteria

Effects of different atmospheres on the LAB numbers in meat stored at 4ºC are given on Figure 4.2. The numbers of LAB were increased in meat packages at different atmospheres during storage period at 4°C. Growth of LAB was lower in packages in the packages containing CO_2 and vacuum than packages containing O_2. LAB numbers increased similarly in all packages during first two days of storage. After that at the 5th day, number of LAB was slightly higher in $30\%CO_2 + 30\%O_2 + 40\%N_2$ than $60\%CO_2 + 40\%N_2$. In $100\%CO_2$ package LAB numbers increased by 1.89 log after 5 days. At the end of 12 days storage, increase in LAB numbers was higher in air packages than the others. This increase in air packages was about 5.52 logs. Numbers of LAB in meats increased at $100\%CO_2$ packages by 2.96 logs after 12 days. Significant differences ($p<0.05$) were observed in LAB numbers in meats stored by packaging with different atmospheres.

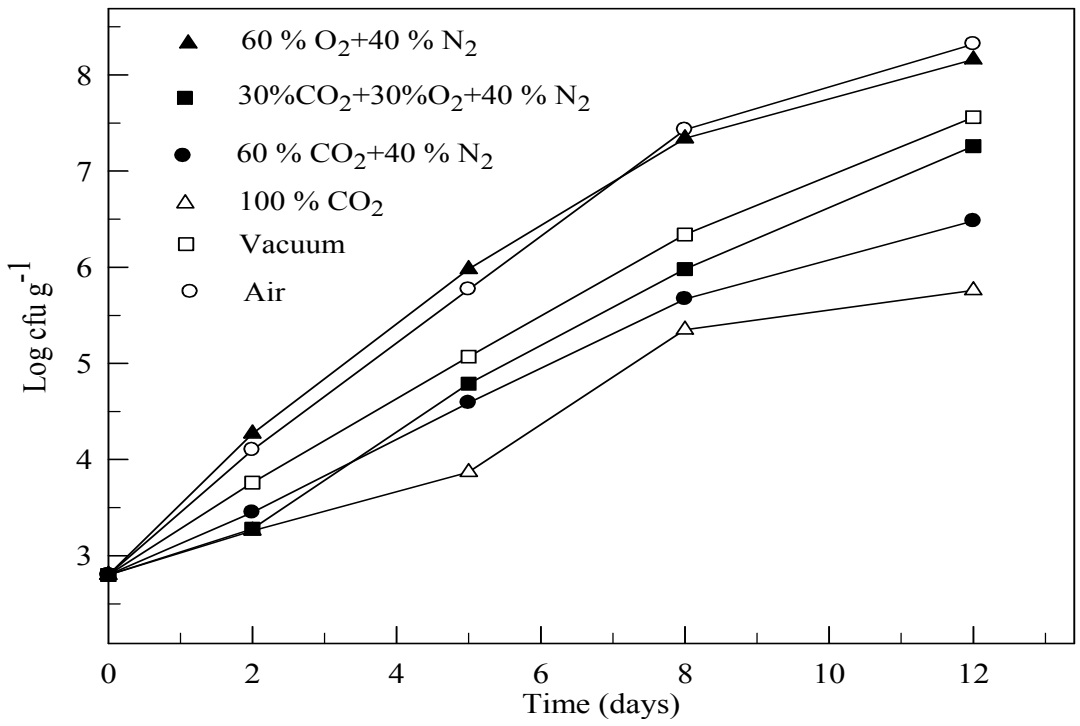

Figure 4.2 Growth of lactic acid bacteria in meat packaged with different gas atmospheres (4°C)

Cayre et al. (2005) found an increase in numbers of LAB with vacuum packages during storage time (at 0°C for 75 days at 8 and 15°C for 52 days). This increase was about 5 logs.

4.1.1.3.3. *Listeria monocytogenes*

Effects of different gas atmospheres on *L. monocytogenes* in meat stored at 4°C showed in Figure 4.3. *L. monocytogenes* number was not increased during first days of storage in meats packaged with different atmospheres. All of the packages had an initial number of *L. monocytogenes* as 4.3 log cfu/g. During the first 2 days of storage, numbers of *L. monocytogenes* were similar and remain constant. There was an increase in the number of bacterium during the storage period in packages containing air, vacuum and O_2 (30% O_2 and 60% O_2) but this increase was lower in 30% O_2 packaged meats. After 2 days, *L. monocytogenes* numbers started to increase in O_2 containing packages (60% O_2 + 40% N_2 and air) and vacuum packages. This increase was 2.08 log in air packages, 2.05 in 60% O_2 + 40% N_2 packages and 0.96 log in vacuum packages at the end of 12 days of storage. Vacuum packages can be considered as not containing O_2 but O_2 would be entered in these packages due to the O_2 permeability of package material from air.

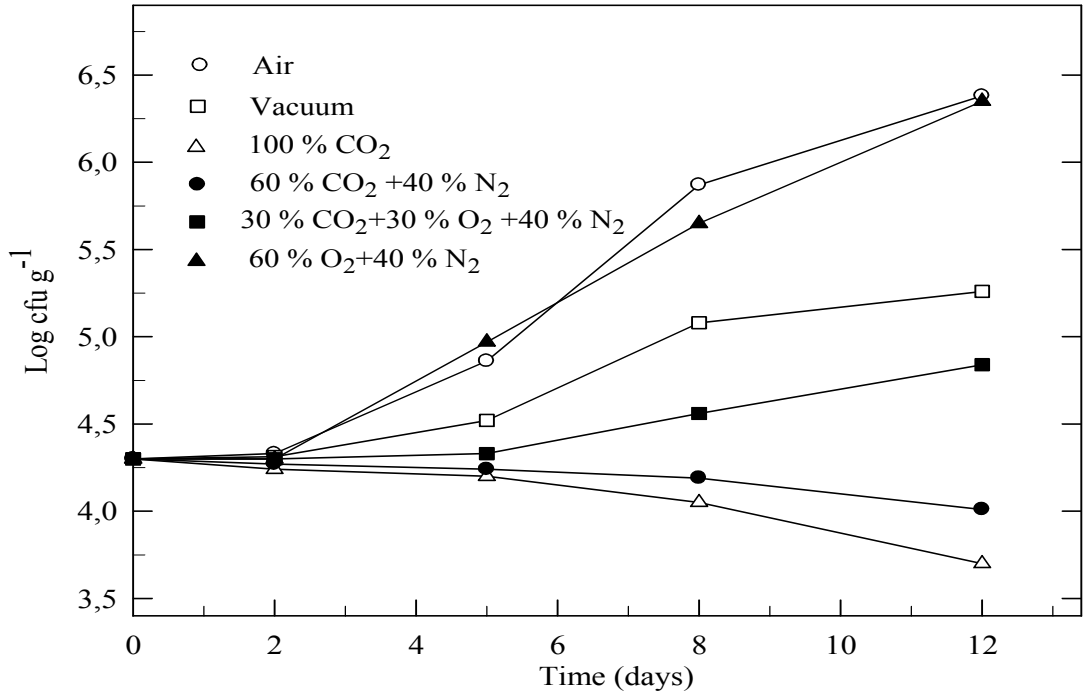

Figure 4.3 Survival of *L. monocytogenes* in meat packaged with different atmospheres stored at 4°C

On the other hand CO_2 containing packages (100% CO_2, 60% CO_2 + 40% N_2, and 30% CO_2 + 30% O_2 + 40% N_2) remained constant until 5th day but after that number of *L. monocytogenes* decreased by 0.5 and 0.2 logs at the end of 12 days of storage in meat packaged with 100% CO_2 and 60% CO_2 + 40% N_2, respectively. Numbers of *L. monocytogenes* started to increase by 0.5 log in meats packaged with 30% CO_2 + 30% O_2 + 40% N_2. *L. monocytogenes* counts with different package atmospheres showed significant differences ($p<0.05$).

Nissen et al. (2000) packaged meat with different gas atmospheres and stored at 4 and 10°C, while the decrease in the number of *L. monocytogenes* was about 1 log in meat packages without oxygen (60% CO_2 + 40% N_2), it was slightly lower in meat packages containing high levels of O_2 (70% O_2 + 30% CO_2). Michaelsen et al. (2006) packaged meat with different gas atmospheres and stored at 4^{o}C and 10^{o}C. They described that numbers of *L. monocytogenes* in meats stored at 4^{o}C for 28 days were decreased but it was increased in the packages containing air and O_2. The increase in the numbers of *L. monocytogenes* was higher in meats stored at 10^{o}C than 4°C. Franco-Abuin et al. (1997) found that number of *L. monocytogenes* in meat packaged with 100% CO_2 was decreased by 0.5 log cfu/g, while in meats packaged with 65% CO_2 were increased little and in meats packaged with 20% CO_2 the numbers of *L. monocytogenes* were increased by 1.5 log.

Rutherford et al. (2007) observed that numbers of *L. monocytogenes* were increased during the storage for 15 days at 12ºC and this increase was 5.5 log, also it was 3 log at 7ºC, and 2.0 log at 3ºC. These increases were higher at packages containing air and O_2 than packages containing CO_2. They also recorded that numbers of *L. monocytogenes* were decreased in the presence of CO_2 in packaged meat

L. monocytogenes is a psychotrophic pathogen. They can grow in refrigerated temperature (Erkmen and Bozoglu, 2008). Growth or survival level of *L. monocytogenes* in meats can be lowered or prevented with packaging in the presence of CO_2 over 50%.

A relationship between numbers of *L. monocytogenes* and amount of CO_2 in the survival rate was determined during storage period. Carbonic acid formation from CO_2 and amount of acid produced by LAB would affect the survival of *L.*

monocytogenes since pH of the medium was lowered more in meat packaged with higher concentration of CO_2 (Table 4.1). Depending on this reduction in pH, higher decreases in the numbers of *L. monocytogenes* and AB were observed. In the packages without CO_2, LAB produced higher amount of acid but this was not enough to lower pH as required and so *L. monocytogenes* and AB numbers were high at the end of 12 days of storage. pH level of meat packages without CO_2 (60% O_2 + 40% N_2) decreased from 5.60 to 5.46 and it was decreased from 5.60 to 5.10 in meat packages with 100% CO_2 during 12 day of storage period.

4.1.2. Microbiological and chemical changes during manufacturing and storage of sucuk

4.1.2.1. Chemical changes in sucuk

4.1.2.1.1. Water activity changes in sucuk

Sucuks made with and without starter culture were prepared in this research and kept in an air conditioned (humidity and temperature) chamber for fermentation (3 days) and ripening (5 days). During processing (fermentation and ripening) sucuks were not packaged. They were packaged with different atmospheres after ripening (5 days). Changes in water activities (a_w) during fermentation, ripening, and storage times of sucuks made with and without starter culture, are showed in Figure 4.4. During fermentation, relative humidity (RH) of fermentation room was kept at 90%, resulting less water loss in sucuk. At the end of fermentation time reduction in a_w of sucuk was very slow. This reduction was determined approximately 0.02 %. Ripening was done for 5 days in a_w between 80% and 60%. The moisture reduction was about 0.928 % in sucuks made with starter culture and 0.934 % in sucuks made without starter culture at the end of 5 days of ripening. Sucuks were packaged with different gas atmospheres by using barrier film packages at the end of ripening and stored at 4°C. During storage period, any effects of modified atmosphere conditions on a_w were not determined ($p>0.05$). But significant differences of a_w were observed between sucuks made with and without starter culture ($p<0.05$). For this reason, only the average a_w level of sucuks made with starter and packaged with five different atmospheres and sucuks made without starter culture were given in Figure 4.4. Sucuks made without starter culture were packaged with only air. During first eight days of storage time (16^{th} day), reduction of a_w was continued like in ripening stage of sucuks made with starter culture. But this reduction was slower. However, slower

reduction was determined in sucuks made without starter culture. At the end of 8 day of storage, water activities were determined as 0.913 and 0.924 % in sucuks made with and without starter culture, respectively. The final a_w levels in sucuks made with and without starter culture at the end of 22 days of storage time were determined as 0.909 % and 0.912 % respectively. The moisture content of sucuks made with starter culture was lower than the control sucuks made without starter culture. The lowest a_w requirement for growth of pathogenic bacteria is range from 0.920 to 0.930 (Franco-Abuin et al., 1997), and the optimum growth is 0.970 (Petran and Zottola, 1989). Partially all microorganisms can grow when a_w is 0.980 (Franco-Abuin et al., 1997). The interaction of starter culture activities with ripening and storage at low RH had significant ($p<0.05$) effect on a_w value of sucuks. Five sucuk samples from each of four different brands that are served for sale in markets bought and a_w were analyzed. A_w of commercial sucuks that are served for sale in markets were between 0.8918 % and 0.9272 %. A_w that were determined in our research were between these a_w values. This showed that sucuks which we produced were not different from commercial sucuks according to a_w levels.

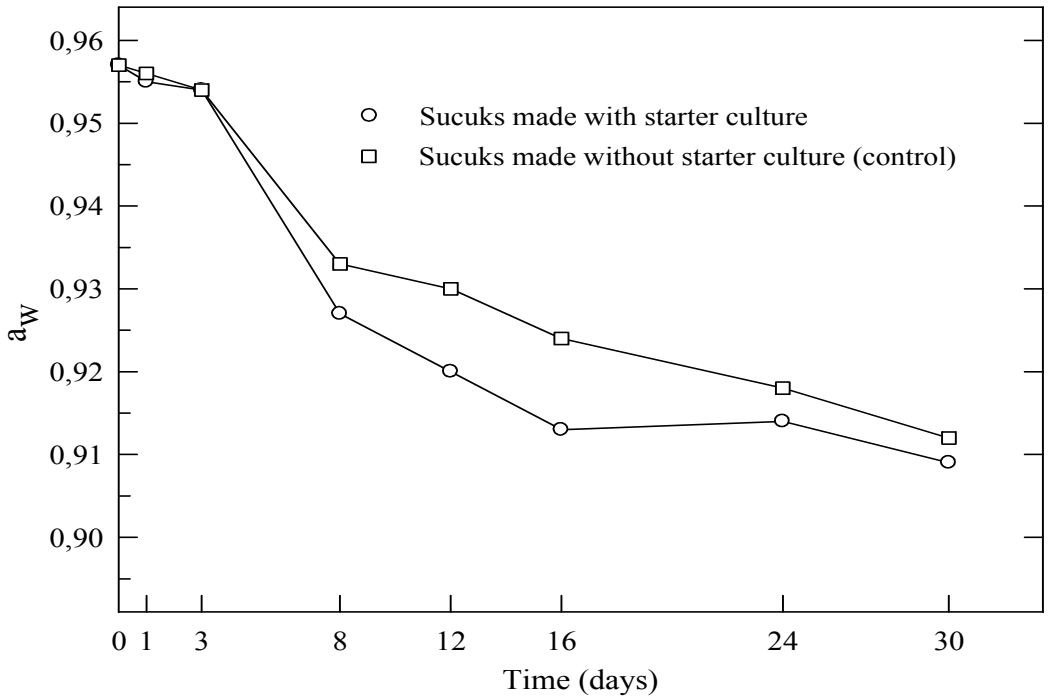

Figure 4.4 Changes in water activities during manufacturing and storage at 4°C periods of sucuks made with and without starter culture

Lahti et al. (2001) determined a_w in fermented sausages that they produced at the end of 14 days of processing as 0.92 % and at the end of 35 days of processing as 0.86 %.

Soyer et al. (2005) found a_w of sucuks approximately 0.92 % at the end of 9 days of ripening stage of sucuks made without starter culture. Kaban and Kaya (2006) determined a_w of sucuks between % 0.892 and % 0.905. Kaban and Kaya did not observe any difference at a_w between sucuks made with and without starter culture. In our research, a higher reduction at a_w was observed in sucuk made with starter culture and this reduction continued during ripening and storage periods. The main reason of this reduction in a_w is the increasing water vapor evaporation rate caused by LAB in sucuks made with LAB. Since water holding capacity of food was decreased by decomposition of sucuk components. The humidity of cold air storage room was changed between 74.9 and 77.2 % during storage periods. Barrier films of sucuk packages (polyethylene/polyamide PE/PA) have a permeability at 25^oC for water vapor: 8.5 g $m^{-2}day^{-1}$ (PE/PA) at 25^oC; which was taken from manufacturer's data. For this reason water loss of sucuks continued during the storage period.

Revilla and Quintana (2005) determined the a_w of sausages that they produced without starter culture as 0.942 % at the end of 3 days processing, 0.935 % at the end of 12 days processing, and 0.91 % at the end of 22 days processing. Degenhardt and Anna (2007) expressed that a_w of Italian types of sausages were decreased during storage at 12°C, and at the end of 21 days of storage period a_w of sausages were between 0.883 % and 0.897 %.

4.1.2.1.2. Changes in pH of sucuks

Changes of pH during fermentation, ripening and storage time (at 4^oC) in sucuks made with and without starter culture packaged with different gas atmospheres were shown in Figure 4.5. After preparation of sucuk dough (before addition of starter culture), pH of sucuk dough was determined as 5.68. During 3 days of fermentation of sucuks, pH was rapidly decreased. pH of sucuks that were made with starter cultures after 3 days of fermentation was reduced to 5.04, on the other hand pH of sucuks that were made without starter culture was reduced to 5.43. During ripening period, pH of sucuks made with starter culture nearly remained constant, near 5.03. pH of sucuks packaged with high level of CO_2 was reduced during 4 days of storage, but then pH increased and at the end of 22 days of storage pH of sucuks packaged with 100% CO_2 and 60% CO_2 + 40% N_2 were determined as 5.04 and 5.06, respectively (Figure 4.5). On the other side, pH of packages containing low level

CO_2 (20% CO_2 + 50% O_2 + 30% N_2), vacuum, and air increased and at the end of 22 days of storage pH of these packages were 5.20, 5.14, and 5.23 respectively. pH of control sucuk made without starter culture increased to 5.42 during storage. Significant statistical differences at the changes of pH of sucuks packaged with different gas atmospheres were determined ($p<0.05$). The decline in the pH value during the fermentation period is very important due to the formation of desired quality and safety point in sucuk such as the inhibition of undesired bacteria, rate of conversion of color and formation of desired flavor.

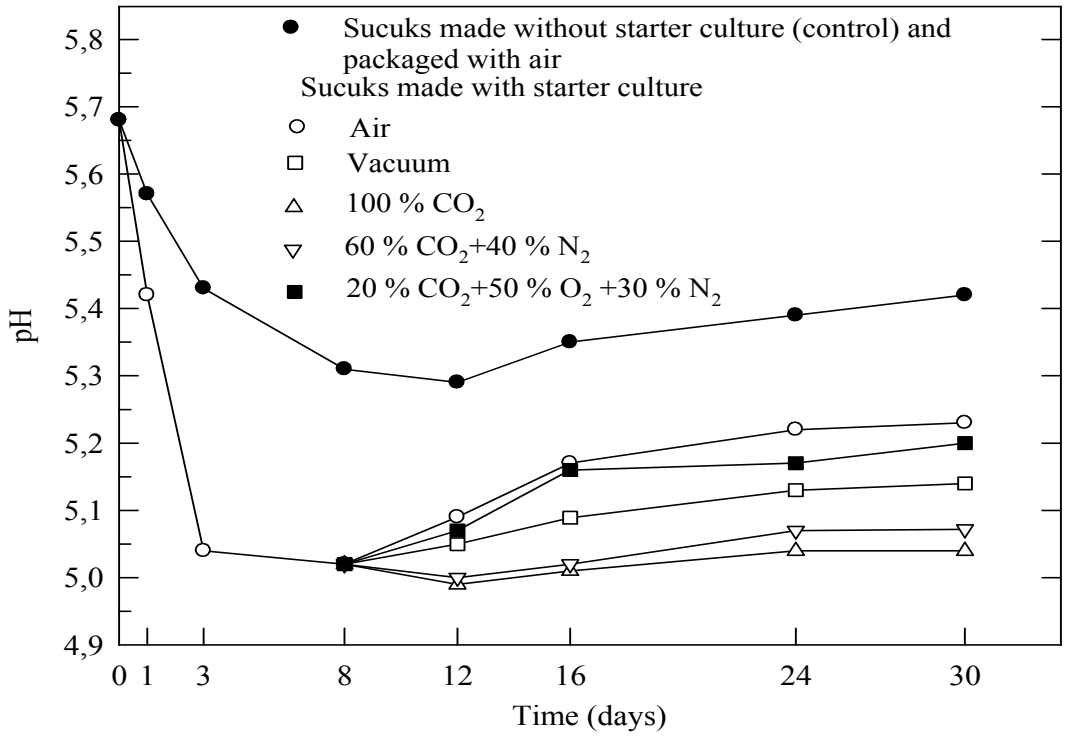

Figure 4.5. Changes of pH during manufacturing and storage (at 4°C) in sucuks made with and without starter culture packaged with different modified atmosphere.

Verluyten et al. (2004) determined that pH of Belgium type sausages decreased during storage period (80 days), and this reduction was higher at 20th and 30th days of storage. Degenhardt and Anna (2007) described that pH reduction of Brazilian type sausages continued until 14th day and then pH increase was observed until 21st day. Tyopponen et al. (2003) determined that pH of Northern Europe type sausages decreased to 4.6 during 7 days of fermentation time and increased to 5.2 during storage period. Kaban and Kaya (2006) determined that pH of sucuks that they produced decreased to 4.6 at the end of fermentation (3 days) and minor increase at

pH was determined during storage (11 days). Again these researchers described pH of sucuks produced without starter culture was higher (5.0) than sucuks made with starter culture (4.6). Lahti et al. (2001) described that pH of sausages decreased to 4.63 during 3 days of fermentation and increased during storage period and the pH at the end of 49 days of storage was about 5.4. According to the Turkish Standard Institute (Anonymous, TS 1070, 2002) pH for high quality sucuk should be in the range of 4.7-5.4. The sucuks prepared with starter culture were found to be close to range 5.04-5.23. So, it can be concluded that pH results were found to be in the range of the Turkish standard values. But the sucuks made without starter culture were ranged from 5.41 to 5.52 during 20 days of storage. According to these results, starter culture use in sucuk making is very important in order to get a high quality fermented sucuk.

4.1.2.1.3. Organoleptic properties of sucuk

Panelist scores of smell (odor), texture (cutting property), color, taste, and general acceptance of sucuks that we produced in this research and three commercial sucuks from local markets, were given in Table 4.3. In our research, panelists gave highest general acceptance score to sucuks made without starter culture packaged with 100% CO_2. Other sucuks that we produced "with and without starter culture packed with 100% CO_2" and "without starter culture and packed with air" gave general acceptance scores above 6. On the other hand, general acceptance values of commercial sucuks were between 2.6 and 4.9. Again depending to other factors like odor, color, and taste our sucuks were higher scored than the commercial VP sucuks. Commercial sucuks scores were only higher at cutting property than sucuks that we produced. The biggest reason of these differences is addition of water holding additives such as; soy flour, wheat flour (in Suprex) and alkaline phosphates (sodium tripolyphosphate, sodium hexametaphosphate, sodium acid pyrophosphate, sodium pyrophosphate, monosodium phosphate and disodium phosphate) to commercial sucuks, and this is also reduced acceptance quality of color, odor, and taste of sucuk.

Table 4.3 Panelist sensory scores (from 10 points) of odor, texture (cutting property), color, taste, and general acceptance quality (GAQ) of sucuks

Sucuk	Odor	Texture (cutting)	Color	Taste	GAQ
Produced with starter culture[1]	7.5	2.6	7.4	6.4	7.2
Produced without starter culture[2]	7.6	3.0	7.3	5.8	7.8
Produced without starter culture[3]	6.1	2.4	6.0	5.6	6.3
Commercial sucuk 1[4]	4.2	5.9	4.8	3.7	4.9
Commercial sucuk 2[4]	2.8	6.1	3.1	2.3	3.3
Commercial sucuk 3[4]	2.9	5.7	2.9	1.7	2.6

[1]%100 CO_2 package, [2]%100 CO_2 package, [3]Air packaged and [4]Vacuum packaged

4.1.2.1.4. Head space gas composition of sucuk

Head space gas compositions of sucuk packages were measured by Oxybaby (gas measurement device) and CO_2 and O_2 % results were recorded and showed in Table 4.4. During fermentation, ripening and storage gas compositions of packages changed because of O_2 consumption of aerobic microorganisms and as a result of that CO_2 production. O_2 in air packages was consumed almost completely at the end of 24 days of storage.

Table 4.4 Head space gas compositions of sucuks stored at 4°C during fermentation, ripening and storage periods.

	Days of storage							
	3 days		8 days		16 days		24 days	
MAP	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)
Air	0.1	19.5	16.8	12.5	20.7	8.5	24.9	0.2
Vacuum	2.1	19.3	2.7	18.9	2.6	19.1	2.9	15.6
100 % CO_2	94.6	1.4	89.6	2.6	71.4	1.6	58.8	1.1
60% CO_2+40%N_2	56.1	5.7	54.7	5.1	43.0	4.0	45.8	1.4
20%CO_2+50%O_2+30 %N_2	16.6	47.3	22.4	43.0	28.7	40.1	40.6	24.5

The headspace gas composition was changed fairly throughout storage. This can be attributed to the presence of competing microflora in the sucuks and metabolic activities of microflora of sucuks packaged under different levels of gases. Permeability characteristic of packaging film may also played important role in the changes of gas composition in headspace of package. Headspace CO_2 decreased in packages packed with 100 and 60% CO_2 throughout storage due probably to the dissolution of CO_2 in the aqueous phase of the product. On the other hand CO_2 concentration was initially decreased (3 days) and then increased in headspace of sucuks packaged with 20% CO_2. Headspace gas composition showed similar attributes in the same types of MA packing therefore only average value was reported for same type of packages.

4.1.2.2. Microbiological changes of sucuk

In this research, sucuks were made with and without *L. monocytogenes* addition to sucuk dough. Sucuks were exposed to microbiological analysis according to AB, *L. monocytogenes* and LAB counts during fermentation, ripening and storage periods.

4.1.2.2.1. Aerobic bacteria

Figure 4.6 shows changes in the numbers of AB during fermentation, ripening and storage (4^oC) periods (stored at different modified atmospheres) sucuks. During fermentation of sucuk (3 days), a significant increase was observed in the number of AB count, this increase was about 0.55 log. During ripening at different modified atmospheres (MAs), AB rapidly decreased from 7.95 to 6.10 log cfu/g and this reduction continued during storage at different MAs. But different reduction numbers were determined in sucuks at different gas atmospheres during storage period. At the end of the 22 days of storage, the highest reduction was determined by 5.65 log in sucuk packaged with 100 % CO_2. In other sucuks these reductions were; 4.89 log for VP, 4.16 log for 60% CO_2 + 40% N_2, 3.28 log for 20% CO_2 + 50% O_2 + 30% N_2. In packages containing air, reduction of AB was 2.58 log.

Soyer et al. (2005) determined that numbers of AB increased during fermentation (3 days) by 3.5 log cfu/g of sucuk made without starter culture and during further steps increase in the numbers of AB remained constant. In our research, decrease in the numbers of AB during ripening and storage was observed. The reason of this

different results may be due to the use of different starter culture and additives that used for making sucuk.

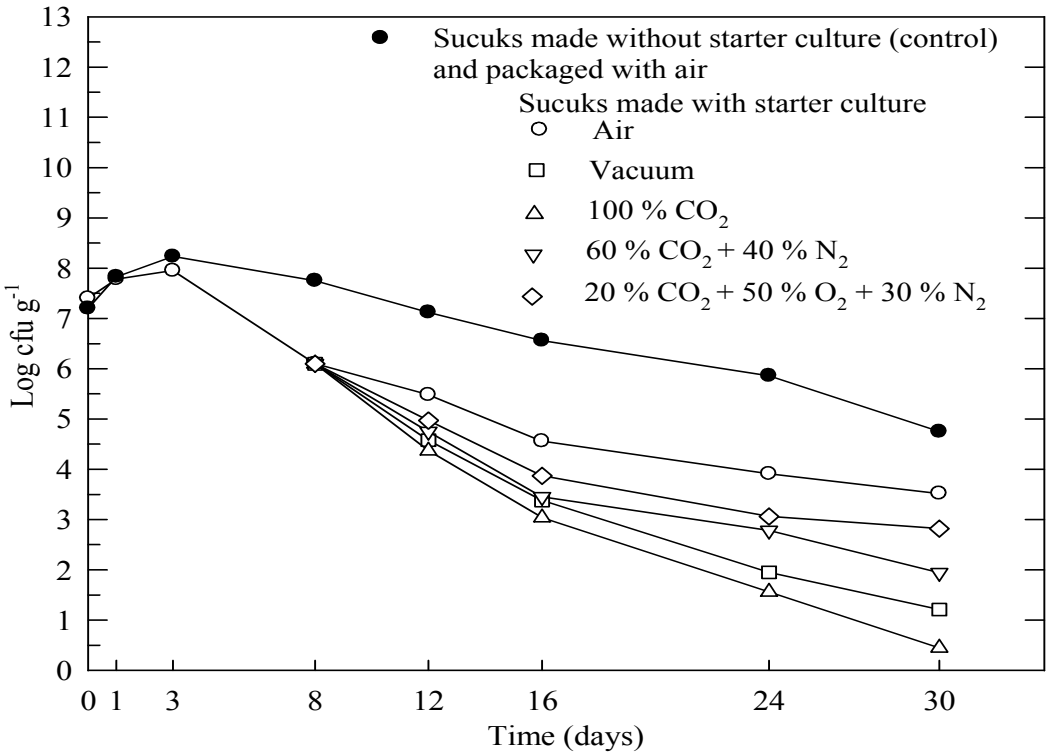

Figure 4.6 Effects of different modified atmospheres on aerobic bacteria in sucuks made with and without starter culture during manufacturing and storage (4^oC)

4.1.2.2.2. *Listeria monocytogenes*

Survival of *L. monocytogenes* in sucuks made with starter culture during fermentation, ripening, and storage with different MAs at 4^oC showed in Figure 4.7. During fermentation of sucuk (3 days) a slow decrease was observed in the numbers of *L. monocytogenes*, this decrease was 0.34 log. During ripening, *L. monocytogenes* numbers rapidly decreased and this reduction was 1.7 log at the end of 8 days. Reduction in the numbers of *L. monocytogenes* continued during storage period. Different reduction amounts were determined in sucuks at different MAs during storage period. After 8 days of storage (16^{th} day) decrease was slowed but continued. At the end of the 22 days of storage, the highest reduction was determined as 4.36 log in package of 100% CO_2.

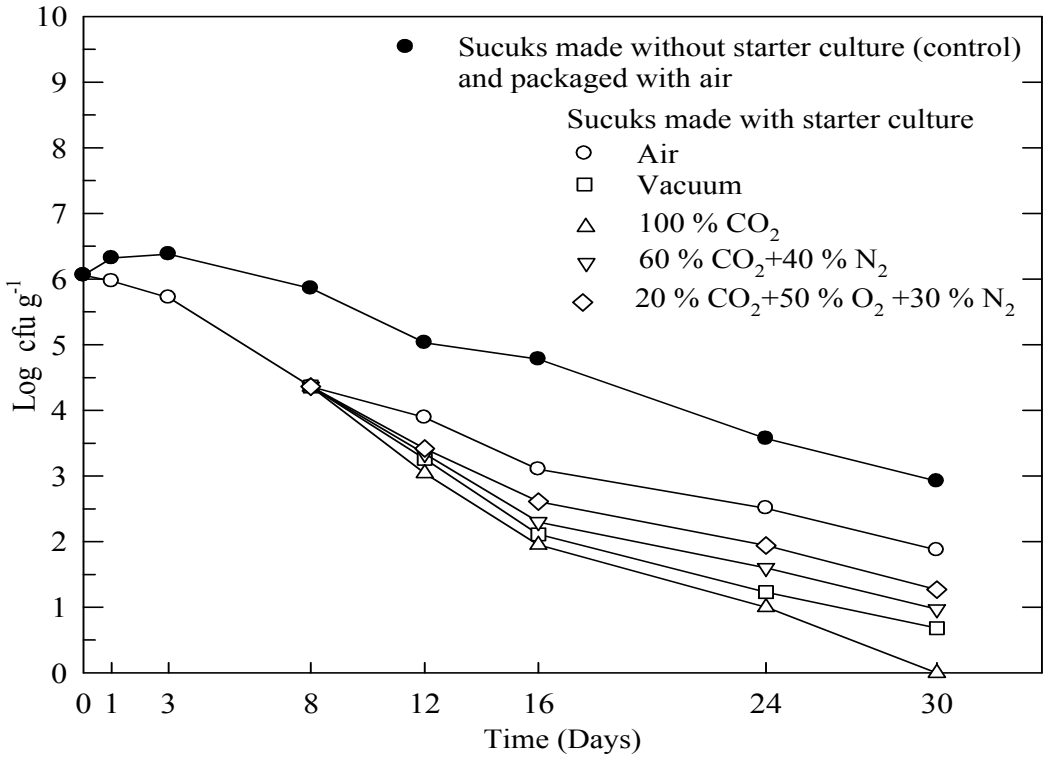

Figure 4.7 Survival of *L. monocytogenes* in sucuks made with and without starter culture during fermentation, ripening, and storage (at 4°C) with different atmospheres

In other packages these reductions were; 3.68 log in VP, 3.39 log in packages containing 60% CO_2 + 40% N_2, and 3.09 log in packages containing 20% CO_2 + 50% O_2 + 30% N_2. In sucuk package with air, the reduction of *L. monocytogenes* numbers was 2.49 log.

Mattila et al. (2003) determined that *L. monocytogenes* numbers decreased about 2 log cfu/g during 6 days of processing of sucuks made with starter culture (*Lactococcus plantarum*). At the end of 21 day storage it was recorded that *L. monocytogenes* was able to survive with 2.8 log cfu/g . Degenhardt and Anna (2007) reported that numbers of *L. monocytogenes* decreased during storage of Italian type sausages due to low acidity. Tyopponen et al. (2003) determined that *L. monocytogenes* in Northern Europe type sausages was inactivated completely at the end of 21 days of storage. These researchers added *L. monocytogenes* into sausage by 3 log cfu/g. In our research, at the end of 21 days of process, about 1.5 log cfu/g bacteria survived. When compared with Northern Europe type sausages, in our research, the number of *L. monocytogenes* added to sucuk initially was two times

more than Northern Europe type sausage. Nissen and Holk (1998) studied Norway fermented sausage and they determined that numbers of *L. monocytogenes* decreased during fermentation and storage. This reduction was 1.5 log at the end of 21 days of sausage processing. They reported a lower inactivation of bacteria than in our research. The possible reason of that may be use of different *L. monocytogenes* strains, addition of different additives in the production of sucuk dough, use of different starter cultures and different a_w levels. Again Lahti et al. (2001) determined that reduction in numbers of *L. monocytogenes* in sucuks made with starter culture occurred rapidly and this decrease was 3.5 log at the end of 49 days of storage.

4.1.2.2.3. Lactic acid bacteria

Effects of manufacturing and different MAs on LAB in sucuks made with starter culture and stored at 4^{o}C were showed in Figure 4.8. During fermentation of sucuk (3 days) a high increase was observed in the numbers of LAB count and this increase was about 1.55 log. During ripening (5 days), LAB decreased slightly as 0.14 log. After packaging of sucuks in MA during 4 days of storage, LAB numbers remained constant but decrease in numbers of LAB was observed in sucuks packaged with air. Especially between 12 and 16 days of process LAB decreased in all sucuks but in sucuks packaged with air numbers of LAB increased. During further days of storage, a slow decrease in the number of LAB was observed in all sucuks. But these reductions changed by 0.15 and 0.67 log from start of storage to end of 22 days. Different gas atmospheres did not affect the LAB numbers significantly ($p>0.05$).

Kaban and Kaya (2006) determined that numbers of LAB increased during fermentation of sucuk (3 days) and it remained constant during further ripening (11 days) at 9 log cfu/g, this increase was higher in sucuks made with starter culture then made without starter culture. Degenhardt and Anna (2007) recorded that LAB numbers increased during fermentation of sausages and then remained almost constant during storage period.

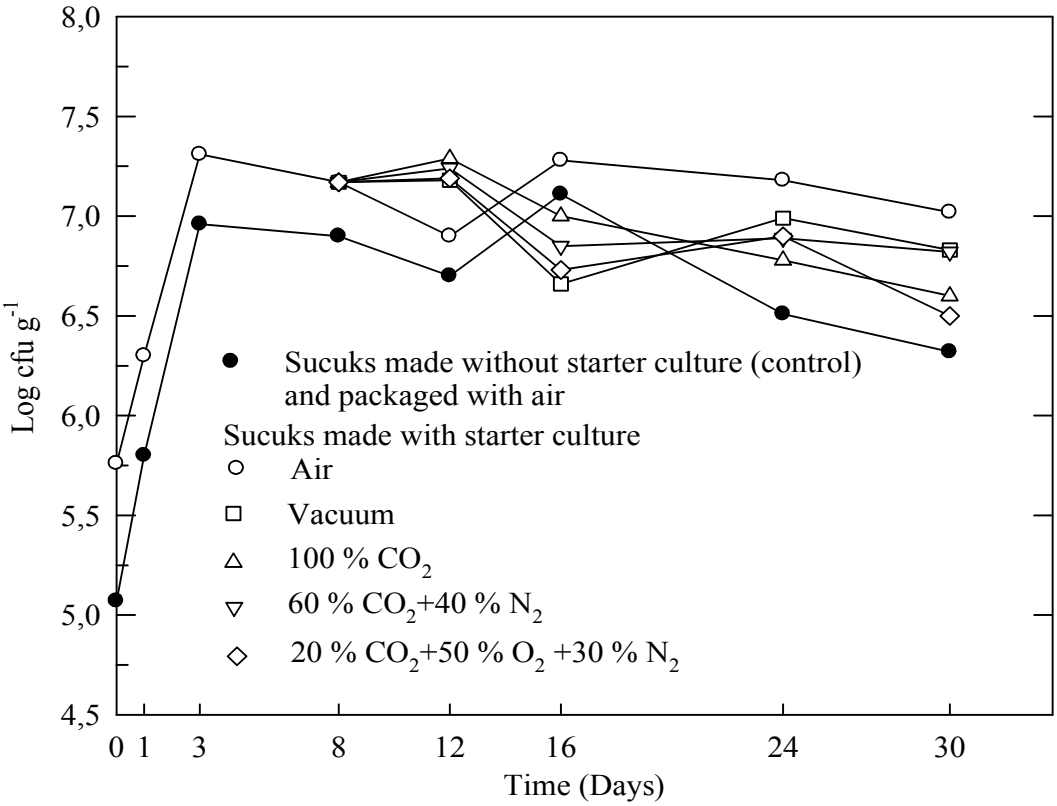

Figure 4.8. Effects of manufacturing and different modified atmospheres on lactic acid bacteria in sucuks made with and without starter culture and stored at 4°C

4.1.3. Microbiological and chemical changes in white cheese

4.1.3.1. Chemical changes in white cheese

4.1.3.1.1. Changes in pH

Changes of pH during storage (4°C) period in cheeses packaged with different MAs were shown in Table 4.5. In all cheeses, pH values decreased during first 7 days of storage and then started to increase. While pH of cheeses packaged with air decreased from 5.43 to 5.35, pH of cheeses packaged with 100% CO_2 decreased from 5.43 to 5.03. Similar decrease was determined in cheeses packaged with 30% CO_2. Here it is clearly seen that the formation of carbonic acid from dissolved CO_2 decreases the pH. Another reason of reduction of pH during first 7 days of storage may be because of developing microbial (LAB) activity in cheeses. But after 7 days of storage, nitrogenic compounds that released from activation of microorganisms in cheeses increased pH.

Table 4.5. Changes of pH during storage (4°C) time in cheeses packaged with different modified atmospheres.

	pH of cheeses in different packages					
Days	**Air**	**Vacuum**	**100% CO_2**	**100% N_2**	**30% CO_2/ 70% N_2**	**Control[1]**
0	5.43	5.43	5.43	5.43	5.43	5.43
2	5.39	5.32	5.07	5.40	5.38	5.28
7	5.35	5.26	5.03	5.25	5.32	5.24
15	5.38	5.34	5.09	5.37	5.39	5.42
30	5.42	5.37	5.10	5.41	5.40	5.40

[1] cheese packaged with air and without contamination of *L. monocytogenes*

Samelis et al. (2003) recorded a 0.53 pH unit reduction in vacuum packaged Greek type White cheeses that is produced from whey during 45 days of storage at 4°C. The initial pH of these cheeses was determined as 6.88. On the other hand, Juric et al. (2003) determined an increase in pH of semi hard cheese that packaged with different modified atmospheres from an initial pH 5.37, during 21 days of storage this increase reached to pH 5.46. Highest CO_2 amount used for packaging of these cheeses was 20%. Also as a result of being semi hard cheese (harder than White cheese) LAB could not grow and physicophilic bacteria developed and pH increased after 7 days. Gonzalez-Fandos et al. (2000) packaged Cameros type cheeses with different MAs and during 30 days of storage period, pH of cheeses was decreased continuously. The highest reduction was observed in cheeses packaged with 100% CO_2 as 5.9. These researchers determined that there was not any pH reduction effect of CO_2 in packages containing CO_2 below 40%. In our research, any significant ($p>0.05$) difference was not observed between packaged cheeses with different MAs.

Whitley et al. (2000) found that pH of cheeses packaged with different MAs decreased during 6 days of storage and further 4 days of storage pH of cheeses was remained constant. They expressed the reduction of pH of cheeses due to LAB inactivation and formation of carbonic acid from CO_2.

4.1.3.1.2. Changes in water activities

There was not any significant ($p>0.05$) differences in a_w of cheeses packaged with different MAs at 4°C. A_w changed between 0.94 and 0.93 during 30 days of storage. The initial a_w value of cheese was 0.9338.

4.1.3.2. Microbiological changes in Cheese

4.1.3.2.1. Aerobic bacteria

Changes in the numbers of AB in cheese packaged with different MA conditions during storage at 4°C were shown in Figure 4.9. In this research, numbers of AB decreased in all cheeses. This reduction was by 1.82 log in packages containing air, by 1.60 log in packages containing 100% N_2, by 2.30 log in packages containing 30% CO_2 / 70% N_2, by 2.39 log in vacuum packages, and by 2.76 log in packages containing 100% CO_2 respectively. On the other side, AB reduction in cheeses that were not contaminated with *L. monocytogenes* was 2.32 log. As seen, cheeses packaged with CO_2 had higher reduction in AB than packaged with O_2 and air.

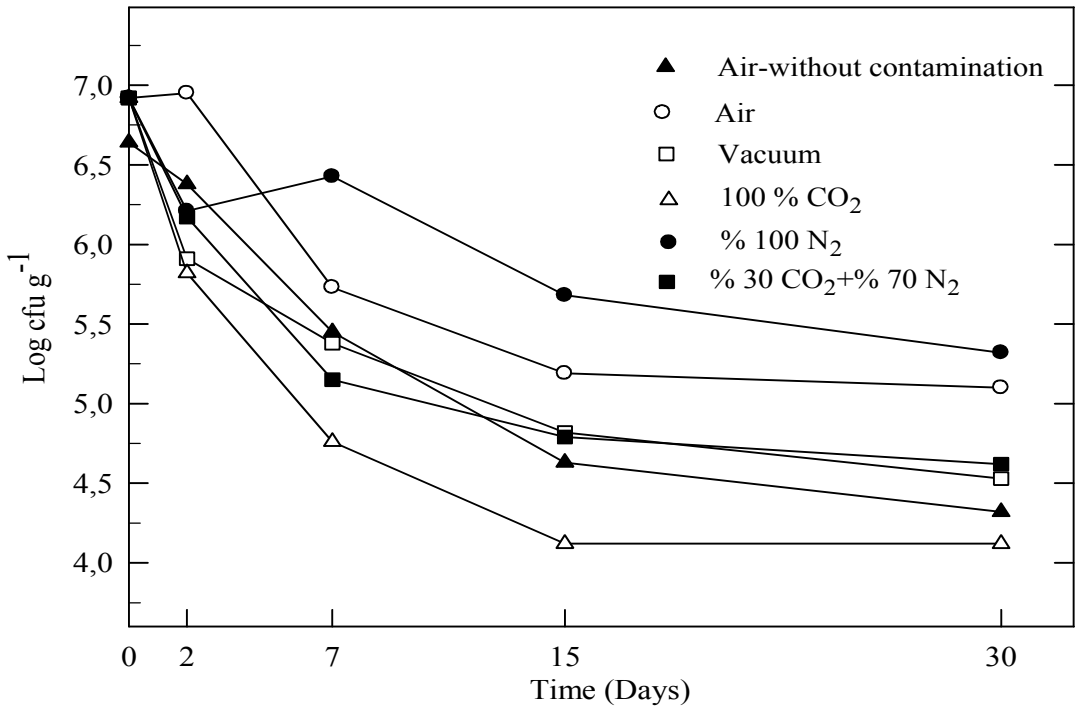

Figure 4.9. Changes in the numbers of aerobic bacteria in cheese packaged with different modified atmosphere conditions during storage at 4°C

Packages with CO_2 atmosphere showed more antimicrobial effect than other MAs. This antimicrobial effects of CO_2 due to carbonation of medium was clearly explained in many researches, by high pressure CO_2 researches (Erkmen, 2000, 2001a, b; 2003).

4.1.3.2.2. *Listeria monocytogenes*

Survival of *L. monocytogenes* in cheese packaged with different MAs and stored at 4°C was given in Figure 4.10. Numbers of *L. monocytogenes* decreased in all cheeses. During first 2 days, this reduction was occurred rapidly. The reductions in the numbers of *L. monocytogenes* were about 1.88 log in packages containing air, about 1.78 log in packages containing 100% N_2, about 2.06 log in packages containing 30% CO_2 / 70% N_2, about 2.51 log in vacuum packages, and about 2.96 log in packages containing 100% CO_2.

Cheeses packaged with CO_2 had higher reduction in numbers of *L. monocytogenes* than packaged with O_2 and air. The reason is reduction in pH because of formed carboxylic acid on the surface of cheeses.

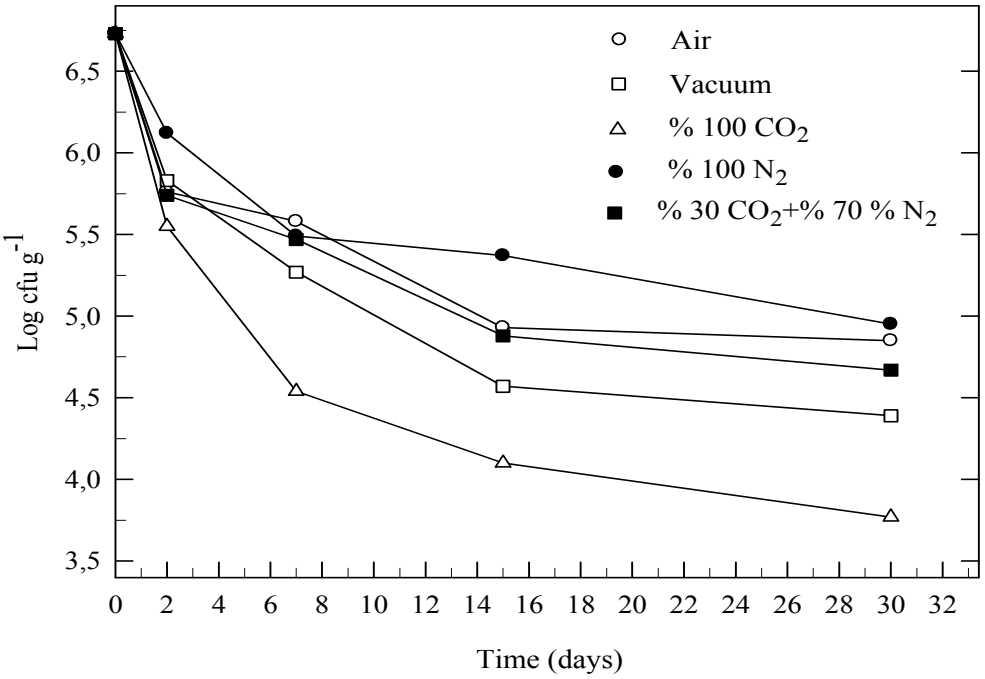

Figure 4.10 Survival of *L. monocytogenes* in cheese packaged with different modified atmospheres and stored at 4°C

Olarte et al. (2002) researched growth of *L. monocytogenes* in fresh Goat cheeses packaged with different MA conditions and stored for 28 days at 4°C. In this research, numbers of *L. monocytogenes* increased both in cheeses packaged with and without gas during storage time and this increase was about 4.0 log cfu/g. In our research, numbers of *L. monocytogenes* decreased during storage period. The reason of this difference may be due to pH of Goat cheeses that is above 6.2 and no usage of salt during making cheese. pH of cheeses in our research was 5.1 in average and salt amount was about 2.5%.

4.2. Survival of *Salmonella* Typhimurium in meat and sucuk

In this research, effects of modified atmosphere on *S.* Typhimurium MY, and AB at 4 and 12°C storage temperatures in meat and sucuk packaged with different modified atmospheres were studied. Also pH of sucuk was analyzed during storage.

4.2.1. Microbial changes in meat

The initial numbers of *S.* Typhimurium and AB were approximately 3.60 and 3.85 log cfu g^{-1} of meat respectively. Control meat, and sucuk meat and ingredients showed no *S.* Typhimurium, confirming that the samples were not cross-contaminated.

4.2.1.1. Aerobic bacteria

Changes of AB in meats during storage (4°C) periods depending on MA, are given in Figure 4.11. AB increased during the storage periods of meats. The increase in the number of AB in meats packed with air was 3.85 logs units after 12 days. The lowest number of increase was observed in meat packaged with 60 and 100% CO_2 packaged meats and the increases were about 1.88 and 2.24 logs respectively. After 22 days of storage, 2.93 and 3.36 logs increase was observed in meats packed with vacuum and 20% CO_2/30% O_2/50% N_2 respectively. On the other hand, effect of different MAs (CO_2, O_2 and N_2) on AB in meat stored at 12°C are given in Figure 4.12.

The increase in the number of AB in meat stored at 12°C was similar to meat stored at 4°C. These increases changes between 2.87 and 6.29 log. The lowest increase in the number of AB was observed in vacuum (2.87 log), the increase was 3.23 log in 100% CO_2 and 3.91 log in 60% CO_2.

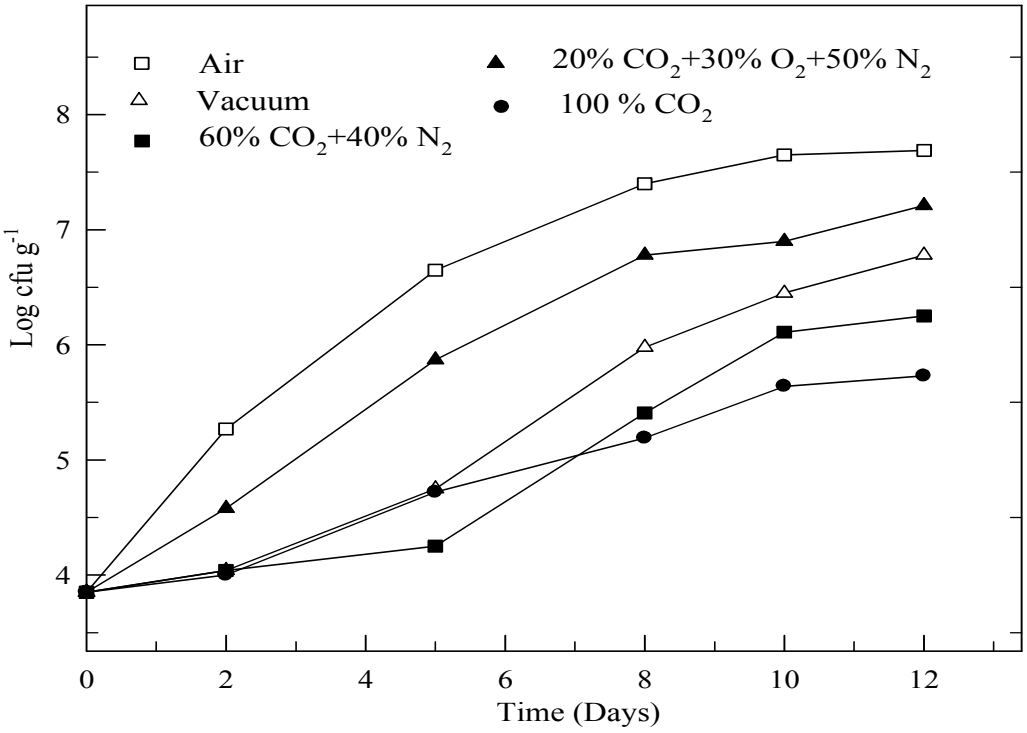

Figure 4.11 Effects of different modified atmospheres on aerobic bacteria in meat stored at 4°C

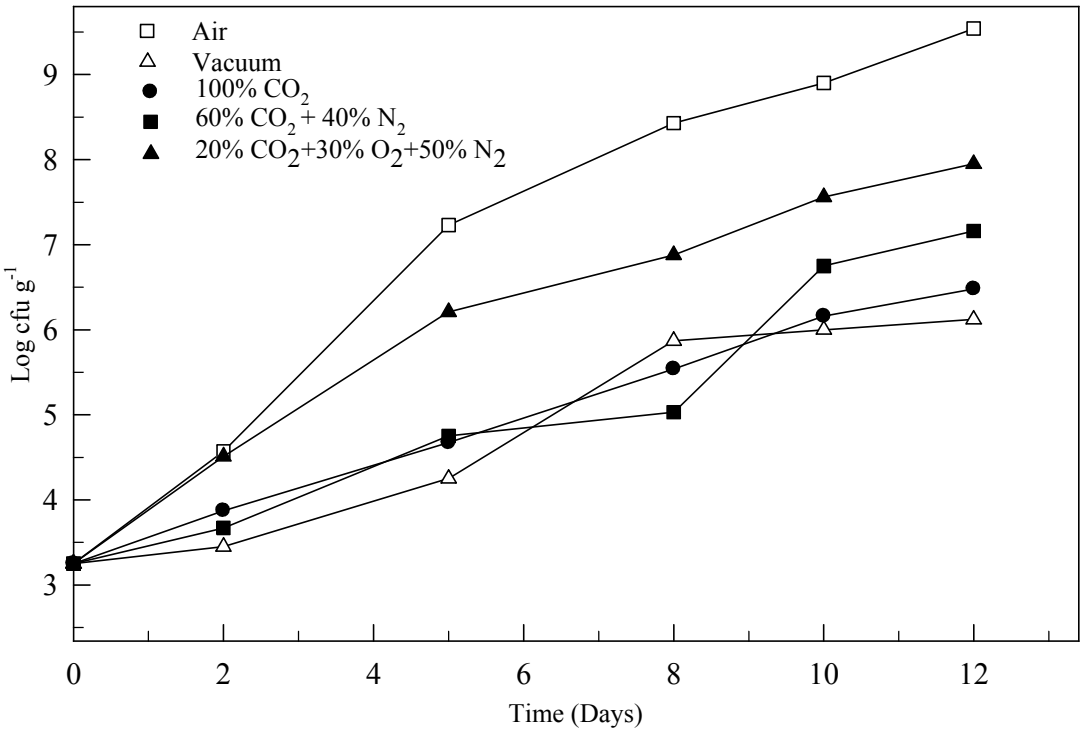

Figure 4.12 Effects of different modified atmospheres on aerobic bacteria in meat stored at 12°C

Numbers of AB in meats increased during storage periods at both 4 and 12°C. The increase in the numbers of AB was higher at 12°C compared to 4°C. The highest AB number in meats stored at 4°C was in air by 3.85 logs, but in meats stored at 12°C this increase in air was 6.29 logs.

4.2.1.2. *Salmonella* Typhimurium

The results of effects of different MAs on survival of *S.* Typhimurium in meat stored at 4°C were shown at Figure 4.13. During 12 days of storage the numbers of *S.* Typhimurium were decreased in all packaged meats. Higher number of decrease was observed in meat packaged with high level of CO_2 (60 and 100 %) during storage.

S. Typhimurium was reduced by 0.68, 1.10 and 1.37 logs unit g^{-1} in meat packaged with vacuum, 60% and 100% CO_2, respectively, while *S.* Typhimurium was reduced by 0.17 log in meat packaged with air. The decrease in the number of *S.* Typhimurium in meat packaged with 100% CO_2 and stored at 4°C was higher during first 5 days of storage but this decrease was slowed down after 5 days until 12 days of storage period.

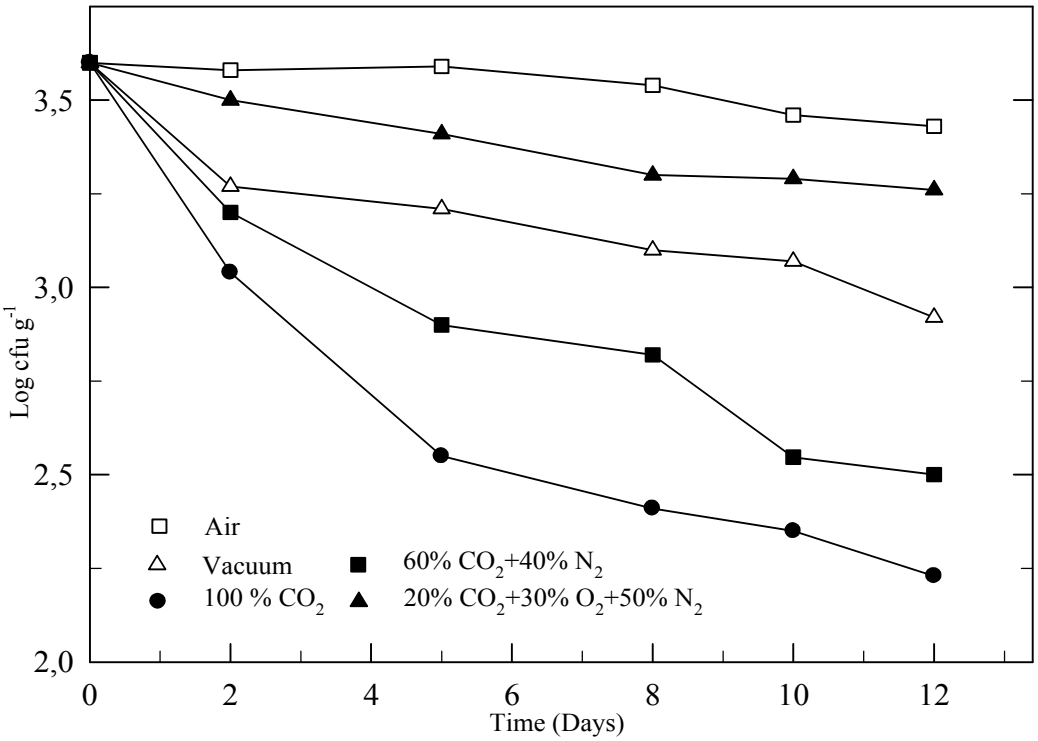

Figure 4.13 Survival of *Salmonella* Typhimurium in meats packaged with different modified atmospheres and stored at 4°C

On the other side, the numbers of *S.* Typhimurium were increased in all meat packages stored at 12°C during 12 days of storage (Figure 4.14).

The increase in the numbers of *S.* Typhimurium was between 0.8 and 5.4 logs in packaged meats. At the packaging with vacuum and 100 % CO_2, the increases in the numbers of *S.* Typhimurium were nearly 0.8 and 1.24 log, respectively.

Growth of AB in VP and MAP meat at low temperature observed in this study was also in agreement with the reports of other researchers (Bodnaruk and Draughon, 1998; Gill and Badoni, 2002; Tsigarida et al., 2000).

In this study, growth of *S.* Typhimurium in packaged meats was inhibited at 4°C and decreased slowly during storage periods, claim to what is found in other studies at 4.4°C (Hintlian and Hotchkis, 1987), at 5°C (Skandamis et al., 2002) and *S.* Typhimurium did not grow in foods stored below 5°C (Drosinos et al., 2000; Rao and Sachindra, 2002; Michaelsen et al., 2006).

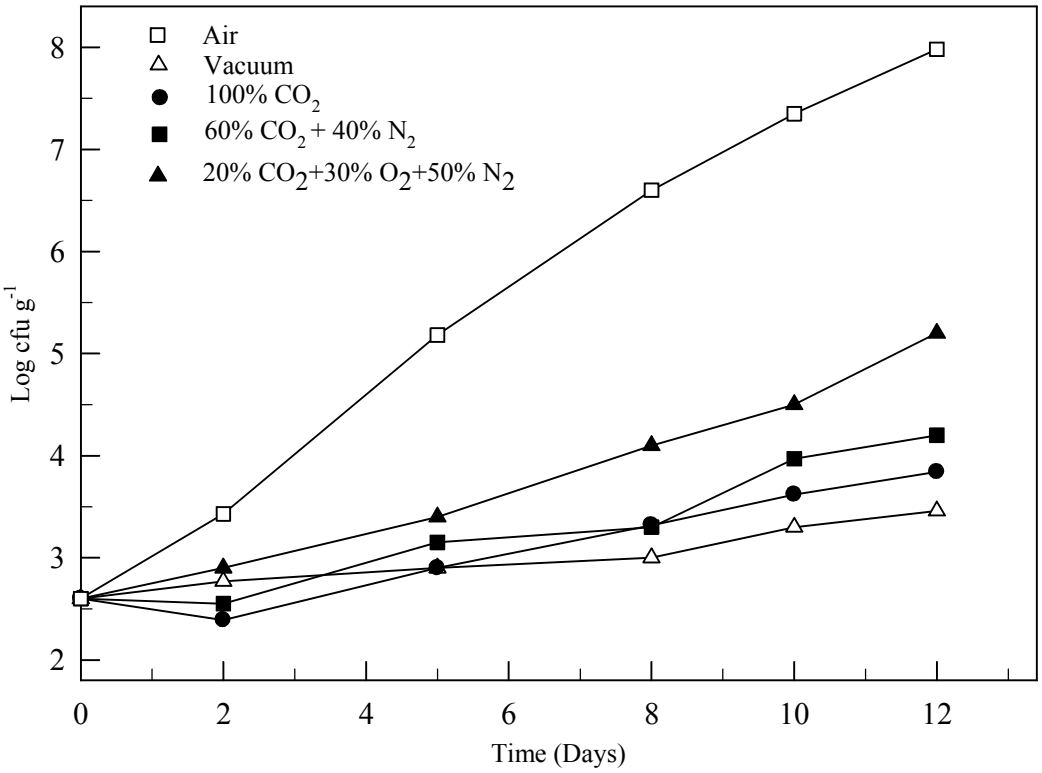

Figure 4.14 Survival of *Salmonella* Typhimurium in meats packaged with different modified atmospheres and stored at 12°C

Salmonella survived for 5 weeks in vacuum-packaged meat at 2°C (Rao and Sachindra, 2002). During storage of minced chicken meat for 28 days at 2°C, lower counts of *Salmonella* have been observed in CO_2 packs than in air packs (Baker et al., 1986).

Meat is a high-risk raw material because pathogens may be mixed into the ground product which may not be sufficiently heated before consumption. To inhibit growth of spoilage bacteria and increase shelf life, MAP is often used to retailers. However, there is a possibility that some pathogenic bacteria may be less inhibited by MAP (Hintlian and Hotchkiss, 1986). In this study, the high CO_2 concentration was inhibited the bacterium and reduced the growth of AB which was also reported by Dixon and Kell (1989). Nissen and Holck (1998) reported that vacuum and CO_2 packing have an inhibitory effect on *Salmonella* and the degree of inhibition increases as the temperature decreases.

High level of CO_2 (100 and 60%) atmosphere showed higher effect on the inhibition of *S.* Typhimurium and AB in meat than the low level CO_2 (20 %) and vacuum. The differences in AB populations in meats stored with air and gas packaging or VP become important after 12 days of storage, probably due to the increasing generation time in the presence of CO_2 at low temperature. CO_2 dissolves in water to form carbonic acid which reduces pH of meat (Rao and Sachindra, 2002). The effects of the MAs on microorganisms in meat seems to be responsible for the data obtained, together with a probable contribution from pH which in turns is likely to be influenced by the gas atmosphere. It is known that CO_2 has an antimicrobial effect (Farber, 1991).

4.2.1.3. Mold and yeast

Mold and yeast (MY) numbers at both 4ºC and 12ºC during the 12 days incubation were recorded. At the first day, the number was 1.3 log cfu/g but at the end of 2 days storage, all mold and yeast were inhibited completely. Because of this, results were not given. On the other hand MY numbers showed little increase about 0.8 log and reached 2.1 log cfu g^{-1} at the end of 12 days storage of meats packaged with air.

4.2.2. Chemical and microbial changes in Sucuk

4.2.2.1 Chemical changes in Sucuk

4.2.2.1.1 Changes in pH

Changes in pH during manufacturing and storage of sucuks with or without starter culture at different atmospheres are shown in Figure 4.15. Within the 3 days of fermentation, the pH decreased from 5.72 (initial) to 5.36 and approximately 4.78 in sucuks made without and with starter culture, respectively. There were significant ($p<0.05$) differences in pH between sucuks made with and without starter culture. The pH values of sucuks made with starter culture were slightly increased during drying and storage, and stabilized around 4.99 after 22 days of storage.

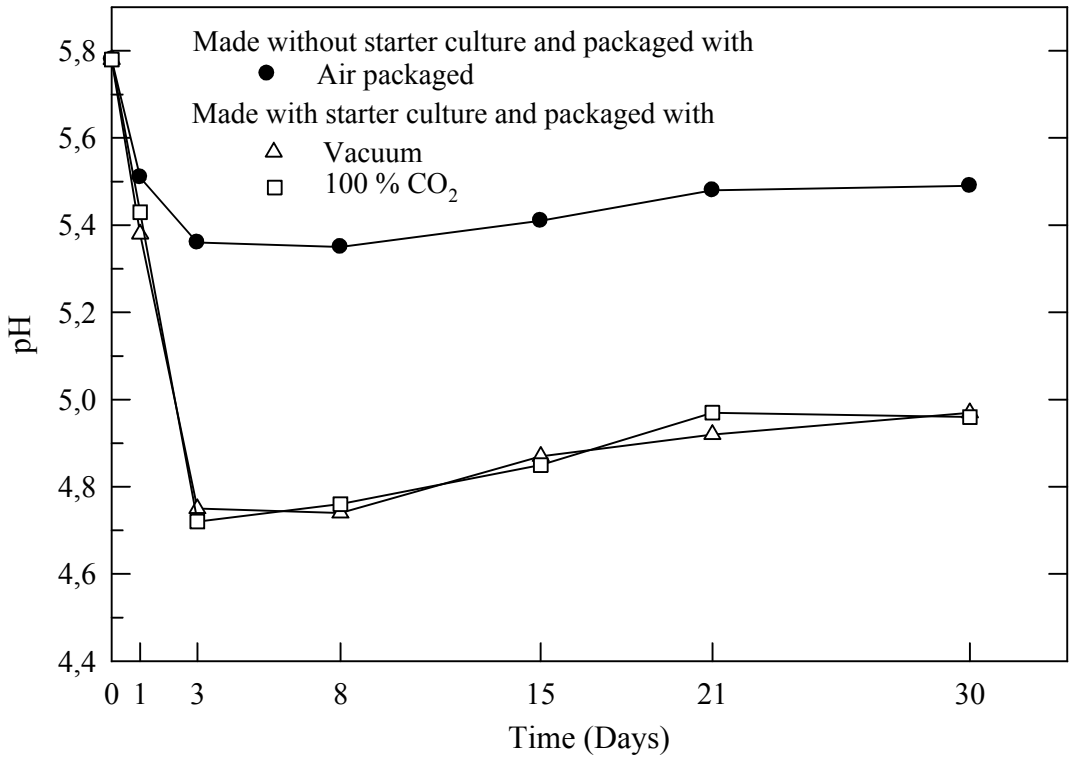

Figure 4.15 Changes in pH of sucuk made with and without starter culture and packaged with different modified atmospheres and stored at 4°C

4.2.2.2. Microbial changes in sucuk

4.2.2.2.1 Aerobic bacteria

During the first one day of the fermentation there was a slight increase in the number of AB (Figure 4.16) in sucuks made with starter culture. The number of AB decreased rapidly during fermentation (3 days) as the pH decreased rapidly. After

packaging and during storage (22 days) at 4°C, the decreases were continued. The survival of AB after 22 days of storage was 2.49 and 2.84 log cfu g^{-1} in sucuks made with and without starter culture, respectively, packaged with air. The survival of AB was lower in sucuks packaged by high level of CO_2 (60 and 100 %) than the other atmospheres. The numbers of AB decreased by 4.75 log in 100% CO_2, 4.4 log in vacuum and 3.96 log in 60% CO_2 packages.

There are significant ($p<0.05$) differences between sucuks packaged with air and MA packages.

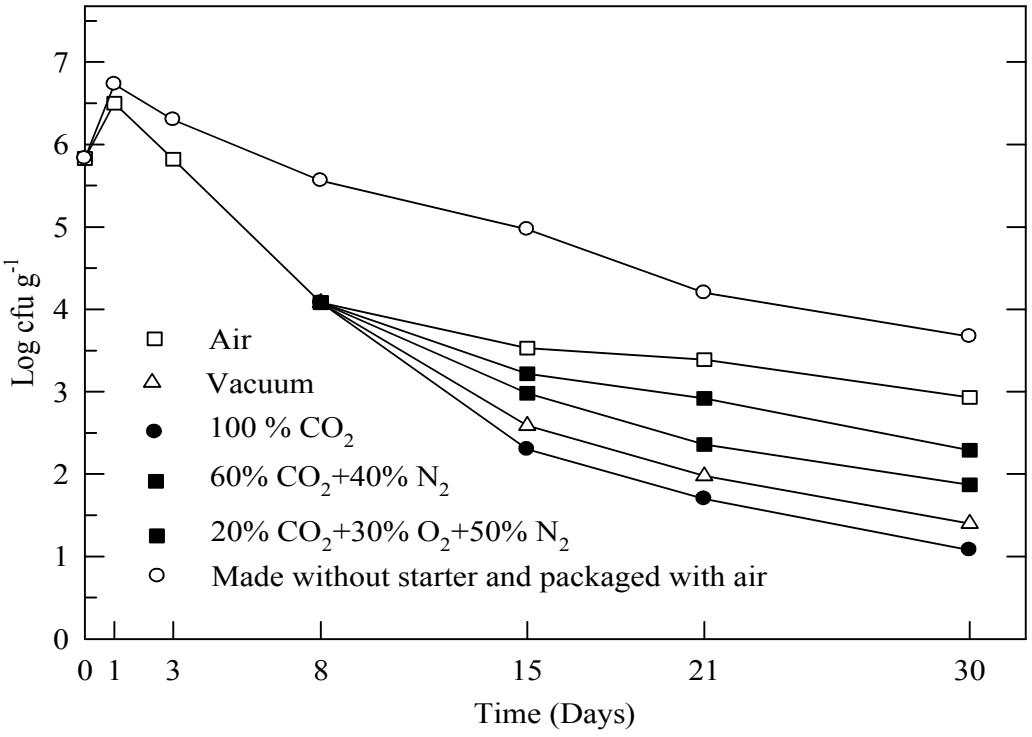

Figure 4.16 Effect of different modified atmospheres on aerobic bacteria in sucuks made with and without starter culture and stored at 4°C

On the other hand, numbers of AB increased in air packaged sucuks stored at 12°C during 30 days of processing (Figure 4.17). At 8 days of fermentation and ripening AB numbers increased from 3.7 to 7.59 log cfu/g. After packaging (8 days) during storage (at the 15 days) AB numbers decreased in vacuum (1.56 log), in 100% CO_2 (1.14 log), and in 60% CO_2 (0.78 log). An at the end of 22 day of storage the highest decrease were seen in 100% CO_2 packages as 0.47 log. Sucuks made with starter culture and packaged with air had an increase about 2.5 log during 22 day of storage.

Bu sucuks made without starter culture and packaged with air increased during 30 day of processing and this increase was about 6 log unit.

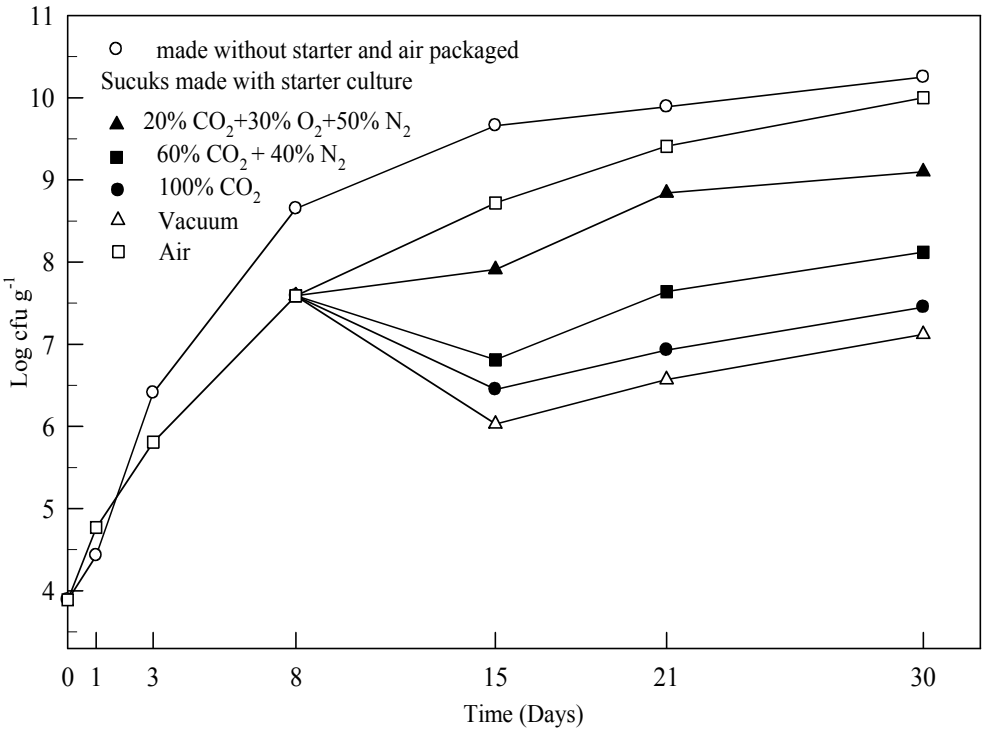

Figure 4.17 Effect of different modified atmospheres on aerobic bacteria in sucuks made with and without starter culture and stored at 12°C

4.2.2.2.2. Lactic acid bacteria

The numbers of LAB during manufacturing and storage of sucuks made with or without starter culture packaged with different atmospheres are shown in Figure 4.18. Before fermentation, the number of endogenous LAB in the sucuk made without LAB was 5.78 log cfu g^{-1}. During fermentation, the counts of LAB grew at a faster rate in the sucuks. After 3 days of fermentation, the counts of LAB increased by 3.18 logs of sucuks. During the drying and storage period, LAB were slightly reduced between 1.18 and 0.54 logs.

At the beginning of fermentation of sucuk with starter culture, the numbers of inoculated LAB increased and this increase was approx. 3.17 logs in 3 days and later the numbers of LAB slightly decreased. The fermentation of carbohydrates to lactic acid decreases the pH value of the sucuk. The lower external pH disturbs the

homeostasis of the bacterial cell (Leistner, 2000), including pathogenic and spoilage bacteria, and therefore restricts their growth.

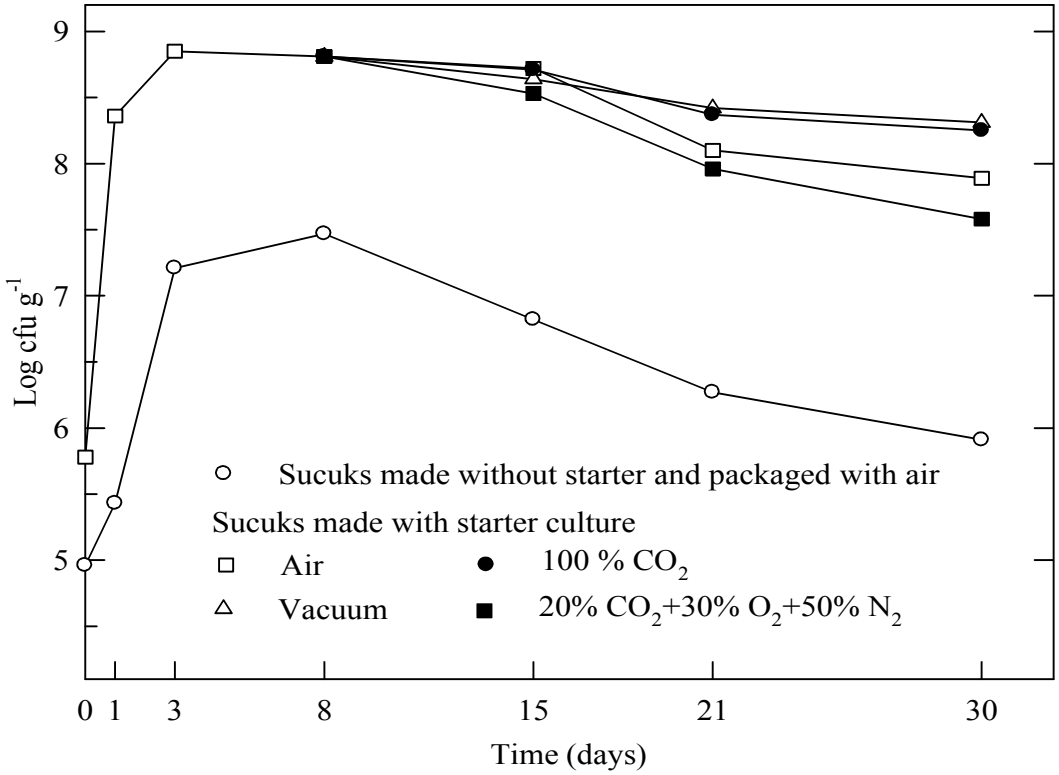

Figure 4.18 Effect of fermentation, ripening and different modified atmospheres on lactic acid bacteria in sucuks made with and without starter culture and stored at 4°C

Furthermore, the decrease in pH causes a decrease in the water binding capacity of the meat, which accelerates the drying process of sucuk. The drying process results in a low water activity of the end product, where only a few foodborne pathogens may be able to survive (Glass et al., 1992; Hugas et al., 1995).

4.2.2.2.3. *Salmonella* Typhimurium

The effect of different gas compositions on the *S.* Typhimurium added to packaged sucuk dough at 4ºC and 12ºC was observed in this research too. Figure 4.19 shows the effect of different modified atmospheres on *S.* Typhimurium in sucuks stored at 4ºC.

During the first one day after the fermentation there was a slight decrease (from 5.62 to 5.58 log cfu g^{-1}) in the number of *S.* Typhimurium (Figure 4.18) in sucuks made

with starter culture. The numbers of *S.* Typhimurium were rapidly decreased during ripening periods as the pH was decreased rapidly (from 5.72 to 4.78).

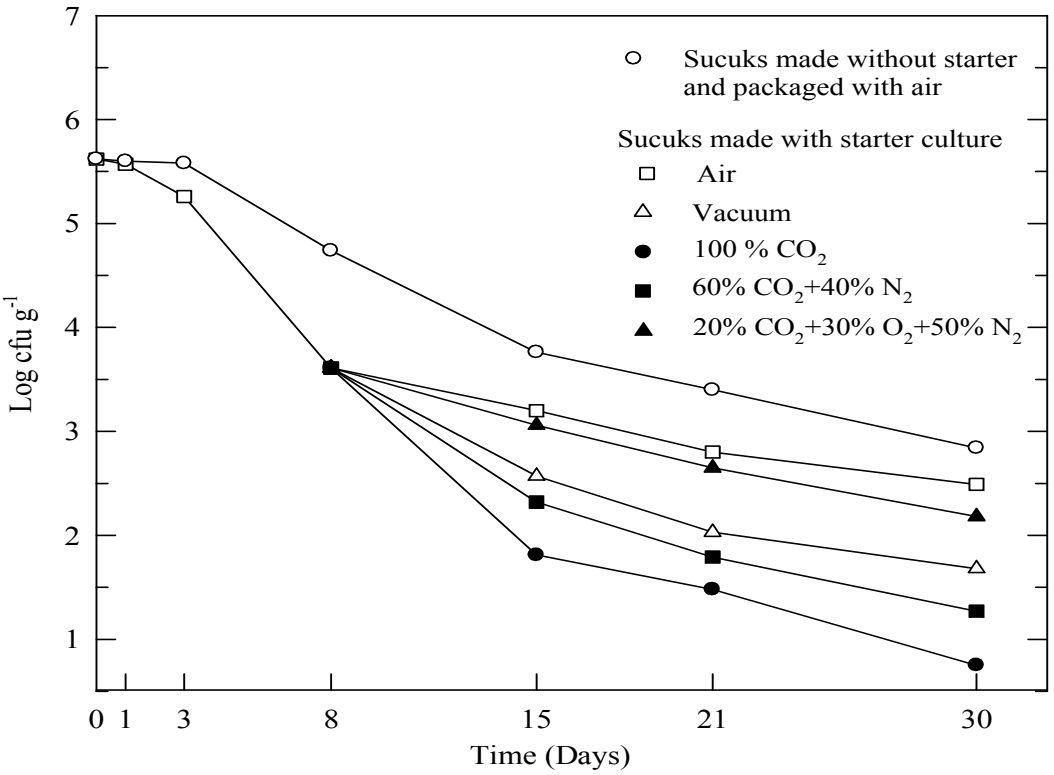

Figure 4.19 Survival of *S.* Typhimurium in sucuks made with and without starter culture during fermentation, ripening and storage at 4°C with different modified atmospheres

After packaging and during storage at 4°C of sucuks, the reductions in the number of *S.* Typhimurium were continued. After 22 days storage, the survival of *S.* Typhimurium was 1.27 and 0.75 log cfu g^{-1} in 60 and 100% CO_2 packaged sucuks, respectively, while 1.68 and 2.18 logs cfu g^{-1} survived in sucuk packaged with vacuum and 20% CO_2/30 %O_2/50 %N_2, respectively. The survival of *S.* Typhimurium was 2.49 log cfu g^{-1} in air packaged sucuk made with starter culture after 22 days of storage. On the other hand, the survived number of *S.* Typhimurium was 2.85 logs cfu g^{-1} in sucuks made without starter culture (Figure 4.19) due to insufficient reduction in pH (from 5.72 to 5.36) during fermentation (Figure 4.15). There were significant ($p<0.05$) differences in the number of *S.* Typhimurium between sucuks packaged in the presence of high (100 or 60 %) and low (20 %) concentration of CO_2, and between sucuks packaged in the presence of high CO_2 and

air. These indicated that additional positive antimicrobial effect was obtained by using CO_2 in packaging atmosphere.

Survival of *S.* Typhimurium in sucuks made with and without starter culture during fermentation, ripening and storage at 12°C with different gas atmospheres is shown in Figure 4.20.

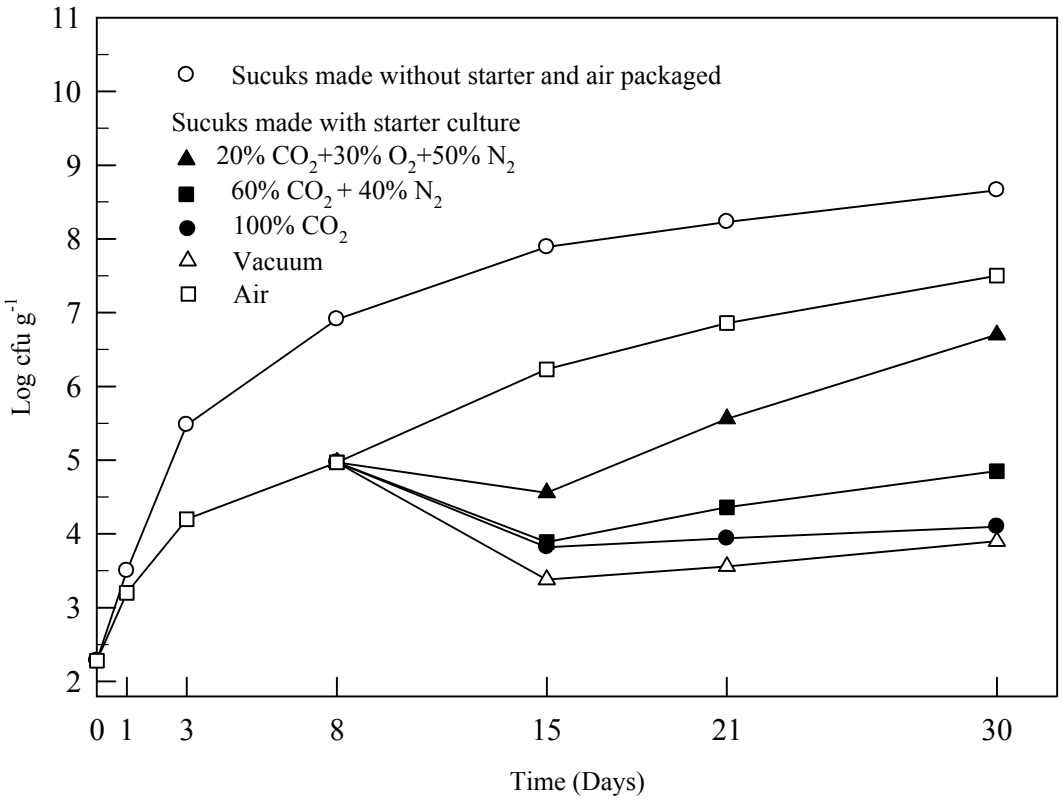

Figure 4.20 Survival of *S.* Typhimurium in sucuks made with and without starter culture during fermentation, ripening and storage at 12°C with different modified atmospheres

During fermentation and ripening numbers of *S.* Typhimurium increased 4.63 log in sucuks made without starter culture and packaged with air, 2.69 log in sucuks made with starter culture and packaged with air. After ripening, during 7 days of storage (15 days) numbers of *S.* Typhimurium in all MA packages decreased, the highest decrease was seen in vacuum packages by 1.6 log. The decrease in 60 and 100% CO_2 packages was 1.15 log (between 8^{th} and 15^{th} days). After 15^{th} days *S.* Typhimurium numbers increased until the end of storage period (22 days of storage). But in total storage period numbers of *S.* Typhimurium decreased (22 days of storage) in sucuk packages containing 60 and 100% CO_2 by 0.28 and 0.96 logs, respectively. On the

other side, *S.* Typhimurium numbers increased in sucuks packaged with low level CO_2 (20% CO_2) by 2.15 log between 15^{th} and 30^{th} days.

S. Typhimurium numbers decreased in sucuks packaged with vacuum and 100% CO_2 at 4ºC. On the other hand, package atmosphere with O_2 showed increase at *S.* Typhimurium counts. So it can be understood as CO_2 showed antimicrobial effect on *S.* Typhimurium. This effect has seen when CO_2 is dissolved in water and turned to carbonic acid. Carbonic acid lowers the pH of the medium and this change decreases or inhibits the growth of *S.* Typhimurium. Microbial cell walls are not permeable to ions, but permeable to organic acids like carbonic acid. As a result of this, decomposition of carbonic acid in the cytoplasm cause immediate decrease at the pH of cytoplasm (Erkmen, 2000, 2001a, b; 2003).

In the literature, it was described that storage of foods by changing the package atmosphere had different effects on microorganisms (Nissen et al., 2000; Tasso and Boziaris, 2002; Cayre et al., 2005). Immediate decrease of pH in and out of the microbial cell was effective on the vital functions of cell; enzyme activation, protein and ions. That effect inhibited the growth of microorganisms (Bodnaruk and Draughon, 1998; Kimura et.al., 1999; Nissen et al., 2000; Francis and O'Beirne, 2001). Nissen et al. (2000) studied the effect of *Yercinia enterocolitica*, *Listeria monocytogenes*, *S.* Typhimurium and *Escherichia coli* on the minced meat packed with different gas compositions and stored at 10ºC. At the end of the research, it was found that growth of microorganisms was increased but none were higher than the counts of microorganisms at the air packed meat.

Hintlian et al. (1987) determined that inhibition of microorganisms, in cooked roasted meats packaged with vacuum, changed depending to medium and microorganism type. Piagentini et al. (1997) determined the inhibition effect of CO_2 on AB by changing the medium atmosphere. Similar researches were done on; fungi (Suhr and Nielsen, 2005), *Staphylococcus aureus* (Kimura et al. 1999), *Listeria monocytogenes* (Franco-Abuin et al. 1997; Bourke and O'Beirne, 2004), *Brochothrix thermosphacta* (Cayre et al., 2005), *Y. enterocolitica* (Pin et al., 2000; Bodnaruk and Draughon, 1998), *Salmonella* (Drosinos et al., 2000; Tasso and Boziaris, 2002; Skandamis et al., 2002), *Escherichia coli* (Nissen et al., 2000) and AB (Panague et al., 2002; Lyhs et al., 2001; Ho et al., 2003) with different foods. Effect of MAP on

microbiological and chemical changes was observed in meat and roasted meat, and in these researches, as a result of changing the medium atmosphere at the presence of CO_2, MAP was found effective on growth of TAB and slowing at growth was determined (Hintlian et al., 1987; Gill and Badoni, 2002). By changing the medium atmosphere as decreasing or absence of O_2, growth of fungi inhibited and reductions were determined (Hintlian et al., 1987; Westall and Filtenborg, 1998; Suhr and Nielsen, 2005).

CONCLUSION

In our research and in literature knowledge changing package atmosphere causes inhibition of growth of microorganisms at different factors. These factors can be listed as; types of microorganism, types of food, composition of gases in package atmospheres, pH, water activities and existing inhibitor. It is seriously needed to obey the hygiene and sanitation rules in production areas and during process to obtain low numbers of microorganisms in foods. When meat, sucuk and cheese stored by changing package atmosphere and reached to the customers, growth of both pathogen and spoilage microorganisims, spoilage of foods, and creating threat to public health can be prevented. Especially application of modified atmosphere with addition of CO_2 to the package atmosphere can cause antimicrobial effect on microorganisms.

Growth of AB in meats contaminated with *S.* Typhimurium and *L. monocytogenes* and stored at 4°C was observed. AB numbers were increased in both meats contaminated with *S.* Typhimurium and *L. monocytogenes*. Also during storage at 12°C AB numbers in meats contaminated with *S.* Typhimurium increased too. AB numbers in sucuks contaminated with *S.* Typhimurium decreased in all packages stored at 4°C. But AB numbers in sucuks contaminated with *S.* Typhimurium stored at 12°C increased a lot. Sucuks contaminated with *L. monocytogenes* and stored at 4°C were also observed according to AB numbers. In all sucuk packages stored at 4°C, AB numbers were decreased.

When we compare two pathogens interested in this research (*L. monocytogenes* and *S.* Typhimurium), in meat and sucuk packaged with different MAs, both pathogenic bacteria; *L. monocytogenes* and *S.* Typhimurium numbers were decreased in sucuks packaged with different modified atmosphere and stored at 4°C. In meats *L. monocytogenes* numbers were decreased in CO_2 containing packages but increased in O_2 containing (Low CO_2) packages. On the other hand in meats stored at 4°C *S.* Typhimurium numbers were decreased in all packages.

S. typhimurium numbers in meat and sucuk stored at 12°C were increased in both meat and sucuk packages. So in order to protect meat and sucuk from pathogenic bacteria growth, meat and sucuk should be kept at low temperatures such as 4°C. Refrigerator temperature changes between 10 to 15°C, so meat and sucuk should be stored at freezing temperature such as 4°C and below. According to the results of our research, the importance of both packaging with CO_2 and storage temperature was observed. If meat and sucuk are produced at sterile conditions and microbial contamination kept low during production stored at 4°C, meat and sucuk were determined as applicable for public health.

Bacterial spoilage of sucuk dough had seen more rapidly than meat cuts. This high rate of spoilage was caused because of increased surface area by mincing the meat before preparing the sucuk dough mixture. General risks of L. monocytogenes and S. Typhimurium contamination for researched foods (meat, sucuk and cheese) in literature can be listed as: MAP foods stored at high temperatures, usage of non-processed foods that increases microbial load and contamination, not storing foods at cold (≤ 4°C), not obeying rules of hygiene and sanitation in food production and not cleaned materials used for food production like forks, blades properly.

During developing technologies for production of safe foods, modified atmosphere packaging always took an important place. As we look future, MAP can give hope for prolonging shelf life of various foods depending on its history. Similar to other preservation techniques MAP cannot be enough for 100% safety alone. The combined use of several preservative methods is known as “hurdle” or “inhibitory factor” concept. This concept may also be applied to MAP of sucuk, as the change in gaseous atmosphere combined with other hurdles, such as, preservatives, low pH, low a_w, low temperature, fermentation, etc., can exert a strong pressure on the developing microflora in sucuk. Even though dry-fermented sucuk is generally regarded as safe and stable products, our results emphasize the necessary for good manufacturing practices during production to assure product safety. These safe practices are especially important for all pathogens have been reported to cause disease, in some cases even when ingested in very low numbers. This research also emphasizes the prevention of contamination from cattle and other carrier species

from farms and hygienic processing conditions in slaughterhouse and sucuk manufacturing plants.

Consequently, VP or MAP can be seen as (i) a means of preventing the growth of *L. monocytogenes*, *S. typhimurium* and AB in meats, (ii) as a more effective system for control of meat spoilage and increase of safety, (iii) reducing *L. monocytogenes* and *S. typhimurium* in sucuks during manufacturing and storage, and (iv) providing safety against pathogens with removal of air or with introducing CO_2. It can be said that modified atmospheres were inhibitory to food-borne pathogens and may provide added safety to meat products and cheese. So with its antimicrobial effects, MAP technique which has an advantage of keeping foods fresh, also it can be used as preservative and protective packaging against pathogens. By using supporting materials such as, antimicrobial packaging materials, time-temperature indicators, MAP can be more effective at preservation in food industry.

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