EFFECTS OF YEAST SPECIES ON QUALITY CHARACTERISTICS OF FERMENTED TURKISH SAUSAGES

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ABSTRACT

EFFECTS OF YEAST SPECIES ON QUALITY CHARACTERISTICS OF FERMENTED TURKISH SAUSAGES

In this study, the effects of yeast species in combination with starter cultures on the chemical, physical, microbiological, organoleptic and aroma characteristics of fermented Turkish sausages (sucuk) during processing and storage were investigated. *Debaryomyces hansenii* and *Yarrowia lipolytica* were used as yeast species. During processing a decrease in moisture content and water activity and an increase in protein and fat contents were determined. The pH values of sausage samples decreased while titratable acidity values increased during ripening. Thiobarbituric acid values of samples having *Y.lipolytica* in combination with starter cultures increased during ripening and decreased during storage. Non-protein nitrogen contents of sausage samples showed an increase during processing while protein solubility of all samples decreased during ripening and storage. Moreover, addition of yeast species did not show any difference in protein solubility. According to sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles, sarcoplasmic and myofibrillar proteins were not affected by the addition of yeast species. Addition of yeast species did not have any important effect on the color of sausage samples during fermentation and ripening. Total aerobic mesophilic bacteria and lactic acid bacteria counts increased, but *Enterobacteriaceae* and yeast counts decreased in all samples. Moreover, micrococci/staphylococci counts decreased in all samples. It was determined that an important part of the volatile fraction in all sausage samples is composed of terpenes. Samples having *Y.lipolytica* in combination with starter cultures had the lowest lipid oxidation derived volatile compounds. According to the sensory analysis, samples having yeast species had the highest overall acceptability scores.

ÖZET

MAYA TÜRLERİNİN FERMENTE TÜRK SUCUKLARININ KALİTESİNE ETKİLERİ

Bu çalışmada, starter kültürlerle birlikte kullanılan farklı maya türlerinin üretim ve depolama sırasında fermente Türk sucukların kimyasal, fiziksel, mikrobiyolojik, duyusal ve aroma karakteristikleri üzerine etkileri araştırılmıştır. Maya türleri olarak *Debaryomyces hansenii* ve *Yarrowia lipolytica* kullanılmıştır. Üretim süresince sucuklarda nem miktarı ve su aktivitesi değerlerinde düşüş, protein ve yağ miktarlarında ise artış gözlemlenmiştir. Olgunlaştırma süresince sucukların pH değerleri düşerken titre edilebilir asitlik değerleri artmıştır. *Y. lipolytica* içeren örneklerin tiyobarbiturik asit değerleri olgunlaşma süresince artmış ancak depolama sırasında düşüş göstermiştir. Sucukların protein olmayan azot değerleri olgunlaşma süresince artarken, protein çözünürlüğü değerleri üretim ve depolama boyunca düşüş göstermiştir. Ancak maya ilavesi protein çözünürlüğü üzerinde önemli bir fark yaratmamıştır. Sodyum dodesil sülfat-poliakrilamid jel elektroforezi sonuçlarına göre maya ilavesinin sarkoplasmik ve myofibriller proteinlerdeki değişmelere etkisinin olmadığını göstermiştir. Maya ilavesi sucukların renk değerleri üzerinde üretim süresince önemli bir etki göstermemiştir. Toplam aerobik mezofilik bakteri ve laktik asit bakteri sayılarında artış, *Enterobacteriaceae* ve maya sayılarında azalma olmuştur. Bununla birlikte, *mikrokoklar/stafilokoklar* tüm örneklerde azalma göstermiştir. Tüm sucuk örneklerinde uçucu bileşik fraksiyonun önemli bir kısmını terpenlerin oluşturduğu tespit edilmiştir. Lipid oksidasyonu sonucu oluşan bileşiklerin starter kültürlerle birlikte kullanılan *Y. lipolytica* ilave edilen örneklerde diğer örneklere kıyasla daha düşük seviyelerde olduğu tespit edilmiştir. Duyusal analiz sonuçlarına göre maya ilave edilen örneklerin daha yüksek genel beğeni puanları aldığı belirlenmiştir.

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LIST OF ABBREVIATIONS

cfu	: Colony Forming Units
GC/MS	: Gas Chromotography/Mass Spectrometry
LAB	: Lactic Acid Bacteria
min	**:** Minute
µl	**:** Microliter
MEB	: Malt Extract Broth
MRS	: Man, Rogosa, Sharp
MS	: *Micrococcus/Staphylococcus*
RH	: Relative humidity
rpm	: Revolutions per minute
SDS-PAGE	: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SPME	: Solid-Phase Micro Extraction
TAMB	: Total aerobic mesophilic bacteria

CHAPTER 1

INTRODUCTION

Sausage is a very popular fermented meat product in Turkey and can also be found in countries located in Balkans, Middle East and Caucasus (Ercoşkun et al., 2009).

Sausage is a product produced from a mixture of meat (beef, sheep and/or water buffalo meat), fat (beef fat and sheep tail fat), salt, sugar, garlic, spices and seasonings and this mixture is stuffed into casings where fermentation is carried out until a semi or dry product is obtained (TSE, 2002). Sausage formulation can change from factory to factory, but a typical formulation consists of 80 % beef meat, 10-20 % tail fat, 2-3 % salt, 0.05 % nitrate or 0.01-0.015 % nitrite and 0.1-3 % starter culture (Bozkurt and Erkmen, 2006). After filling this mixture into natural or artificial casings, sausage is hung up to ferment at 22 °C–23 °C by either microorganisms naturally present or added starter cultures and allowed to dry for several weeks at ambient temperature and humidity (Ensoy et al. 2010).

Numerous chemical and biochemical changes occur during ripening of fermented sausages that determine the flavor of the final product. Glycolysis, proteolysis, lipolysis and lipid oxidation are the main reactions carried out by endogenous meat enzymes and microbial enzymes.

In traditionally-produced sausages, fermentation is carried out by chance inoculation under natural climatic conditions (Ertaş and Göğüş, 1980). However, modern meat industry have modified the traditional method and started to produce sausage by using starter culture and applying heat due to the long processing time, variability in final product and dependence on natural climatic conditions (Soyer, Ertaş and Üzümcüoğlu, 2004).

Lactic acid bacteria and Gram positive catalase positive bacteria are the main starter cultures having technological importance in sausage fermentation. Lactic acid bacteria contribute to flavor by producing lactic acid, while Gram positive, catalase positive bacteria play a role in lipolysis, proteolysis, degradation of peroxides and color stability (Garcia-Varona et al., 2000).

However, the characteristics of sausages produced with the addition of starter cultures and heat application are quite different from the naturally fermented ones with respect to taste and flavor. Currently, there is a much higher market share for the sausages produced with starter cultures and heat application than the naturally fermented sausages. Nevertheless, there is a strong demand for the naturally fermented sausages by consumers (Kaban and Kaya, 2007). In this sense, different starter culture combinations have tried in order to obtain the flavor similar to those of traditional sausages. Studies carried out with different yeast species have indicated that they can positively contribute to flavor development and stabilization of red color due to their proteolytic, lipolytic activities and ability to degrade peroxides. *Debaryomyces hansenii* is one of the yeast species isolated from fermented sausages and it is used in starter preparations due to its occurrence in traditional sausages and its positive effect on flavor and color. *Yarrowia lipolytica* has also frequently been isolated from fresh beef and sausages. Due to its lipolytic and proteolytic activities, this species can have a high technological potential (Gardini et al., 2001).

The objective of this study is to investigate the effect of *Debaryomyces hansenii* and *Yarrowia lipolytica* on the chemical, physical, microbiological, organoleptic and aroma characteristics of Turkish fermented sausage “Sucuk” during processing and storage at 4 °C.

CHAPTER 2

LITERATURE REVIEW

2.1. Fermented Turkish Sausage

“Sucuk” is a term used for spicy, uncooked, cured, dry fermented Turkish sausage, which is very popular meat product in Turkey and some other countries located in Balkans, Middle East and Caucasus (Kılıç, 2009).

Sausage is produced from ground meat (beef, lamb, mutton or mixture of them), sheep tail fat and ingredients such as nitrite and/or nitrate, potassium sorbate, and ascorbic acid with numerous species including cumin, garlic, salt, pimento, black and red pepper. Manufacturing of the sausage varies regionally and there are different formulations. A typical sausage formula which is preferred by many consumers is as follows: 90 kg meat is mixed with 10 kg tail fat, 2 kg table salt, 0.4 kg sugar (sucrose), 1 kg garlic, spices (cumin, cinnamon, allspice, clove, red pepper, and black pepper), 0.033 kg $NaNO_3$, 0.005 kg $NaNO_2$ (Bozkurt and Erkmen, 2006). This mixture is stuffed into natural or artificial casings and subjected to fermentation by either microorganisms naturally present or added starter culture and allowed to drying process at rooms with controlled temperature and humidity. After drying, it is stored at refrigeration temperature (Ensoy et al., 2010). According to the Turkish Food Codex, sausage should consist of maximum 40 % moisture, maximum 40 % fat and pH value should not exceed 5.4. The pH value of thermally processed sausages should not exceed 5.8. For both cases, residual nitrite level should be lower than 50 ppm (Anon. 2000). Table 2.1 shows the microbiological criteria for fermented sausages according to Turkish Food Codex. Erkmen and Bozkurt (2004) determined the sausage composition as; 40 % moisture, 33 % fat, 16 % protein, 4 % ash, and 3 % NaCl.

Table 2.1. Microbiological criteria for fermented meat products

	n	c	m	M
Molds	5	2	10^2	10^3
S.aureus	5	2	10^2	10^3
Salmonella spp.	5	0	0/25g-ml	
L.monocytogenes	5	0	0/25g-ml	
E.coli O157:H7	5	0	0/25g-ml	

Salt is used in sausage formulation due to its effect on the organoleptic characteristics of the product and antibacterial properties. It also lowers the water activity and is able to solubilize salt-soluble proteins (mainly sarcoplasmic and myofibrillar proteins), which are able to create a protein gel that assures the cohesion of the mixture during ripening of the product. Sucrose, dextrose, corn syrup are the sugars which are most commonly used in sausages. They are used as carbon sources by lactobacilli and enhance the acidification step. Sodium nitrite or nitrate used in sausage manufacturing is the most significant curing agent, which has a positive effect on the color formation and flavor development. It also prevents lipid oxidation and growth of anaerobic microorganisms such as *Clostridium botulinum*. Another ingredient used in sausage formulation is ascorbic acid. The main function of ascorbic acid is to prevent oxidation due its antioxidant activity and improve color stability. Black and red pepper, cumin, garlic and pimento are the spices generally used in sausage production. They provide desirable taste and odor (Cocolin and Rantsiou, 2006).

There are two major types of casings which are used in the manufacturing of sausage: Natural and artificial casings (Honikel, 1989). Natural casings are made up of small intestines whereas artificial casings are cellulose, polyvinyl chloride (PVDC), PVDC/fibrous, and collagen casings (Oliphant, 1998). In both cases, they have permeability to moisture and air. As compared with artificial casings, natural casings allowed more proteolytic acitivity to occur however, artificial casings encourages a more rapid pH decline (Ockerman and Basu, 2007).

2.2. Processing of Sausage

2.2.1. Traditional Processing

In the traditional method, sausage formulation can vary from region to region. However general formulation consists of beef meat is mixed with tail fat, salt, sugar, garlic, spices (cumin, black and red pepper, pimento) and vegetable oil (Bozkurt and Erkmen 2006). Antimicrobials and antioxidants are not added into the sausage dough and after stuffing into natural casings, fermentation occurs by microorganisms initially contaminated. Apart from the contribution of the raw materials to the initial contamination with the technologically important microorganisms, it should be pointed out that, in the past years, it has been repeatedly indicated that the processing plant is playing a crucial role in the enrichment of important biota for the production of fermented sausages. Traditional sausages are ripened and dried under climatic conditions during September and December (Soyer, Ertaş and Üzümcüoğlu, 2004). In these seasons, temperature varies from 10 °C to 15 °C and relative humidity ranges between 50 % and 80 %.

2.2.2. Commercial Processing

Traditional method has been modified by the manufacturers owing to the long processing time and dependence on the natural climatic conditions. Sausage formulation again could be changed from factory to factory. As distinct from traditional processing, starter cultures, antimicrobials such as nitrite, nitrate, potassium sorbate, and also antioxidants such as ascorbic acid are used in commercially produced sausages (Soyer, Ertaş and Üzümcüoğlu, 2004). Fermentation and drying (ripening) proceed under controlled temperature and relative humidity in ripening cabinets (Coşkuner, Ertaş and Soyer, 2008). In contrast to the traditional method, sausages are exposed to heat above 40 °C after a short fermentation period of about 12 to 24 hours (Tayar 1989; Öztan 1993). A general commercial sausage production is given in Figure 2.1.

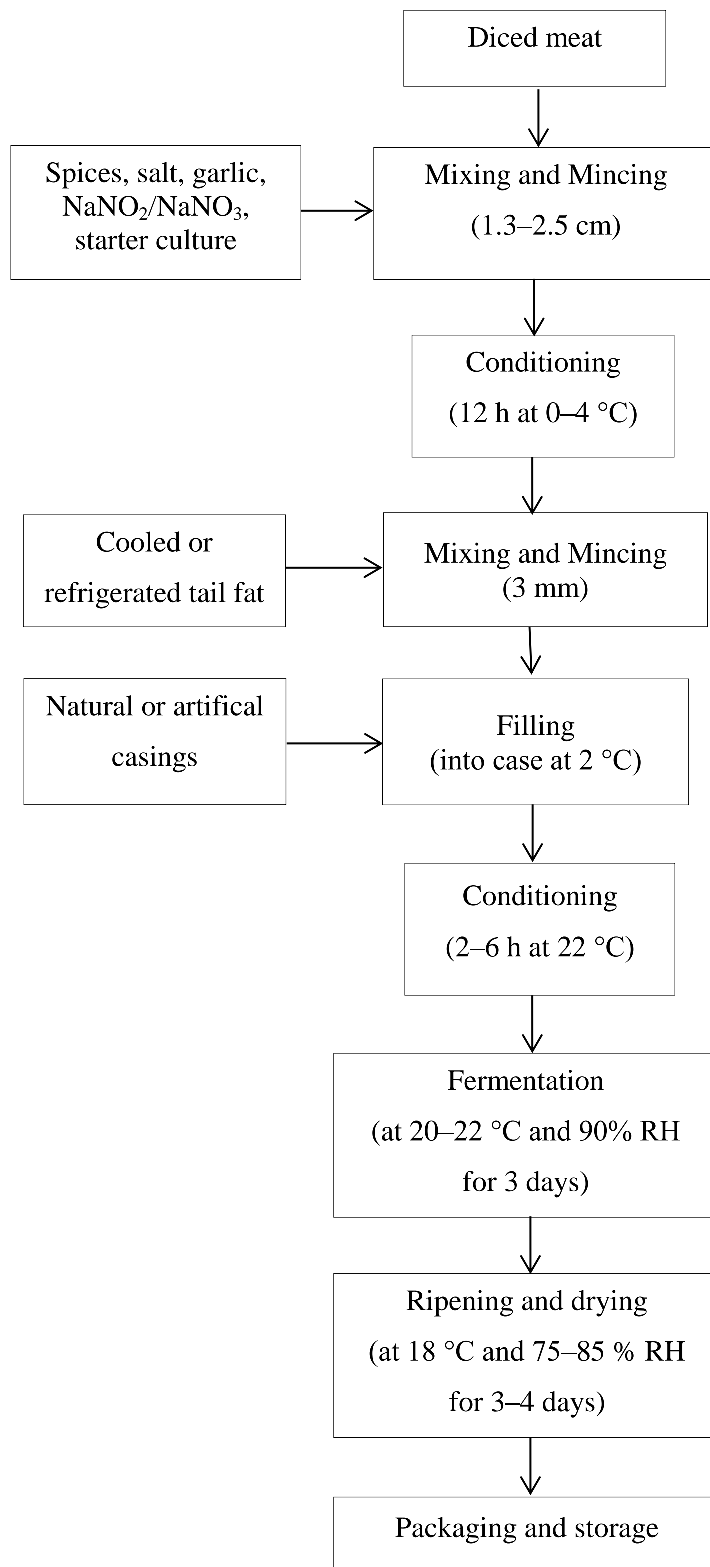

Figure 2.1. General production flowchart of sausages
(Source: Erkmen and Bozkurt, 2004)

2.2.3. Fermentation and Ripening

Fermentation is the metabolic process in which carbohydrates and related compounds are oxidized with the release of energy. The glycogen content of the muscle determines the content of fermentable sugars. As a rule, meat with a pH above 5.9 contains lactate and sugar in small quantities for safe fermentation; it holds water tightly and provides a better condition for growth of acid-labile bacteria (Ordonez et al. 1999).

In the ripening of sausage, both fermentation and drying occur. During fermentation, lactic acid bacteria consume carbohydrates, which naturally found in meat and added to sausage mix (Öztan, 1999).

Fermentation is naturally started by lactic acid bacteria. The breakdown of carbohydrates causes an increase in the quantities of lactic acid, lactates or other organic acids. During fermentation, pH drop occurs due to the acid formation and water is removed from the sausage. At the end of the ripening, desired changes occur in color, consistency, and flavor of sausage (Öztan, 1999).

2.3.4. Packaging and Storing

Sausage is packaged mainly in order to prevent physical and chemical changes and deterioration caused by microorganisms. If the product does not go for sale immediately, it is generally stored at 65–70 % RH, at temperatures lower than 10 °C, and at an air flow rate of 0.05 m/s (Öztan, 1999).

2.3. Starter Cultures

The basic role of starter cultures is to drive the fermentation process. In the past, the use of starter cultures in the production of sausages was not practiced and fermentation process was carried out by microorganisms that are naturally inoculated (Weber, 1994). Production of sausage with chance inoculation took a lot time and caused wide variation in physical, chemical and microbiological properties of final product. Hence, starter cultures were started to use by modern plants during the last decade (Yaman, Gökalp and Çon, 1998). They provide some technological advantages such as rapid and uniform acidification, good texture and slice-ability, production of

desirable flavor compounds, enhanced safety, good color formation and stability, better control over the fermentation process and unique product characteristics (Arihara et al., 1998; Sameshima et al., 1998).

The selection of suitable starter cultures is crucial to produce high quality sausage and ensure product safety (Kaban and Kaya, 2006). The commercial starter cultures that are frequently used in production of sausage are selected in accordance with their fermentative, proteolytic and lipolytic characteristics. Nevertheless, they are not always suitable to use in sausage production, since they may lead to loss of some desirable sensory characteristics. The specific strains of bacteria selected as good starter cultures should have salt tolerance, ability to grow in the presence of 80 to 100 ppm nitrite and be homofermentative. They should not have proteolytic and lipolytic activities and produce undesirable odors and off-flavors (Bozkurt and Erkmen, 2002a).

Lactic acid bacteria and gram positive catalase positive cocci are the basic groups of bacteria which have technological importance in fermentation and drying of sausage. Microbial strains belonging to the genera *Lactobacillus* (*L. plantarum*, *L. pentosus*, *L. curvatus*, and *L. sake*), *Pediococcus* (*P. pentosaceus* and *P. acidilactici*), *Micrococcaceae* (*Staphylococcus* and *Micrococcus*) have the most relevant role in fermentative process and ripening but also yeasts such as *Debaryomyces* spp. (*D. hansenii*) and molds (*Penicillium* spp.) can be involved (Anon. 2002, Gardini et al., 2001). In the U.S., *L. plantarum*, *P. pentosaceus,* or *P. acidilactici* are the frequently used starter cultures in order to obtain a pH 4.6–5.1 at 32 °C. In Europe, the most used starter cultures include *S. xylosus, S. carnosuss* and to a lesser extent *Micrococcus* spp. to achieve a pH 5.2-5.6 at a temperature of below 24 °C (Ockerman and Basu, 2007).

Lactic acid bacteria are mainly responsible for acidification by fermenting carbohydrates into lactic acid (Hammes and Knauf 1994; Hammes et al. 1990). Moreover, they enhance taste and aroma by producing acetic acid, ethanol, acetoin, pyruvic acid and carbon dioxide in small quantities (Bacus 1986; Demeyer 1982) and they preserve the hygienic quality against contaminated foodborne pathogens and microorganisms to the sausage mixture (Schillinger and Lücke, 1989). Antimicrobial activity of them is related to their capability of producing different types of acid (Yaman, Gökalp, Çon, 1998). Taking into consideration the LAB ecology, *L. sake*, *L. curvatus* and *L. plantarum*, which are reported as the main LAB isolated from fermented sausages produced in different countries, are the best adapted *Lactobacillus* spp. to meat fermentation (Urso et al., 2006a). Their initial counts (10^2-10^3cfu/g) reach

values of 10^7-10^8cfu/g in the first three days of fermentation and their counts remain rather stable in the course of the ripening period (Dorisinos et al., 2005). *Pediococcus* spp. are more commonly used in fermented sausages in the United States, where they are added as starter cultures to accelerate the acidification of the meat batter. Bacteria of the genera *Staphylococci* and *Micrococci* are Gram positive catalase positive and *S. xylosus*, *S. saprophyticus*, *S. carnosus* and *M. varians* are the species isolated from sausage (Erkmen and Fadıloğlu 2001; Kaban and Kaya 2008). They contribute to the final characteristic of the product by releasing low molecular weight compounds such as peptides, amino acids, aldehydes, amines, and free fatty acids through the action of their proteolytic and lipolytic enzymes (Demeyer et al. 1986; Schleifer 1986). The numbers of these bacteria show an increase in the first days of ripening period because of the high water activity level in sausage, and then decrease throughout the further ripening and storage (Gökalp et al. 1999). They are also involved in the development and stability of the red color via the formation of nitrosomyoglobin by nitrate reductase activity. *Micrococci* are poor acid producers in fermented sausages, which have extremely long fermentation periods because they grow aerobically. Due to the ability of some *Micrococci* such as *M. varians* to reduce nitrate to nitrite, they are components of starter cultures when nitrate is curing agent. Their use enhanced color characteristics and produced detectable flavor differences (Talon et al., 1999).

Molds, in particular, the species belonging to the genus *Penicillium*, are involved in the development of the organoleptic profile of fermented sausages by virtue of their lipolytic activity that is mainly involved in the aroma formation process. Since molds are rigorously aerobic organisms, they grow only on the surface of the sausages, where they create a homogeneous white mycelium that characterizes certain productions in the south of Europe (Lopez-Diaz et al., 2001). *P. nalgiovense* and *P. chrysogenum* are the most important species during sausage production, and they are frequently used as starter cultures (Leistner, 1990).

Yeasts are present during sausage fermentations in lower numbers as compared with lactic acid bacteria and Gram positive catalase positive bacteria. They can reach the values of 10^6 cfu/g in fermented sausages, even they are not added as starter cultures in traditional methods of spontaneous fermentation (Cocolin et al., 2006; Encinas et al., 2000). The predominant yeast species that are isolated from fermented sausages are *Debaryomyces* (especially *D.hansenii*), *Rhodotorula*, *Hansenula*, and *Torulopsis*. However, the composition and development of the mycoflora are dependent on the

nature of the product, the processing time and the ripening conditions. For instance, in a study carried out with Spanish fermented sausage, it is stated that the most abundant species is *Debaryomyces* and it is followed by *Rhodotorula*, *Candida*, *Pichia*, *Yarrowia* and *Trichosporon* (Dura et al., 2004b).

Several studies carried out with different yeast species (mainly *Debaryomyces hansenii*) have indicated that the use of yeasts as starter culture in fermented sausages contributes to the development of the typical sausage flavor due to their ability to degrade peroxides, proteolytic and lipolytic acitvities and the stabilization of reddening reaction by consuming oxygen (Olesen and Stahnke, 2000; Patrignani et al., 2006). NaCl tolerance, ability to grow at low water activities and at low pH values are the desired properties of yeast starter cultures in relation to meat fermentation (Jessen, 1995). However, it must be regarded that yeasts can inhibit the indigenous staphylococci and therefore it is adequate to use yeasts in combination with microorganisms having nitrate reductase activity to avoid color defects (Dura et al., 2004; Patrignani et al., 2006).

The proper contribution of yeasts to fermented sausage characteristics is influenced by numerous factors involving the species such as garlic, strain characteristics, their sensitivity to the stringent ripening conditions and their interaction with other components of microbiota (Olesen and Stahnke, 2000; Flores et al., 2004). Olesen and Stahnke (2000) pointed out that *D. hansenii* has very low effect on the aroma formation, due to the poor survival rate in the sausages produced with garlic powder having fungistatic effect. However, not all the authors have detected the lack of *Debaryomyces* survival in sausages while several authors have observed a reduction in yeast population during ripening (Encinas et al., 2000; Flores et al., 2004). In another study, an initial proliferation and a later reduction has been detected particularly in the presence of *Lactobacillus curvatus* and *Kocuria varians*. Thus, not only the presence of spices but also the existence of other starter cultures can affect yeast survival (Gehlen et al., 1991).

Table 2.2. Starter cultures and their roles in fermentation
(Source: Cocconcelli and Fontana, 2008)

Species	Functional Technological Properties for Meat Fermentation	Quality Characteristics
Lactobacillus sakei	Decrease of pH value Catalase activity Flavor development Amino acid metabolism Antioxidant properties Bacteriocin production	Preservation Firmness (Consistency) Aroma formation
Lactobacillus curvatus	Decrease of pH value Proteolytic activity Antioxidant properties Bacteriosin production	Preservation Firmness (Consistency) Aroma formation
Lactobacillus plantarum	Decrease of pH value Antioxidant properties Bacteriocin production	Preservation Firmness (Consistency)
Pediococcus spp. *(P. acidilactici,* *P. pentasauces)*	Acidification Bacteriocin production	Preservation Firmness (Consistency)
Staphylococcus spp. (S. xylosus, S. carnosus,	Proteolysis Amino acid catabolism Lipolysis Antioxidant properties Nitrate reduction	Color Aroma formation Preservation
Micrococcus varians	Nitrate reduction	Color Preservation
Debaryomyces hansenii	Oxygen consumption Peroxide destruction Proteolysis Lipolysis	Delay rancidity Aroma formation Color stability
Penicillium nalgiovense	Oxygen consumption Peroxide destruction Proteolysis Lipolysis	Color stability Delay rancidity Aroma formation

2.4. Color Formation

The characteristic color of fermented meat products is associated with the interaction between meat pigments and the products that result from the reduction of nitrate or nitrite added to sausage batter. Nitrate in itself does not produce the red color, but it must first be reduced to nitrite in the presence of enzyme nitrate reductase that is produced by the *Micrococcaceae*. The nitrite form is reduced to nitric oxide immediately after preparation of sausage mix due to acidification caused by lactic acid bacteria and nitric oxide finally reacts with meat pigment myoglobin in order to form nitrosomyoglobin, which yield a red color. In addition to microorganisms, additives such as ascorbic acid are involved in the reduction of nitrite to nitric oxide (Ordonez et al., 1999).

The color formation in fermented sausages is closely related with the pH (Öztan, 1999). The optimum pH value should be between 5.4 and 5.7 to development of color (Kamarei and Karel, 1982).

Discoloration of cured meat can be observed due to the formation of peroxide. This defect can be avoided by the catalase activity of Gram-positive bacteria that protect the color (Nychas and Arkoudelos, 1990).

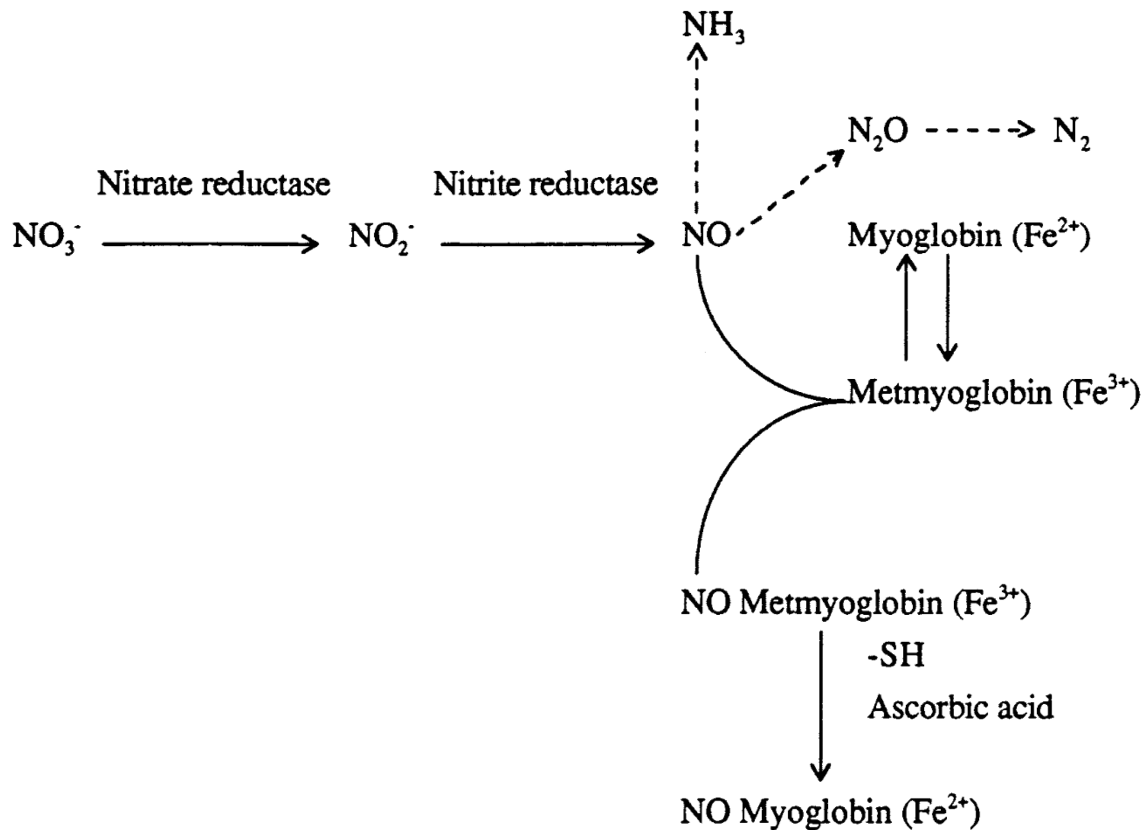

Figure 2.2. Formation of nitrosomyoglobin
(Source: Nychas and Arkoudelos, 1990)

2.5. Flavor Generation in Fermented Sausages

Combination of several elements such as batter ingredients, manufacture technology, activity of tissue enzymes, proteases and lipases and microbial metabolism play role in the generation of aroma compounds in fermented sausages. Flavor generation constitutes a very complex process that involves numerous chemical and biochemical reactions and mainly affects carbohydrates, proteins and lipids (Demeyer and Stahnke, 2002). Carbohydrate degradation, proteolysis, amino acid degradation reactions, Maillard reactions, Strecker degradation reactions, lipolysis and lipid oxidation are the main reactions that occur throughout the ripening as a result of meat endogenous enzymes and microorganisms. Addition of spices is also another important factor which may exert a high impact on the aroma of fermented sausages (Ordonez et al., 1999). Some of the flavor results simply from curing salt and nitrite.

The production of different metabolic products (Table 2.2) is species and strain dependent and thus the flavor of sausage is also species and strain dependent. Lactic acid bacteria are weakly proteolytic and lipolytic in the conditions of fermented sausages and having effect on proteolysis and lipolysis mainly by producing lactic acid and small amounts of acetic acid, ethanol and acetoin while staphylococci show an effective metabolism of lipids (Cocconcelli and Fontana, 2008). Hence, the use of starter cultures in different combinations produces important differences in volatile compound profiles and thus has a different impact on flavor (Berdague, Montel and Talon, 1993).

Some model systems for the investigation of microbial role during meat fermentation have been used in recent years. Some of these models have been used for the study of lactic acid production and proteolysis of lactic acid bacteria (Sanz et al., 1999b) or proteolysis of *D. hansenii* and *Y. lipolytica* (Santos et al., 2001; Patrignani et al., 2006), while others have been developed to examine the generation of volatiles by *Staphylococcus* strains or by *D. hansenii* (Dura, Flores and Toldra, 2004b).

During the fermentation and ripening, proteins are degraded into peptides, dipeptides and amino acids and lipids into fatty acids via the chemical and enzymatic reactions. Aroma compounds or biogenic amines are generated from further decarboxylation of amino acid, while aldehydes, alkanes, alcohols and ketones are formed as a result of hydrolysis of fatty acids (Montel *et al.* 1996). Thus, the typical aroma of fermented sausages is primarily related with a high number of volatile compounds that are generated during the processing through the following reactions.

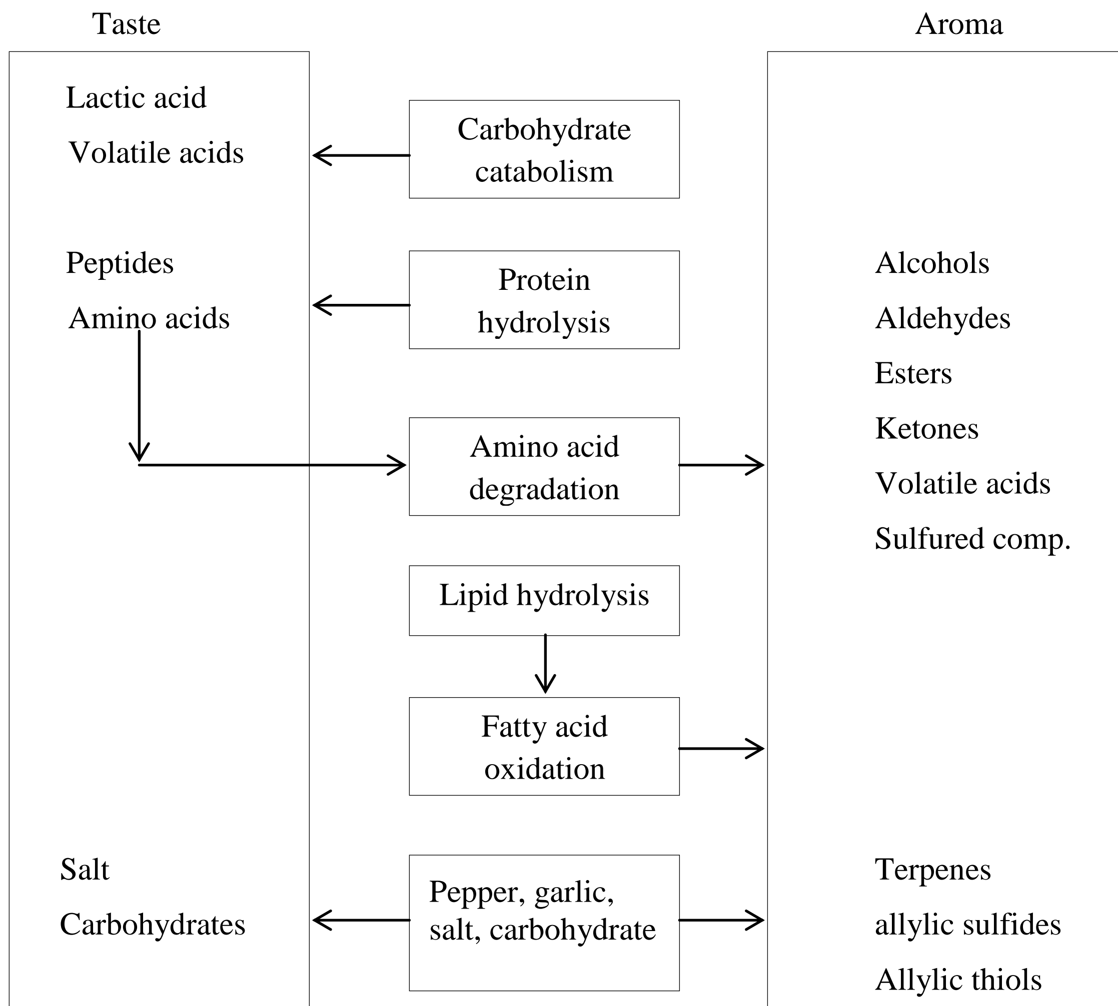

Figure 2.3. Formation of important flavor compounds in fermented sausages (Source: Stahnke, 2002)

2.5.1. Glycolysis in Fermented Sausages

Carbohydrates (glucose occasionally lactose or saccharose) serve as a substrate for biological acidulation yielding lactic acid as the main end product which is responsible for the pH drop. The intensity of pH decrease is dependent on the type of LAB used as starter, the type and amount of added carbohydrates, fermentation temperature and the other relevant processing parameters such as amount of salt, conditions of ripening. The acid taste is a significant component of the overall taste of fermented meat products (Demeyer, 1992; Demeyer et al., 2000). If the starter cultures added to sausage mixture are heterofermentative, some additional end products such as acetate, formate, ethanol and acetoin may be produced and these fermentation products

contribute significantly to flavor and aroma. Acetic acid contributes to acidic taste and also plays an important role in sausage aroma by providing a hint of vinegar. The buttery or dairy product aroma of certain sausages is related to the presence of diacetyl and acetoin (Demeyer and Stahnke, 2002).

2.5.2. Proteolysis in Fermented Sausages

Proteolysis is one of the major degradation mechanisms that influence proteins throughout the ripening process and it is attributed by both to endogenous enzymes and to exogenous enzymes originating from microorganisms (Dalmış and Soyer, 2007). Meat proteins undergo hydrolysis first to polypeptides by the action of endogenous muscle enzymes, such as cathepsins and calpains (Toldra et al., 1992), and then further to smaller peptides by peptidases. The final step in proteolysis phenomena is generation of free amino acid generation from peptides by aminopeptidases and it is attributed to protease enzymes generated via microorganisms as well as enzymes inherent in the meat itself (Hughes et al. 2002). Low-molecular-weight peptides and free amino acids are major components of the non-protein nitrogen (NPN) fraction in fermented meats, and these contribute, directly or indirectly, to generation of volatile and nonvolatile flavor compounds in dry and semidry sausages (De Masi et al., 1990). Candogan et al. (2009) have also reported a large increase in the NPN fraction during fermentation of dry sausages inoculated with *L. sake* and *S. carnosus* and they related it to a corresponding decrease in sausage pH value.

The amino acid generation directly contributes to the basic taste of dry fermented sausages and indirectly contributes to the development of their characteristic aroma, because they are precursors of many volatile compounds such as aldehydes and ketones, which have intense aroma characteristics, have an obvious role in development of flavor (Sanz and Toldra, 1997).

The extent of proteolysis is influenced by several variables such as product formulation, processing conditions and starter cultures. It is generally believed that the use of different starter cultures produces differences in the sensory characteristics of fermented sausages (Erkkila et al., 2000). Martin attributed a greater proteolytic activity to lactobacilli than to micrococci. Similarly, Montel et al., showed that species from the genera *Lactobacillus* and *Pediococcus* produced a rise in the free amino acids as a result

of their peptidase activity. In a study focused on the effect of processing method and starter culture on proteolytic changes in sausage, it is stated that starter inoculated and control sausages (without starter culture) showed intense proteolysis in both the traditional and heat processing methods. However, after heating, intense degradation of proteins was observed in heat-processed samples due to the denaturation (Dalmış and Soyer, 2007). In another study, the effect of *Debaryomyces* spp. on the proteolysis of dry fermented sausages was evaluated and it is concluded that the degradation of myofibrillar proteins was accelerated in *Debaryomyces* spp. inoculated batch at the beginning of the drying stage even though the final sausage, inoculated with *Debaryomyces* spp., had lower myofibrillar proteolysis (Dura et al., 2004).

2.5.3. Amino Acid Catabolism

Free amino acids, which are generated as final products of the proteolysis of muscle proteins, act as substrate for numerous enzymatic reactions, which produce amines, ammonia or various compounds through the microbial metabolism (Toldra 2008). Demeyer et al., (2000) stated that *Micrococcaceae* family (*Staphylococcus* and *Microccoccus*) and *Debaryomyces hansenii* have good ability for such kind of metabolism.

Aldehydes, alcohols and acids play an important role in acquiring flavor and they are produced by the catabolism of amino acids in particular branched-chain amino acids (leucine, isoleucine, valine), aromatic amino acids (phenylalanine, tyrosine, tryptophan), and sulfured amino acids (methionine) (Stahnke 2002).

2.5.4. Lipolysis in Fermented Sausages

Lipids constitute the main components of fermented sausages that are subject to lipolytic and oxidative reactions. Lipolysis plays an essential role in the development of fermented sausage flavor. Lipids are hydrolyzed to free fatty acids, which are substrates for the oxidative changes that are responsible for flavor compounds (Stahnke, 1995). Hydrolysis of triglycerides by both microbial and endogenous lipases is the first step in the lipid breakdown (Molly et al., 1997). For example, Sorensen and Samuelsen (1996)

indicated that the lipases produced by *S.xylosus* and *D.hansenii* are able to hydrolyze the fat primarily throughout the initial stages of processing.

The concentration of free fatty acids in the fat depends on the hydrolytic activity of the lipases, the microbial metabolic processes, and the oxidative reactions that work on the free fatty acids released in the lipolysis and the final products. The further lipolysis regards the releasing of fatty acids that undergo later enzymatic and non-enzymatic oxidative processes yielding, as final products, carbonyls and other low-molecular-weight compounds such as alcohol, carboxylic acids, which are the main flavor compounds of the final sausage (Toldra, 1998).

Enzymatic hydrolysis causes the acceleration of lipid peroxidation during fermentation. Because of the high fat content and low water activity of these products, lipid oxidation is the main factor responsible for loss of quality and leading to oxidative flavors (Melton 1983). Thus, Gray et al. (1996) noticed that lipid oxidation in meat products can be controlled or minimized by the addition of commercial synthetic or natural antioxidants, while Berdague et al. (1993) stated that *Staphylococcus* spp. contribute to oxygen consumption and catalase activity that reduces the rancidity and improves color stability.

Table 2.3. Main products, affecting sensory quality, resulting from biochemical reactions by muscle and microbial enzymes (Source: Toldra, 2008)

Initial substrate	**Type of reaction**	**Final product**	**Effect on sensory quality**
Carbohydrates	Glycolysis-homofermentative	Lactic acid	Taste
Carbohydrates	Glycolysis-heterofermentative	Lactic acid Diacetyl Acetaldehyde Acetoin Short chain fatty acids Carbon dioxide	Taste and aroma
Proteins	Proteolysis	Peptides Free amino acids	Taste
Amino acids	Degradation reactions	Branched aldehydes Branched alcohols Branched acids	Aroma
Amino acids	Deamination	Aldehydes Ketones Acids Ammonia	Aroma
Amino acids	Decarboxylation	Amines	-
Amino acids	Transamination	Other amino acids	Taste
Lipids	Lipolysis	Free fatty acids	Taste
Free fatty acids	Oxidation	Volatile compounds	Aroma

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.3. Yeast Strains

In sausage fermentation, two different yeast strains, *Debaryomyces hansenii* (Y-1448) and *Yarrowia lipolytica* (YB-618), were used: Lyophilized *D. hansenii* and *Y. lipolytica* were obtained from the United States Department of Agriculture, Microbial Genomics and Bioprocessing Research Unit, Peoria, IL, USA.

3.1.4. Activation of Yeast Strains

Lyophilized yeast strains, which were selected due to their proteolytic and lipolytic activities, were grown in Malt Extract Broth (MEB) at 25 °C for 48 hours and sub-cultivated in MEB at 25 °C for 18 hours. They were stored in 15 % glycerol at –80 °C until use. Before sausage processing, yeast strains in stock were transferred to MEB and the same procedure that was mentioned above was applied. After the second incubation period, yeast cells were obtained by centrifugation (4500 rpm, 20 min, 4 °C), washed with sterile phosphate buffer saline twice and re-suspended in sterile distilled water according to the amount of sausage produced.

3.1.5. Sausage Formulation and Processing

Two lots of sausages were produced in Pınar Meat Co. (İzmir, Turkey) as described in Gökalp (1982) with the following modifications. For the production of 1 kg of sausage mixture, 800 g/kg beef meat, 200 g/kg beef meat fat, 10 g/kg salt, 1 g/kg garlic, 4 g/kg sucrose, 2 g/kg red pepper, 5g/kg black pepper, 6 g/kg cumin, 6 g/kg pimento and 150 ppm $NaNO_2$ were used. For each lot, four batches of sausages were

produced. First, control group, batter was prepared without adding starter culture. Then, batter containing only starter cultures (*Pediococcus pentosaceus* and *Staphylococcus carnosus*), batter containing *D. hansenii* with starter cultures and batter containing *Y. lipolytica* with starter cultures were produced. Starter cultures were used as lyophilized form. For each kg of sausage batter, 10 ml sterile distilled water containing about 10^5-10^6 cfu/ml of *D. hansenii* and *Y. lipolytica* was added and steril distilled water (10 ml/kg of sausage mixture) was added to both control group and the batch produced with only starter cultures. The mixture was minced by using a meat grinder (3 mm) and each of sausage batter (250 g) was stuffed into collagen casings (ø 34 mm). Table 3.1 shows the ripening program applied to sausages. At the end of the ripening, sausages were vacuum-packed and stored at 4 °C for 3 months.

Table 3.1. Fermentation and ripening program for sausage samples

Temperature (°C)	Relative humidity (%)	Duration
25	95	Until the pH reached 5.4-5.2
23	92	Until the pH reached 5.2-5.0
18	91	Maximum 6 hours
17	86	Maximum 6 hours
16	72	Until the moisture decreased to % 40 or below

3.1.6. Sampling

Sausages were analyzed before stuffing (day 0), at the 1st, 3rd, 6th and 9th days of ripening period and at the 1st, 2nd and 3rd months of storage. Two samples from each group were taken and analyzed at these periods.

3.2. Methods

3.2.1. Proximate Analyses

3.2.1.1. Moisture Content

Moisture content of sausage samples was determined by drying samples to constant weight in an oven at 105 ºC. Sausage sample (3 g) was crushed with 20 g sea sand and glass stick after adding methanol in dried and pre-weighed dish. The difference in weight before and after drying for 4-5 hours at 105 °C gives the results of total solid content (AOAC, 2006). Moisture analysis was performed in triplicate for each sausage sample.

$$\% \text{ Moisture} = (\text{loss in weight} / \text{initial sample weight}) \times 100 \qquad (3.1)$$

3.2.1.2. Protein Content

Protein content of sausage samples was determined according to Kjeldahl method (AOAC, 2006). The Kjeldahl tubes containing about 1 g of sample, catalyst, antifoaming agent and 15 ml H_2SO_4 were placed into the digestion unit. A blank containing only these reagents were also prepared. All the tubes were digested at 450 °C for 5 hours (Kjeldatherm and Turbosog, C. Gerhardt GmbH & Co. KG, Germany). After digestion period, the tubes were cooled at room temperature and placed in the distillation-titration unit (Vapodest 50s, C. Gerhardt GmbH & Co. KG, Germany). Distillation with 70 ml of 32 % NaOH was carried on and the distillate was accumulated in a beaker containing 20 ml of boric acid. Titration with 0.1 N HCl was performed and the amount of consumption was recorded. The protein content was calculated according to equation (3.2) with the conversion factor of 6.25.

$$\% \text{ Protein} = [(\text{sample-blank}) \text{ ml HCl} \times F_{HCl} \times 0.014 \times 6.25] / [\text{weight of sample (g)}] \qquad (3.2)$$

3.2.1.3. Fat Content

Fat content of sausage samples was determined according to Soxhelet method and expressed as percentage (AOAC, 2006). Hexane was used as solvent.

3.2.1.4. Salt Content

Salt content was determined according to Mohr method by using ash samples. Ashed samples were dissolved in 100 ml hot distilled water and transferred into an erlenmayer flask by filtering. Same process was repeated five times. Then water level was completed to 500 ml with distilled water at room temperature and 25 ml of this solution was transferred into an erlenmayer flask. K_2CrO_4 (5 % w/v) solution (0.5 ml) was added and titration was performed with 0.1 N $AgNO_3$ until the red color was occurred (Kirk and Sawyer, 1991).

$$\% \text{ Salt} = [(V1-V2)\times 0.585\times F] / P \quad (3.5)$$

V1: Used 0.1 N $AgNO_3$ amount (ml) from experiment with sample solution
V2: Used 0.1 N $AgNO_3$ amount (ml) from experiment with deionized water
P: Sample amount included in titration
F: Factor of 0.1 N $AgNO_3$

3.2.1.5. Ash Content

Ash content of sausage samples was determined according to AOAC, 2006. Three g of sausage sample was weighed into a dried and pre-weighed porcelain crucible. Sample was placed into a muffle furnace (Protherm, Turkey) and was incinerated at 550 °C until the sample residue became light gray-white. After cooling in a desiccator, weight was recorded. Ash content was calculated as in equation (3.3).

$$\% \text{ Ash} = [\text{weight of residue (g)} / \text{weight of sample (g)}]\times 100 \quad (3.3)$$

3.2.2. The pH and Titratable Acidity

The pH and titratable acidity of sausage samples were determined in duplicate. Ten g of sample was mixed with 100 ml of distilled water using a blender and the pH of the slurry was measured with a pH meter (AOAC, 2006).

Titratable acidity was determined by titration of slurry with 0.1 N NaOH to an end point of pH 8.30. The acidity was expressed as percent lactic acid.

% lactic acid = [0.1 N NaOH amount (ml) x 0.009 x 100] / [amount of sample (g)] (3.4)

3.2.3. Water Activity

The water activity of samples was measured with water activity meter (Hygrolab V3, Bassersdorf, Germany). Ten gram sample was weighed into the sample cup and placed into Hygrolab water activity meter. Measurements were done at room temperature. When the partial pressure in the air above sample is unchanged, water activity is read from the monitor as % relative humidity of air ×100.

3.2.4. Weight Loss

Two strings of sausage from each treatment were weighed just before being placed in the fermentation cabinet. The same strings were re-weighed on day 1, 3, 6 and 9. Weight loss was expressed as the percentage of the initial weight.

3.2.5. Thiobarbituric Acid (TBA) Analysis

Thiobarbituric acid (TBA) analysis was performed according to the method of Tarladgis et al. (1960) modified by Shahidi et al. (1985).

Ten g sausage sample was blended with 70 ml distilled water and transferred to a Kjeldahl flask by washing with additional 27.5 ml distilled water. 2.5 ml 4 N HCl and a small amount of antifoam A (Merck) were added to the mixture and then heated with an electrical heater until 50 ml distillate was collected. Five ml distillate was transferred

to a tube and 5 ml TBA reagent was added. Tube was stoppered and immersed in a boiling water bath for 35 minutes. Distilled water – TBA reagent blank was prepared and treated like the samples. After cooling the tubes, optical density of the samples were read against the blank at a wavelength of 532 nm (Shimadzu UV-2450, Japan). Readings were multiplied with the factor 8.1 and the results expressed as mg malonaldehyde per 1 kg meat.

3.2.6. Non-Protein Nitrogen (NPN) Content

Non-protein nitrogen (NPN) content of the samples was determined according to the method of Hughes et al. (2002).

Ten g of sausage sample was homogenized with 20 ml of 2 % TCA for 1 min using an Ultra Turrax (IKA T25, U.S.A.) at 13,500 rpm. The homogenate was then centrifuged at 10,000*g* for 20 minutes at 4 °C. The nitrogen content of the supernatant was analyzed using the Kjeldahl method (AOAC, 2006).

3.2.7. Protein Solubility

Protein solubility was determined according to the method of Decker, Xiong, Calvert, Crum, and Blanchard (1993) modified by Dalmış and Soyer (2007). A 2 g portion of sausage sample was homogenized with 20 ml of 1.1 N KI in 0.1 N K_2HPO_4 (pH 7.4) for 20 seconds at 13,500 rpm using an Ultra Turrax (IKA T25, U.S.A.) and centrifuged at 6000*g* for 15 minutes. Protein concentration of supernatant was determined by Bradford assay. Protein solubility was calculated as:

$$\% \text{ Solubility} = [\text{protein concentration in supernatant (mg/ml)}] / [\text{original protein concentration(mg/ml)}] \times 100 \quad (3.6)$$

3.2.8. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was applied to sarcoplasmic and myofibrillar protein extracts. Sarcoplasmic protein extracts

were prepared according to the method of Toldra, Rico, and Flores (1993). Four grams of sausage was homogenized with 40 ml of 0.03 M potassium phosphate buffer (pH 7.4) for 2 minutes using an Ultra Turrax (IKA T25, U.S.A.) at 13,500 rpm. The homogenate was centrifuged for 20 minutes at 10,000*g* at 4 °C. The supernatant included the sarcoplasmic proteins. Myofibrillar proteins were extracted from the resultant pellet by homogenizing with a solution containing 8 M Urea and 1% (w/v) β-mercaptoethanol for 2 minutes using an Ultra Turrax (IKA T25, U.S.A.). The homogenate was recentrifuged under the same conditions and the supernatant contained the myofibrillar proteins.

SDS-PAGE was carried out according to Laemmli, (1970) and all solutions used in this analysis were given in Appendix B. PROTEAN II XL (20x22 cm) system (Bio-Rad, U.S.A.) was used. 12% separating gel was poured between the glass plates which were arranged by using 1 mm spacers. After separating gel was polymerized, 4% stacking gel was poured and 25 well comb was inserted. Sarcoplasmic and myofibrillar protein extracts were mixed with sample buffer in a ratio of 1:1, according to nanodrop (NanoDrop 8000, Thermo Scientific, U.S.A.) measurements and were heated in a boiling water bath for 10 min. Molecular weight marker with a 10-170 MW range (Fermentas, Canada) was used. After loading 20 µl/well of each sample and 2 µl of marker to the wells, the gels were placed in tank and 1X running buffer was loaded. The lid of the tank was closed and the system was connected to a power supply (EC 3000 XL, Thermo Scientific, U.S.A) and a cooling unit (PolyScience, U.S.A). After electrophoresis, the gels were stained with Comassie Brilliant Blue R-250. The gels were destained using 40% methanol and 10% acetic acid.

3.2.9. Volatile Compound Analysis

The changes in volatile composition of sausage samples during ripening and storage were investigated with GC/MS (Trace GC Ultra/ISQ, Thermo Scientific, U.S.A.). For the extraction of volatile compounds, solid-phase micro extraction (SPME) method was used. For this purpose, a fiber, provided by Supelco (57348-U, PA, USA) coated with the following sorbent material: Divinylbenzene/Carboxen/Polydimethyl-siloxane and 30 m × 0.2 µm i.d. TR-5MS column (Thermo scientific, U.S.A.) with 0.25 µm film thicknesses were used. Samples, vacuum-packed and stored at -20 °C for volatile compound analysis, were defrosted at 4 ºC prior to analysis. The casings of

samples were removed and the samples were minced. Two grams of minced samples were weighed into a 15 ml headspace vial, and a PTFE silicone septum was immediately sealed with an aluminum crimp seal. Sample was equilibrated at 60 ºC for 30 minutes. Then, fiber was inserted into headspace of the vial using SPME fiber holder. After 30 minutes, the fiber was inserted into the gas chromatography injector port and held for 5 minutes for desorption of absorbed molecules. The temperature of the injector port was 250 °C. Carrier gas (He) flow rate was 1 ml/min. Oven temperature was programmed as: 40 °C for 5 minutes then the temperature was raised to 165 ºC (5 ºC/min, held 5 minutes) to a final temperature 240 °C (30ºC/min). Volatile compound fractions were expressed as percentage area.

3.2.10. Color Measurement

Color measurements were carried out using a Minolta CR-400 reflectance colorimeter (Osaka, Japan). CIE L^*, a^*, b^* color values were measured (L^*: lightness; a^*: redness; b^*: yellowness). Color readings were taken at three points on the central part of the cut surface of the two slices.

3.2.11. Sensory Analysis

Sensory evaluation was conducted at the end of ripening period (9th day) and 1st, 2nd, and 3rd months of the storage by a 9-member trained panel.

Hedonic scale from 1(worst) to 5 (best) was used for sensory evaluation. Panel members were selected based on willingness to participate and time available. Table 3.2 shows the attributes which were evaluated by panelists. Samples were served both as raw and cooked. Surface color, cut surface color, outer surface appearance, and cut surface appearance were evaluated in raw sausages while texture and flavor were evaluated in cooked sausages.

The panelists were provided with water and bread between samples. The sausages were coded with three-digit numbers and presented in plastic plates. Panelists evaluated each sausage twice. Duplicate samples were served in different sessions.

Table 3.2. Sensory evaluation sheet

Name: Date:

Age:

Samples	Surface color	Cut surface color	Outer surface appearance	Cut surface appearance	Texture	Flavor	Overall acceptability

3.2.12. Microbiological Analysis

3.2.12.1. Sampling

The sausage casing was removed aseptically. Ten gram portions were aseptically cut from each sausage and homogenized with 90 ml sterile peptone water in a stomacher for 1 minute. Subsequent serial dilutions were prepared in sterile 0.1 % peptone water for microbial analysis. Pour plate method was used for total aerobic mesophilic bacteria, lactic acid bacteria and *Enterobacteriaceae* enumerations and spread plate method was used for enumerations of yeast and mold and *Staphylococci/Microccocci*. Two measurements were carried out and average values were represented. After the incubation, the plates with colony forming units (CFU) ranging from 30 and 300 were selected for enumeration. After colony counting, the numbers were expressed in log $CFUg^{-1}$.

3.2.12.2. Enumeration of Total Aerobic Mesophilic Bacteria

Plate Count Agar (PCA) was used for enumeration of total aerobic mesophilic bacteria count. Plates were incubated at 30 °C for 72 hours (Soyer et al., 2004).

3.2.12.3. Enumeration of Lactic Acid Bacteria

Man, Rogosa, Sharp (MRS) agar was used for enumeration of lactic acid bacteria. Plates were incubated at 30 °C for 48-72 hours in anaerobic jars (Dura et al., 2004).

3.2.12.4. Enumeration of *Enterobacteriaceae*

Double layer violet red bile agar was used for the enumeration of *Enterobacteriaceae*. Plates were incubated at 37 °C for 24 hours in anaerobic jars (Patrignani et al., 2006).

3.2.12.5. Enumeration of *Staphylococcus/Micrococcus*

Mannitol salt agar and spread plate method was used for enumeration. Plates were incubated at 35 °C for 48 hours (Patrignani et al., 2007).

3.2.12.6. Enumeration of Yeasts

Yeast extract glucose choloramphenicol agar and spread plate method was used for yeast and mold enumeration. Plates were incubated at 25 °C for 72 hours (Olesen et al., 2000).

3.2.13. Statistical Analysis

Mean values and standard deviations were calculated for all the determined parameters. Pearson's correlation coefficients were also calculated to determine linear relations between the some characteristics of sausages. Analysis of variance (ANOVA) was performed to investigate the differences ($p<0.05$) in characteristics of sausages during ripening and storage. Minitab (Minitab, State College, PA) software (version 16.0 for Windows) was used for statistical analyses (Winner 1971).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Proximate Composition of Sausage Samples

The effect of different yeast species used in combination with starter cultures on physical, chemical, microbiological, organoleptic and aroma characteristics of Turkish fermented sausages were evaluated during ripening and storage. Table 4.1 shows the composition of sausages at the end of 9 days of ripening.

Moisture content of sausage samples varied from 27.17-32.53%. Yeast inoculated batches had higher moisture content than the other groups. As moisture content decreased, protein content of products generally increased. The protein content of sausage samples changed between 24.94 and 26.66%. As the moisture content decreased, the fat content and salt contents of sausages also increased. Fat content of sausage samples was between 37.64 and 44.75%. According to Turkish Food Codex (TFC), the proportion of moisture content to protein content and the proportion of fat content to total protein content should be lower than 2.5. In addition, protein content of fermented Turkish sausages should be minimum 16 %. As the salt content increased, ash content also increased. Salt content of all sausage samples was below 5 % which is the limit determined by TSE 1070. In conclusion, all groups were in agreement with TFC.

Table 4.1. Proximate composition of sausage samples (%)

Groups	Moisture content	Protein content	Fat content	Salt content	Ash content
Control	27.17±0.02^{a}	26.66±0.20^{a}	42.52±0.06^{b}	2.42±0.07^{a}	3.65±0.06^{b}
Starter	26.49±0.02^{a}	24.94±0.61^{c}	44.75±0.05^{a}	2.80±0.04^{b}	3.82±0.02^{a}
D.hansenii	32.53±0.04^{a}	26.42±0.63^{ab}	37.64±0.64^{d}	2.62±0.04^{c}	3.41±0.01^{d}
Y.lipolytica	31.92±0.04^{a}	25.35±0.61^{bc}	39.22±0.56^{c}	2.39±0.05^{a}	3.51±0.05^{c}

a-d : Means having different letters within each column denote significant difference at $p<0.05$.
Data are mean values ± S.D.

4.1.1. Moisture Contents of Sausage Samples

The moisture contents of sausage samples during processing (during fermentation and ripening) are shown in Table 4.2 and the moisture content of samples during storage are indicated in Table 4.3. The moisture contents for control, starter culture, *D. hansenii* inoculated and *Y. lipolytica* inoculated groups were 60.58%, 61.73%, 59.07%, and 60.49% before stuffing, respectively and 59.92%, 60.21%, 55.65%, 55.74% after fermentation, respectively.

From the initial day of processing to the 6th day of the ripening, moisture content of all groups showed a slight decrease whereas, the yeast inoculated batches indicated significantly ($p<0.05$) higher moisture content than the control and starter inoculated batches at 9th day of ripening. During processing, proteins are denatured due to the decrease of pH value below 5.3 by action of lactic acid bacteria. Due to protein denaturation, water holding capacity of proteins decreases and drying in sausages occur (Toldra et al. 2001). Moisture content and type of batch × processing time interactions found insignificant ($p>0.05$) during ripening and storage.

At the end of the ripening process, moisture contents of sausage samples changed between 26.49% and 32.53%. Turkish Food Codex states that proportion of moisture content of ripened sausages to protein content should be lower than 2.5. All groups were in agreement with TFC. The results of yeast-inoculated batches were also in agreement with the previous studies. Coşkuner et al. (2008), Ercoşkun et al. (2009) and Kaban (2004) found the moisture content of traditionally produced sausage as 32.80%, 32.48% and 28.07%, respectively.

Table 4.2. Moisture contents of sausage samples during ripening

Groups	Before stuffing	Day 1	Day 3	Day 6	Day 9
Control	60.58±0.05^{A}	59.92±0.04^{A}	47.97±0.07^{B}	38.94±0.10^{B}	27.17±0.02^{C}
Starter	61.73±0.04^{A}	60.21±0.05^{A}	45.04±0.08^{B}	34.34±0.07^{C}	26.49±0.02^{C}
D.hansenii	59.07±0.03^{A}	55.65±0.05^{AB}	49.58±0.02^{B}	36.16±0.04^{C}	32.53±0.04^{C}
Y.lipolytica	60.49±0.06^{A}	55.74±0.09^{A}	50.31±0.06^{A}	36.92±0.09^{B}	31.92±0.04^{B}

A-C: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=3)

The decrease in moisture content values continued during storage. At the 30th day of storage, moisture contents ranged between 25.92 % and 28.86 %. From the beginning of storage to the 60th day of storage, moisture contents showed slight decrease. At the last day of storage, moisture contents reduced to 23.37% for control batch, 24.54 % for starter batch, 26.73 % for *D. hansenii* batch and 26.39 % for *Y. lipolytica* batch. During storage, no significant differences were observed between batches ($p>0.05$).

Table 4.3. Moisture contents of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	27.17±0.02^{B}	27.06±0.02^{B}	27.00±0.01^{B}	23.37±0.02^{A}
Starter	26.49±0.02^{A}	25.92±0.01^{A}	25.59±0.01^{A}	24.54±0.03^{A}
D.hansenii	32.53±0.04^{A}	28.74±0.04^{AB}	28.57±0.01^{AB}	26.73±0.01^{B}
Y.lipolytica	31.92±0.04^{A}	28.86±0.02^{B}	26.78±0.02^{B}	26.39±0.01^{B}

A-B: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=3)

4.1.2. Protein Content of Sausage Samples

Table 4.4 and Table 4.5 show the protein content of sausage samples during ripening and during storage, respectively.

Before stuffing, control group had significantly ($p<0.05$) greatest protein content. During ripening, protein content of all groups increased depending on the drying. The initial protein contents were 16.37-17.36 % and increased to 24.94-26.66 %. At the end of the ripening, control and *D.hansenii* inoculated batches had similar results, which were significantly different from starter batch and *Y. lipolytica* inoculated batch. The interactions between protein content and type of batches × time were significant ($p<0.05$) during both ripening and storage periods.

Table 4.4. Protein contents of sausage samples during ripening (%)

Groups	Day 0	Day 9
Control	17.36±0.37^{aB}	26.66±0.20^{aA}
Starter	16.90±0.55^{abB}	24.94±0.61^{cA}
D.hansenii	16.99±0.16^{abB}	26.42±0.63^{abA}
Y.lipolytica	16.37±0.42^{bB}	25.35±0.61^{bcA}

a-c : Means having different letters within each column denote significant difference at p<0.05.
A-B: Means having different letter within each row denote significant difference at p<0.05
Data are mean values ± S.D. (n=2)

Ercoşkun (2009) found the protein content of traditionally produced sausage as 20.97%. Gökalp (1982) and Soyer (1989) obtained 27.30-30.0 % protein in ripened sausages. The differences between protein contents in these researches were probably due to the differences of moisture content and fat content in the formulation (Gök, 2006). Sausages having minimum 22% protein can be classified as high quality sausage (Bozkurt and Belibağlı, 2012).

Table 4.5. Protein contents of sausage samples during storage (%)

Groups	Day 9	Day 30	Day 60	Day 90
Control	26.66±0.20^{aA}	26.97±0.52^{abA}	26.32±0.64^{aA}	23.27±0.43^{aB}
Starter	24.94±0.61^{cC}	27.37±0.69^{aA}	27.43±0.39^{aA}	23.71±0.49^{aB}
D.hansenii	26.42±0.63^{abA}	25.83±0.43^{bA}	24.04±0.53^{bB}	25.91±0.48^{bA}
Y.lipolytica	25.35±0.61^{bcA}	24.55±0.36^{A}	23.38±0.54^{bB}	24.97±0.49^{bA}

a-c : Means having different letters within each column denote significant difference at p<0.05.
A-C: Means having different letter within each row denote significant difference at p<0.05
Data are mean values ± S.D. (n=2)

During storage, protein contents changed depending on solid content of sausage samples. At the end of the storage period, protein contents were 23.27-25.91%. The differences between batches were statistically important (p<0.05) during storage.

4.1.3. Fat Content of Sausage Samples

Fat contents of all sausage samples determined during processing were shown in Table 4.6. In addition, fat contents of sausage samples determined during storage were indicated in Table 4.7.

Fat content of sausage samples was between 18.09-21.55 % before stuffing and increased to 37.22-44.92 % at the end of the 9 days of ripening depending on drying. Difference between treatments and the interaction of type of treatment × ripening time were found to be significant ($p<0.001$). According to Turkish Food Codex (TFC), the proportion of fat content to protein content should not exceed 2.5. Fat contents of all groups were in agreement with TFC.

Table 4.6. Fat contents of sausage samples during ripening (%)

	Day 0	**Day 9**
Control	19.86±0.22^{cB}	42.87±0.06^{bA}
Starter culture	18.09±0.09^{dB}	44.92±0.05^{aA}
D.hansenii	21.55±0.06^{aB}	37.22±0.64^{dA}
Y.lipolytica	20.58±0.03^{bB}	39.22±0.56^{cA}

a-d : Means having different letters within each column denote significant difference at $p<0.05$.
A-B: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

During storage, fat content of all groups increased from the initial values of 37.22-44.92 % to 40.19-45.5 % at the end of the storage depending on drying. The interaction between type of treatment and storage time had significant ($p<0.001$) effect on fat contents of sausage samples.

Table 4.7. Fat contents of sausage samples during storage (%)

Groups	**Day 9**	**Day 30**	**Day 60**	**Day 90**
Control	42.87±0.06^{aB}	40.42±0.13^{cC}	39.98±0.01^{cC}	45.95±0.02^{aA}
Starter culture	44.92±0.05^{bA}	41.27±0.21^{bC}	40.48±0.11^{bD}	44.13±0.06^{bB}
D.hansenii	37.22±0.64^{cC}	39.75±0.10^{dB}	40.29±0.05^{bA}	40.19±0.03^{dA}
Y.lipolytica	39.22±0.56^{dC}	43.09±0.07^{aA}	43.13±0.02^{aA}	41.30±0.08^{cB}

a-c : Means having different letters within each column denote significant difference at $p<0.05$.
A-D: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

4.1.4. Salt Content of Sausage Samples

Salt content of all sausage samples during ripening were shown in Table 4.8 and fat content of all groups during storage were indicated in Table 4.9.

Table 4.8. Salt contents of sausage samples during ripening (%)

Groups	Day 0	Day 9
Control	1.99 ± 0.08^{aB}	2.42 ± 0.07^{cA}
Starter culture	1.97 ± 0.02^{aB}	2.80 ± 0.04^{aA}
D.hansenii	1.98 ± 0.03^{aB}	2.62 ± 0.04^{bA}
Y.lipolytica	2.01 ± 0.03^{aB}	2.39 ± 0.05^{cA}

a-c : Means having different letters within each column denote significant difference at $p<0.05$.
A-B: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

Before stuffing salt content of sausage samples changed between 1.97% and 2.01%. During ripening salt contents of all groups showed an increase depending on dehydration. At the last day of the ripening salt contents were between 2.39-2.80%. These values were lower than the limitation (5%) reported in TSE 1070. Interaction between type of treatment and ripening time had a significant effect on salt content of sausage samples.

Table 4.9. Salt contents of sausage samples during storage (%)

	Day 9	Day 30	Day 60	Day 90
Control	2.42 ± 0.07^{cD}	3.22 ± 0.02^{cC}	3.87 ± 0.01^{bB}	4.43 ± 0.02^{aA}
Starter culture	2.80 ± 0.04^{aD}	3.39 ± 0.04^{aC}	3.91 ± 0.01^{aB}	4.35 ± 0.04^{bA}
D.hansenii	2.62 ± 0.04^{bD}	3.18 ± 0.02^{cC}	3.82 ± 0.01^{cB}	4.14 ± 0.02^{cA}
Y.lipolytica	2.39 ± 0.05^{cD}	3.29 ± 0.01^{bC}	3.88 ± 0.03^{abB}	4.31 ± 0.04^{bA}

a-c : Means having different letters within each column denote significant difference at $p<0.05$.
A-D: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

During storage salt content of sausage samples increased from the initial values of 2.39-2.80 % to 4.14-4.43 %. Difference between the treatments was significant ($p<0.001$). The values at the end of the 90 days of ripening were in agreement with TSE 1070.

4.2. The pH Values of Sausage Samples

The pH values of sausages were followed during ripening and storage periods. Table 4.10 shows the pH values of sausage samples during ripening while Table 4.11 indicates the pH values during storage.

Before stuffing, the pH values of samples were between 5.86 and 5.89 and no significant ($p>0.05$) differences were observed between treatments. The pH showed a reduction in all four batches at the first day of fermentation although the control s showed a weaker decrease that was statistically different ($p < 0.05$). The pH values of yeast inoculated batches were smaller than the others. This is probably because yeast produced additional lactic acid. The fastest drop was observed at the first three days of ripening. From the 3rd day to the 6th day of ripening, the pH values of control, starter and *D.hansenii* batches indicated a slight increase. There were no significant ($p>0.05$) differences between batches at these days. The pH value of *Y.lipolytica* inoculated batch remained constant between day 3 and day 9. Cocolin et al. (2001) stated that the pH values, after the initial decrease produced by fermentation, increased during ripening in all the samples due to proteolytic activity and lactic acid consumption by inoculated or naturally occurring yeasts.

Table 4.10. The pH values of sausage samples during ripening

Groups	Day 0	Day 1	Day 3	Day 6	Day 9
Control	5.86±0.08^{aA}	5.19±0.40^{aB}	4.56±0.08^{aC}	4.60±0.09^{aC}	4.53±0.05^{abC}
Starter	5.87±0.13^{aA}	4.75±0.14^{abB}	4.50±0.03^{aC}	4.55±0.06^{aBC}	4.59±0.04^{abC}
D.hansenii	5.89±0.13^{aA}	4.69±0.19^{abB}	4.51±0.03^{aB}	4.57±0.07^{aB}	4.62±0.05^{aB}
Y.lipolytica	5.89±0.11^{aA}	4.66±0.10^{bB}	4.52±0.02^{aC}	4.52±0.04^{aC}	4.52±0.04^{bC}

a-b: Means having different letters within each column denote significant difference at $p<0.05$.
A-C: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

At the end of the ripening process, control batch showed a reduction, whereas an increment was observed in the other three batches. Differences between the pH values of treatments and the pH value and type of batch × processing time interactions found significant ($p < 0.05$) during ripening.

The standard for traditional sausage (TSE, 2002) states that ripened sausages should have a pH value between 4.8 and 5.4. Our results were not between the limits at the end of the ripening. However, Dalmış and Soyer (2007) found the pH values of sausages, which were produced without heat treatment, 4.76 for control group and 4.62 for starter group. Coşkuner et al. (2008) evaluated the pH values of heat processed and traditionally processed sausages and found the pH value as 4.63 for traditionally processed sausage after seven days of ripening. They also reported that the short fermentation (3 days) and heat processing gave rise to a relatively high pH value in sausages. Bozkurt and Erkmen (2002) reported that the pH of the sausages fermented with starter culture mix containing *Pediococcus acidilactici*, *Lactobacillus plantarum* and *S*taphylococcus *carnosus* had pH value of 4.53 after fermentation, which was in agreement with the present study.

Table 4.11. The pH values of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	4.53±0.05	4.52±0.12	4.52±0.06	4.52±0.08
Starter	4.59±0.04	4.52±0.08	4.57±0.05	4.60±0.04
D.hansenii	4.62±0.05	4.58±0.05	4.59±0.04	4.61±0.05
Y.lipolytica	4.52±0.04	4.59±0.07	4.60±0.04	4.61±0.09

Data are mean values ± S.D. (n=2)

During storage, a slight increase were observed in inoculated batches that is not statistically important ($p>0.05$). At the end of the storage the pH values were ranged between 4.52 and 4.61. The pH value and type of batch × processing time interactions found insignificant ($p>0.05$) during storage. Keller and Acton (1974), Gökalp (1982), Kaya (1992), Vural (1998) reported that the decrease in pH values caused the protein denaturation and denatured proteins buffered the acidity of the medium. As a result, a slight increase could be observed in pH of sausages.

4.3. Titratable Acidity Values of Sausage Samples

Table 4.12 shows the titratable acidity values of different batches as percent lactic acid during ripening. Titratable acidity values of different batches during storage were shown in Table 4.13.

Titratable acidity increased from the initial values of 0.115-0.175 % to 1.456-1.639 % at the end of the ripening. It has been observed that the interactions between type of batches and time did not have a significant effect on the % lactic acid values of batches during ripening. After fermentation (at day 1), the acidity values of samples ranged between 0.761-0.974 % and control group had significantly ($p<0.05$) lower titratable acidity value than the other three batches. Lactic acid, which is formed as a result of carbohydrate degradation, cause the pH drop during processing depending on the increase in titratable acidity.

At day 9, which is the last day of ripening period, titratable acidity values increased to 1.456-1.639 %. *D.hansenii* inoculated batches had the highest titratable acidity value. However, there was no significant difference between batches in terms of titratable acidity. Gök (2006) found the titratable acidity of sausages, which produced by adding antioxidants, between % 0.961-% 0.986 after 12 days of ripening. Glass et al. (1992) reviewed that the titratable acidity values of fermented s had 1.0 % at the end of ripening for 10 days. Ensoy (2004) reported that turkey s had between 1.55 % and 1.59 % titratbale acidity at the end of the ripening period. Salgado et al. (2004) stated that titratable acidity of traditional Spanish sausage (Chorizo de cebolla) increased rapidly at the first seven days of ripening and reached to 7 % at the end of 14 days of ripening. pH values of sausage samples were correlated significantly with acidity values. Correlation factor was found as -0.882 with p value less than 0.001.

Table 4.12. Titratable acidity values of sausage samples during ripening

Groups	Day 0	Day 1	Day 3	Day 6	Day 9
Control	0.175 ± 0.079^{D}	0.761 ± 0.096^{bC}	1.115 ± 0.010^{bB}	1.449 ± 0.049^{A}	1.456 ± 0.144^{A}
Starter	0.150 ± 0.051^{D}	0.903 ± 0.082^{abC}	1.309 ± 0.114^{abB}	1.333 ± 0.144^{B}	1.583 ± 0.164^{A}
D.hansenii	0.107 ± 0.005^{C}	0.910 ± 0.061^{abB}	1.323 ± 0.144^{aAB}	1.511 ± 0.066^{A}	1.639 ± 0.495^{A}
Y.lipolytica	0.115 ± 0.016^{C}	0.974 ± 0.059^{aB}	1.275 ± 0.059^{abAB}	1.482 ± 0.334^{A}	1.530 ± 0.147^{A}

A-D: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

At the beginning of the storage period, titratable acidity values of sausage samples changed between 1.456 % and 1.639 %, decreased during storage and ranged between 1.307-1.450 % at the end of the storage. Titratable acidity and type of batches

× time interactions did not have a significant effect on the % lactic acid values of batches during storage.

Table 4.13. Titratable acidity values of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	1.456±0.144^{B}	1.681±0.012^{A}	1.461±0.034^{B}	1.450±0.123^{B}
Starter	1.583±0.164^{A}	1.537±0.044^{A}	1.529±0.018^{A}	1.448±0.352^{A}
D.hansenii	1.639±0.495^{A}	1.581±0.015^{A}	1.367±0.031^{A}	1.307±0.127^{A}
Y.lipolytica	1.530±0.147^{A}	1.569±0.031^{A}	1.446±0.012^{A}	1.372±0.073^{B}

A-D: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

4.4. Water Activity Values of Sausage Samples

Water activity is a measure of the availability of water for biological functions and relates to water present in a food in free form. Water activity affects the microbial activity during ripening.

Table 4.14 shows the water activity of different batches during ripening while Table 4.15 indicates the water activity of batches during storage.

Before stuffing, water activity values of sausage samples were between 0.955-0.966, decreased gradually depending on drying during ripening and ranged between 0.844-0.900 at the end of the ripening. Control group had the significantly ($p<0.05$) greatest A_w value than the other three groups. After fermentation, yeast inoculated batches had similar water activity values and difference between treatments was significant ($p<0.001$). *D.hansenii* and *Y.lipolytica* inoculated batches had the higher water activity valuesthan control and starter batches ($p<0.01$).

Table 4.14. Water activity values of sausage samples during ripening

Groups	Day 0	Day 1	Day 3	Day 6	Day 9
Control	0.955±0.006^{bA}	0.891±0.009^{cAB}	0.865±0.048^{bB}	0.861±0.06^{aB}	0.844±0.028^{bB}
Starter	0.963±0.004^{abA}	0.927±0.010^{bAB}	0.903±0.022^{abB}	0.899±0.02^{aB}	0.854±0.021^{bC}
D.hansenii	0.966±0.003^{aA}	0.944±0.011^{abAB}	0.934±0.007^{aBC}	0.919±0.01^{aC}	0.894±0.003^{aD}
Y.lipolytica	0.963±0.002^{abA}	0.949±0.001^{aB}	0.948±0.008^{aB}	0.922±0.01^{aC}	0.900±0.004^{aD}

a-c : Means having different letters within each column denote significant difference at $p<0.05$.
A-D: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

All the sausage samples showed a slight decrease during storage period. The difference between treatments was important ($p<0.01$) and interaction of type of batch and time had no significant ($p>0.05$) effect on water activity values of sausage samples.

Table 4.15. Water activity values of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	0.844±0.028^{bA}	0.813±0.0059^{cB}	0.785±0.0031^{bBC}	0.768±0.0032^{bC}
Starter	0.854±0.021^{bA}	0.846±0.0329^{bcA}	0.843±0.0121^{aA}	0.841±0.0208^{aA}
D.hansenii	0.894±0.003^{aA}	0.876±0.0177^{abAB}	0.863±0.0312^{aAB}	0.847±0.0123^{aB}
Y.lipolytica	0.900±0.004^{aA}	0.892±0.0195^{aA}	0.884±0.0306^{aAB}	0.844±0.0213^{aB}

a-c: Means having different letters within each column denote significant difference at $p<0.05$.
A-C: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

Dalmış and Soyer (2007) reviewed that water activity was 0.892 for control and 0.884 for starter group in traditionally produced sausages. Coşkuner et al. (2008) stated that traditionally produced sausages had lower water activity values when compared with heat processed sausages due to the water loss during fermentation and drying and determine 0.924 water activity for traditionally sausages. They also pointed out that at the end of 90 days of storage, water activity indicated a slight decrease depending on storage time. Patrignani et al. (2006) determined water activity values as 0.900 for *D.hansenii* inoculated batch and 0.863 for *Y.lipolytica* batch at the end of 10 days of

processing. They reviewed that water activity of *D.hansenii* inoculated batch was 0.740 and *Y.lipolytica* inoculated batch was 0.726 after 30 days of storage period. They also stated that yeast inoculation increased water activity decrease during ripening. Water activity values of all groups was lower that the values determined by Coşkuner et al. (2008). However, water activity values of yeast inoculated batches were in agreement with Dalmış and Soyer (2007) and Patrignani at al. (2006).

4.5. Weight Loss Values of Sausage Samples

In fermented sausages, weight loss occurs depending on the moisture loss during fermentation and primarily during drying. Weight loss of sausage samples during ripening were shown in Table 4.16.

The weight loss of samples at the first day of ripening ranged between 5.54 % and 6.07 %. Weight losses of treatments were increased from the 3rd day of ripening depending on drying. At the last day of the ripening, weight loss values increased to 38.46 % for control group, 39.32 % for starter batch, 39.49 % for *D.hansenii* inoculated batch and 38.80 % for *Y.lipolytica* inoculated batch. Kaban (2004) stated the weight loss of ripened sausage as 36.33 % while Gökalp found that weight loss of sausage changed between 38.08-43.09 % after ripening. Our results showed similarity to the values that reported in previous studies about sausage.

No significant differences ($p>0.05$) were found in weight loss values between treatments during fermentation and ripening. Weight loss and type of batch × processing time interactions found insignificant ($p>0.05$) during ripening and storage. There is a negative correlation between weight loss and moisture content of sausage (Gökalp et al., 2004) and it is also reported that there is a relationship between ripening time and weight loss (Gökalp, 1986). Weight loss of sausage samples were correalated significantly with moisture content values. Correlation factor was -0.580 with p value less than 0.001.

Table 4.16. Weight loss of sausage samples during ripening (%)

Groups	Day 1	Day 3	Day 6	Day 9
Control	5.54±1.31^{D}	20.65±0.98^{C}	33.05±0.45^{B}	38.46±0.28^{A}
Starter	6.07±1.19^{D}	21.08±0.14^{C}	33.41±1.69^{B}	39.32±0.99^{A}
D.hansenii	5.63±0.53^{D}	21.10±0.29^{C}	33.98±1.44^{B}	39.49±1.14^{A}
Y.lipolytica	5.63±1.74^{D}	19.55±2.60^{C}	32.81±0.29^{B}	38.80±0.38^{A}

A-D: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

4.6. Thiobarbituric Acid (TBA) Values of Sausage Samples

The degree of rancidity in sausage samples was measured by using TBA analysis. Table 4.17 shows Thiobarbituric acid (TBA) values of sausages during ripening while Table 4.18 indicates TBA values of sausage during storage as mg malonaldehyde/kg sausage.

The initial TBA values were 0.14–0.27 mg malonaldehyde (MDA)/kg sausage and increased to 0.48–0.56 mg MDA/kg sausage after 9 days of ripening.

At day 0 (before stuffing) control group had the greatest TBA value while *D.hansenii* inoculated group had the lowest value. Increase in TBA values of sausages was significant ($p<0.001$) during ripening, indicating that oxidative reactions increased as ripening period progressed. Before stuffing, TBA values of control and starter batches were significantly ($p<0.001$) different from the yeast inoculated batches. TBA values and type of treatments × ripening time interactions found significant ($p<0.001$) both at before stuffing and at the last day of ripening.

As shown in the table, lipid oxidation began in the sausage mix before stuffing. Moreover, Gökalp (1982) reported that lipid oxidation started at the beginning of fermentation. TBA analysis is the most commonly used chemical tests for the quality assessment of lipid oxidation in animal product. The significant increases of TBA values might be a result of lipid increase in the samples during drying.

Table 4.17. TBA values of sausage samples during ripening

Groups	Day 0	Day 9
Control	0.27±0.06^{aB}	0.56±0.05^{aA}
Starter	0.19±0.01^{bB}	0.48±0.02^{bA}
D.hansenii	0.14±0.01^{bB}	0.51±0.04^{abA}
Y.lipolytica	0.16±0.03^{bB}	0.48±0.03^{bA}

a-b: Means having different letters within each column denote significant difference at p<0.05.
A-B: Means having different letter within each row denote significant difference at p<0.05
Data are mean values ± S.D. (n=2)

At the end of the ripening, the lowest value recorded was 0.48 mg MDA/kg sausage both for starter batches and *Y.lipolytica* inoculated batches and the significantly ($p<0.05$)highest value was observed in control batch. Bozkurt and Erkmen (2002) reported that catalase produced by starter culture breakdown yields rancid compounds such as peroxides, aldeyhdes and ketones. Thus, sausages made without starter culture had higher TBA values than those made with starter culture. Moreover, Viallon et al. (1996) determined low amounts of lipid oxidation compounds in fermented sausages inoculated with *Debaryomyces hansenii.* Dura et al. (2004) also reported that lipid oxidation is delayed in the presence of yeasts.

Table 4.18. TBA values of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	0.56±0.05^{aD}	1.12±0.01^{aC}	1.43±0.02^{aB}	1.88±0.01^{aA}
Starter	0.48±0.02^{bC}	0.79±0.02^{bB}	0.81±0.01^{bAB}	0.84±0.01^{bA}
D.hansenii	0.51±0.04^{abB}	0.76±0.04^{bA}	0.76±0.01^{bA}	0.78±0.01^{cA}
Y.lipolytica	0.48±0.03^{bB}	0.53±0.01^{cA}	0.48±0.01^{cB}	0.39±0.02^{dC}

a-c: Means having different letters within each column denote significant difference at p<0.05.
A-D: Means having different letter within each row denote significant difference at p<0.05
Data are mean values ± S.D. (n=2)

During storage, TBA values of all batches, except *Y.lipolytica* batch, increased gradually. TBA index of starter and *D.hansenii* inoculated batches were nearly similar, whereas control batches, which had the highest value, and *Y.lipolytica* batches, which had the lowest value, showed significant ($p<0.05$) differences. The interactions between TBA value and type of batches and storage time was significant ($p<0.001$).

TBA values of *Y.lipolytica* inoculated batches indicated a decline during storage and yeast inoculated batches had lower values than other batches at the end of the storage period. It has been reported that the addition of yeasts as starter culture delayed the lipid oxidation due to their catalase activity (Dura, 2003; Encinas, Lopez-Diaz, Garcia-Lopez, Otero, & Moreno, 2000; Flores et al., 2004; Olensen & Stahnke, 2000). Ercoşkun (2006) stated TBA value of traditionally produced sausages as 0,43 mg malonaldehyde/kg, while Coşkuner (2002) reported as 0,48 mg malonaldehyde/kg. TBA value of *Y.lipolytica* inoculated batch was lower than the values stated in the above mentioned studies about sausage.

4.7. Non Protein Nitrogen (NPN) Content

Changes in NPN content (% of total nitrogen) throughout the ripening and storage stages are shown in Table 4.19 and Table 4.20, respectively.

NPN content of all groups increased gradually during ripening. NPN contents ranged from values of 3.97 % to 5.24 % in the sausage mix before stuffing and from 7.06 % to 8.28 % at the end of the ripening. Before stuffing, starter and *Y.lipolytica* batches had significantly ($p<0.05$) different NPN content when compared with control and *D.hansenii* batches.

At the end of the ripening, NPN contents ranged between 7.06 and 8.28 %. Yeast inoculated batches had higher NPN content as a result of their proteolytic acitivity. Control group had the lowest value. Differences between batches found significant ($p<0.05$) at the last day of ripening. It has been reported that NPN content was one of the proteolytic indexes and it increased as the proteolytic activity increased (Bolumar et al., 2005). Soyer (2005) determined the NPN content as 9.29 % for control group and 10.93 % for starter group of traditionally produced sausages. Our results are lower than the values reported in previous studies about sausage.

Table 4.19. NPN contents of sausage samples during ripening

Groups	Day 0	Day 1	Day 3	Day 6	Day 9
Control	4.78±0.21^{aC}	5.26±0.62^{aBC}	7.10±0.65^{aA}	5.85±0.65^{aB}	7.06±0.13^{cA}
Starter	3.97±0.16^{bC}	4.69±0.28^{cC}	7.03±0.64^{aAB}	6.51±0.50^{aB}	7.51±0.37^{bA}
D.hansenii	4.82±0.06^{ab}	5.81±0.58^{bB}	7.89±0.11^{aA}	6.29±0.73^{aB}	8.28±0.48^{aA}
Y.lipolytica	5.24±0.31^{aC}	5.47±0.39^{bC}	7.23±0.32^{aAB}	6.77±0.29^{aB}	7.75±0.42^{cA}

a-d : Means having different letters within each column denote significant difference at p<0.05.
A-D: Means having different letter within each row denote significant difference at p<0.05
Data are mean values ± S.D. (n=2)

During storage period, NPN content of all groups showed a decrease. At the last day of storage, starter batch had the greatest NPN value while *Y.lipolytica* inoculated batch had the lowest value. The differences in the NPN contents of batches were significant (p<0.05) during storage. Type of batches × time interactions had a significant effect on NPN contents during both ripening and storage periods.

Table 4.20. NPN contents of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	7.06±0.13^{cA}	7.68±0.35^{a}	6.76±0.32^{cA}	6.37±0.28^{bA}
Starter	7.51±0.37^{bA}	7.01±0.34^{a}	7.32±0.51^{ab}	6.88±0.32^{a}
D.hansenii	8.28±0.48^{aA}	7.72±0.10^{aA}	7.77±0.36^{aA}	6.65±0.06^{a}
Y.lipolytica	7.75±0.42^{cA}	7.09±0.49^{b}	6.42±0.19^{cA}	5.93±0.13^{cA}

a-d : Means having different letters within each column denote significant difference at p<0.05.
A-D: Means having different letter within each row denote significant difference at p<0.05
Data are mean values ± S.D. (n=2)

4.8. Total Protein Solubility of Sausage Samples

The changes in total protein solubility of sausage samples during ripening and during storage were shown in Table 4.21 and Table 4.22.

Protein solubility decreased in all treatments during fermentation and ripening. At the beginning of the process, protein solubility of sausage treatments was between 31.73-34.98% and decreased to 18.72-19.53%. This decrease was significant (p<0.01) statistically. Protein solubility was also showed a decrease during storage and at the end of the storage protein solubility of sausage samples was ranged between 14.84-15.43%.

The difference between treatments and the interaction between type of treatment and time did not have an important ($p>0.05$) effect on protein solubility.

Table 4.21. Total protein solubility of sausage samples during ripening

Groups	Day 0	Day 1	Day 3	Day 6	Day 9
Control	31.73±1.29^{A}	27.54±3.19^{AB}	22.74±0.63^{BC}	21.39±1.35^{C}	18.72±0.47^{C}
Starter	33.34±0.44^{A}	27.92±0.81^{B}	23.52±1.75^{C}	23.09±0.59^{C}	19.53±0.78^{D}
D.hansenii	32.39±3.25^{A}	27.33±0.97^{B}	22.81±1.33^{C}	22.61±1.86^{C}	18.81±0.99^{C}
Y.lipolytica	34.98±3.03^{A}	29.03±1.50^{AB}	24.33±2.21^{BC}	21.06±1.76^{C}	19.32±0.84^{C}

A-D: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

Dalmış (2007) reviewed that the protein solubility of traditionally produced sausages decreased during ripening and storage and the protein solubility was 19.13% in control groups and 14.42% in starter culture added groups at the end of the ripening and decreased to 15.98% in control groups, 13.07% in starter culture added groups at the end of the storage period. Klement et al (1973) reported that protein solubility of fermented sausages showed a decrease as a result of pH drop. Our result was in agreement with these studies.

Table 4.22. Total protein solubility of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	18.72±0.47^{A}	16.98±0.87^{B}	15.78±0.85^{B}	15.43±1.04^{B}
Starter	19.53±0.78^{A}	16.67±1.04^{B}	15.31±0.74^{B}	15.25±0.51^{B}
D.hansenii	18.81±0.99^{A}	17.29±0.82^{AB}	16.07±0.88^{BC}	14.84±0.96^{C}
Y.lipolytica	19.32±0.84^{A}	18.18±0.93^{A}	16.36±1.08^{B}	15.09±0.59^{B}

A-C: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=2)

4.9. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

In order to investigate protein hydrolyzation during processing and storage of sausage samples SDS-PAGE was applied to sarcoplasmic and myofibrillar extracts of each sample.

The changes in sarcoplasmic and myofibrillar proteins of sausage samples during ripening and storage were determined with SDS-PAGE method. The SDS-PAGE patterns of sarcoplasmic proteins and myofibrillar proteins were shown in Figure 4.1 and Figure 4.2.

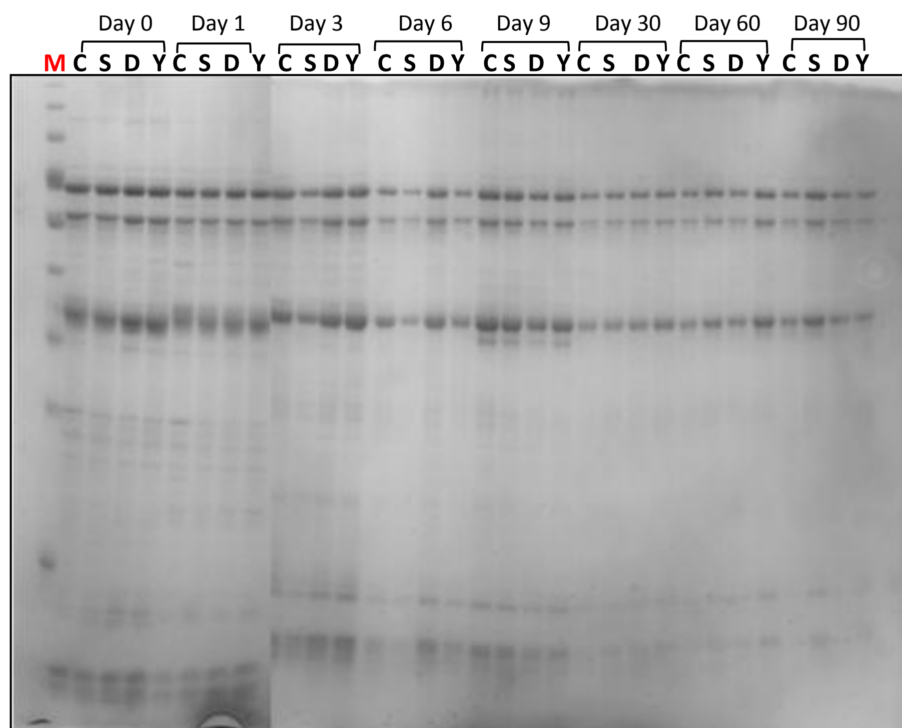

Figure 4.1. SDS-PAGE gel of sarcoplasmic fractions from sausage samples (Lines: **M**: Marker**; C:** Control group**; S:** Starter culture inoculated group; **D**: *D.hansenii* inoculated group; **Y**: *Y.lipolytica* inoculated group

In all sausage samples, there were three intense bands appeared in the ranges 35-70 kDa. In general, sausages produced adding different type of microorganisms showed similar protein patterns. These bands keep their intensities during ripening. However, their intensities decreased slowly during storage. On the other hand, a new band having molecular weight of about 31.52 kDa formed at day 9 in all groups. The bands, which

have molecular weight between 10-12 kDa disappeared after fermentation. By the time, new bands having molecular weight between 11-13 kDa formed in all groups.

Hydrolysis of sarcoplasmic proteins was very similar in all batches. The addition of yeast species did not produce a relevant difference in proteolytic behavior in terms of sarcoplasmic proteins as also reported by Dura et al. (2004) and Patrignani et al. (2007).

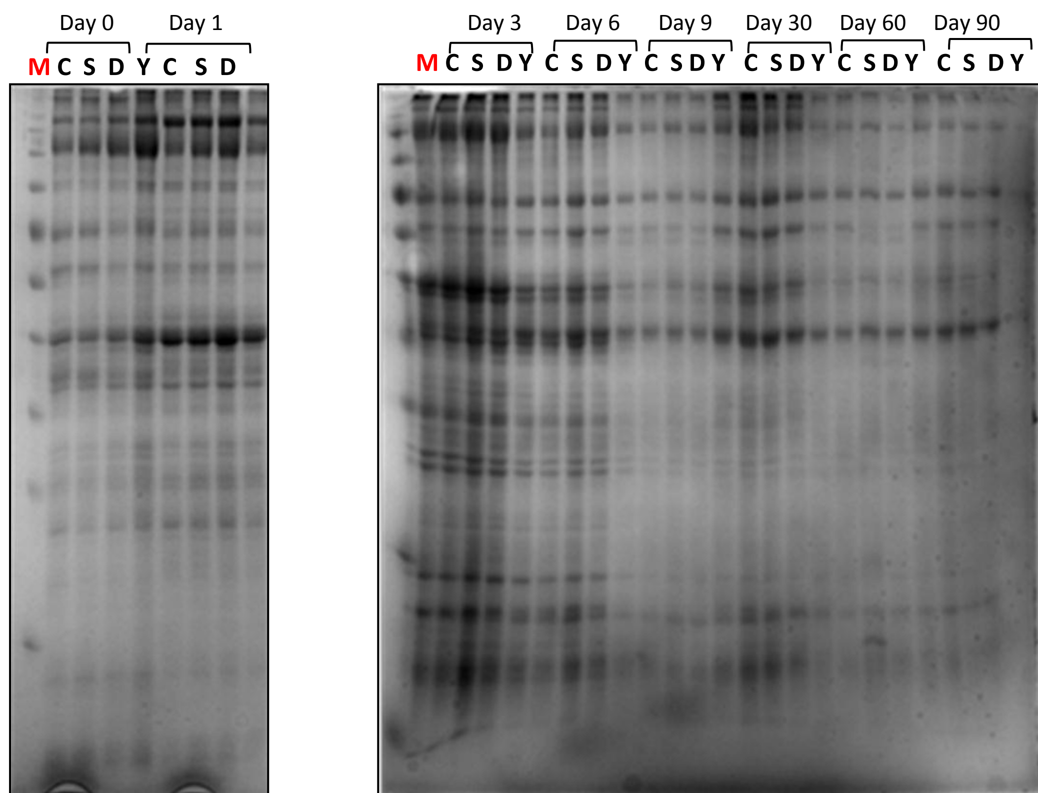

Figure 4. 2. SDS-PAGE gel of myofibrillar fractions from sausage samples (**Lines**: Marker**; C:** Control group**; S:** Starter culture inoculated group; **D**: *D.hansenii* inoculated group; **Y**: *Y.lipolytica* inoculated group

The intensities of high molecular weight proteins (170 kDa and 130 kDa) decreased during ripening. The intensities of proteins having molecular weight of around 70 kDa and 58.52 kDa increased after fermentation while their intensities decreased after ripening (day 9). After fermentation, the bands having molecular weight of about 10.57 kDa and 13.63 kDa degraded and new bands having molecular weight of ≈ 11 kDa, ≈ 13.23 and ≈ 14.34 formed during ripening. In general, there a considerable degradation in myofibrillar ptoeins of sausage, however all groups showed similar protein patterns.

The literature concerning the proteolytic activity of *D.hansenii* in fermented meat is contradictory. Dura et al. (2004) found that *D. hansenii* as a yeast species scarcely contributed to fermented sausage proteolysis. On the contrary, other authors reported important activities on myosin, actin as well as sarcoplasmic proteins in several *D.hansenii* strains (Martin et al., 2002; Rodriguez, Nunez, Cordoba, Bermudez, & Asensio, 1998).

4.10. Color of Sausage Samples

The changes of *L** (lightness), *a**(redness) and *b** (yellowness) values of sausage samples during ripening were shown in Table 4.23, Table 4.24 and Table 4.25, respectively.

*L** values of treatments changed between 47.66-51.15 before stuffing in sausage mix, 46.33-48.12 after fermentation (at day 1) and 44.75-47.06 at the last day of ripening (at day 9). At day 1 (after fermentation) *Y.lipolytica* inoculated batch had the significantly ($p<0.01$) highest *L** value. That means this batch had darker color when compared with other batches. However, yeast inoculated batches had lower *L** values than control and starter batch which did not significant ($p>0.05$). Interactions of type of batch and ripening time did not significantly affect the *L** values of sausage samples.

Table 4.23. *L** values of sausage samples during ripening

Groups	Day 0	Day 1	Day 3	Day 6	Day 9
Control	47.99±3.53	46.33±5.07	48.06±5.34	47.55±3.95	46.72±5.84
Starter	51.15±4.91	48.11±4.23	47.94±2.43	48.46±2.76	47.06±3.13
D.hansenii	47.66±4.33	47.20±4.54	49.09±5.11	45.48±2.93	45.68±4.48
Y.lipolytica	47.75±3.44	48.12±5.48	49.09±2.58	47.68±5.69	44.75±3.40

Data are mean values ± S.D. (n=6)

All the treatments had their greatest *a** values at day 1 and *Y. lipolytica* batch had significantly ($p<0.05$) lowest *a** value. *a** values increased from the initial values of 10.48-11.27 to 15.27-17.97. At the end of the ripening, *D. hansenii* inoculated batch had the significantly ($p<0.01$) lowest value. *a** values of other three batches were similar although starter batch had the highest *a** value. Nitrite reduces to nitrite oxide more rapidly at low pH values and nitrite oxide with myoglobin contributes to red color

formation of sausage. Thus, batches inoculated with starter culture had darker red color. Interaction between type of batch and ripening time had no significant effect on *a** values of sausages.

Table 4.24. *a** values of sausage samples during ripening

Groups	Day 0	Day 1	Day 3	Day 6	Day 9
Control	11.19 ± 0.83^{B}	19.88 ± 2.15^{A}	18.97 ± 3.39^{A}	18.86 ± 2.98^{A}	17.67 ± 1.99^{A}
Starter	10.48 ± 2.15^{D}	20.84 ± 1.93^{A}	19.44 ± 2.06^{AB}	17.40 ± 0.95^{C}	17.97 ± 1.82^{BC}
D.hansenii	10.50 ± 1.93^{D}	20.52 ± 1.80^{A}	17.94 ± 1.73^{B}	19.06 ± 2.03^{AB}	15.27 ± 1.78^{C}
Y.lipolytica	11.27 ± 1.80^{B}	18.65 ± 1.00^{A}	18.54 ± 2.46^{A}	18.47 ± 1.51^{A}	17.02 ± 1.84^{A}

A-D: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=6)

*b** values of treatments did not show a significant ($p>0.05$) difference during ripening. *b** values of treatments decreased from the initial values of 20.99-21.46 to 15.57-17.04.

Table 4.25. *b** values of sausage samples during ripening

Groups	Day 0	Day 1	Day 3	Day 6	Day 9
Control	20.99 ± 1.83^{A}	19.77 ± 1.58^{AB}	18.33 ± 0.82^{B}	18.58 ± 1.90^{B}	16.63 ± 1.22^{C}
Starter	20.99 ± 1.92^{A}	18.89 ± 1.31^{B}	17.97 ± 0.88^{BC}	17.52 ± 1.18^{BC}	17.04 ± 1.78^{C}
D.hansenii	21.46 ± 2.13^{A}	19.34 ± 1.66^{B}	18.27 ± 1.86^{BC}	17.65 ± 0.81^{BC}	16.60 ± 1.27^{C}
Y.lipolytica	22.32 ± 1.27^{A}	18.31 ± 1.31^{B}	19.09 ± 1.04^{B}	16.77 ± 1.06^{C}	15.57 ± 1.34^{C}

A-C: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=6)

The changes of *L** (lightness), *a**(redness) and *b** (yellowness) values of sausage samples during storage were shown in Table 4.26, Table 4.27 and Table 4.28, respectively.

During storage, interaction between type of treatment and time had a significant ($p<0.001$) effect on *L**, *a** and *b** values of sausages. *L** values of sausage increased from initial values of 44.75-47.06 to 45.86-48.21 at the end of the storage.

Table 4.26. *L** values of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	46.72±5.84^{aA}	47.44±3.66^{aA}	40.62±3.39^{bB}	48.21±1.99^{aA}
Starter	47.06±3.13^{aA}	43.63±3.52^{bB}	45.10±2.06^{aAB}	45.98±2.18^{bAB}
D.hansenii	45.68±4.48^{aA}	43.19±3.44^{bB}	43.23±3.34^{abB}	46.78±1.21^{abA}
Y.lipolytica	44.75±3.40^{aA}	43.75±3.00^{abA}	43.08±1.45^{abA}	45.86±2.01^{bA}

a-b: Means having different letters within each column denote significant difference at $p<0.05$.
A-B: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=6)

At the end of the storage period, *D.hansenii* inoculated batch had significantly ($p<0.01$) highest *a** value and yeast inoculated batches had darker red color than other three batches. These results indicated that the formation of nitrosomyoglobin was fast in yeast inoculated batches.

Table 4.27. *a** values of sausage values during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	17.67±1.99^{aA}	17.29±2.44^{aA}	15.20±1.80^{bA}	11.22±5.42^{bB}
Starter	17.97±1.82^{aA}	12.13±2.67^{bcBC}	15.05±0.73^{bAB}	10.36±5.67^{bC}
D.hansenii	15.27±1.78^{bAB}	14.08±2.52^{abB}	18.83±2.64^{aA}	17.36±0.68^{aA}
Y.lipolytica	17.02±1.84^{abAB}	10.30±4.25^{cC}	18.33±1.26^{aA}	14.29±2.62^{abB}

a-c: Means having different letters within each column denote significant difference at $p<0.05$.
A-C: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=6)

*b** values of sausages increased during storage and *D. hansenii* batch took the significantly ($p<0.001$) greatest value at the end of the 90 days of storage.

Table 4.28. *b** values of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	16.63±1.22^{aA}	16.18±1.73^{aA}	15.36±1.04^{cA}	16.56±0.61^{bA}
Starter	17.04±1.78^{aA}	14.79±0.91^{bB}	17.24±1.63^{abA}	16.41±1.34^{bA}
D.hansenii	16.60±1.27^{aB}	16.03±0.82^{aB}	18.54±0.83^{aA}	18.00±1.60^{aA}
Y.lipolytica	15.57±1.34^{aAB}	15.35±1.45^{aB}	16.93±1.21^{bA}	15.47±0.98^{bB}

a-c: Means having different letters within each column denote significant difference at $p<0.05$.
A-B: Means having different letter within each row denote significant difference at $p<0.05$
Data are mean values ± S.D. (n=6)

4.11. Volatile Compound Analysis of Sausage Samples

Table 4.29 shows the changes in quantity of volatile compounds in sausage samples during ripening and table 4.30 indicates the changes in quantity of volatile compounds during storage.

In the ripened sausages, 37 volatile compounds were identified. These compounds were aldehydes, alcohols, acids, ketons, sulfur compounds and terpenes. Above mentioned compounds are formed as a result of lipid oxidation, amino acid oxidation, reactions between amino acids and sugar, animal feed and spices (Blom et al. 1996, Montel et al. 1998). Aldehydes, ketones, alkanes and acids, which are the products of enzymatic reactions and autoxidation of lipids, consist of 60 % of volatile fractions in the sausages produced by without adding spices (Montel et al. 1998).

Aldehydes are the volatile compounds that formed by lipid oxidation. In the ripened sausages, control batch had the lowest percentage of aldehydes (2.29 %). *D. hansenii*, showed with respect to the other batches, greater percentages of aldehydes. (4.55 %). Gök (2006) determined 5.29-6.88 % aldehyde in sausages produced by adding antioxidant compounds and Anserona et al. (2001) determined the aldehyde level of chorizo de Pamplona as 13.8 %. Hexanal, which is one of the lipid oxidation products, has an unpleasant fatty and potato like odor. This compound was determined in all ripened sausages. The lowest level of hexanal was recorded in *Y. lipolytica* batch while highest level was determined in *D. hansenii* batch.

Nonanal is formed as a result of unsaturated free fatty acids oxidation and it has waxy and bitterish odor. Before stuffing it was not determined in control groups however it existed in all batches with different quantities at the end of the ripening. The lowest level was recorded in *Y. lipolytica* batch while the highest level was recorded in *D.hansenii* batch. 2 or 3-methylbutanal and 2-methyl propanal are formed by non-enzymatic strecker degradation of amino acids. Berdague et al. (1993), Stahnke (1995), Montel et al. (1996) reviewed that these volatile compounds have important effect on sausage aroma. However, these compounds were not detected in our sausages. They also stated that the differences in lipid oxidation products are due to the differences in ripening conditions and time. Kaban (2006) also reported that these compounds were not detected in traditionally produced sausages. Aldehydes produce a wide range of flavors and odors. In fact, the saturated aldehydes are reported to enhance the odor,

while 2-enals and 2,4-dienals give the taste and odor sweety, fruity and fatty (Ordonez et al., 1999)

Benzaldehyde which gives the almond oil odor to sausages were detected in all stages of the ripening period and at the end of the ripening starter inoculated batch had the lowest level (0.13 %) and *D.hansenii* batch had the greatest level (0.735 %).

During storage, percentage of aldehyde decreased in all groups. Nonanal, 2-decenal, dodecenal and pentanal were not detected from the 60th day of storage (Table 4.30). However, nonanal was detected on the groups inoculated with *D.hansenii* and its content increased during storage. Hexanal and benzaldehyde were the aldehyde groups detected in all groups at the end of the storage period. The lowest aldehyde level was recorded in sausages inoculated with *Y.lipolytica*.

The addition of *Y.lipolytica* as starter culture reduced the production of aldehydes, which cause the unpleasant aroma formation in sausages.

Another chemical group determined as volatile compound in sausages is acids. Acetic acid, 2-methylpentanoic acid, 2-hydroxypropanoic acid and Hexanoic acid were the detected acids in sausage samples. Acids, which are formed as a result of carbohydrate and amino acid catabolism, gives cheesy odor to sausages (Montel et al., 1998). Acetic acid that is formed by carbohydrate metabolism of homofermantative lactic acid bacteria and Staphylococcus gives sour taste (Berdague et al., 1993). Acetic acid content increased during fermentation and then decreased during ripening and it was not detected at the 6th day of ripening. However, at the end of the ripening it showed a small increase in control, starter and *D.hansenii* batches. The highest level was observed in starter batch (1.06 %) and no acetic acid was detected in *Y.lipolytica* batch. 2-hydroxypropanoic acid in other name lactic acid was produced by the fermentation of carbohydrates by lactic acid bacteria. At the beginning of the process, no lactic acid was detected in control and starter batches and its content increased during ripening in these batches although a small decrease was observed at the 6th day of ripening. *D.hansenii* batch had the highest level of lactic acid at the beginning of the process however, its content showed a decrease during ripening, except in day 6, and had the lowest level at the end of the process. A decrease was observed in lactic acid content of *Y.lipolytica* batch until the middle of ripening and then an increase was observed. Starter batch had the highest level of lactic acid (0.735 %). Because yeasts use lactic acid, the lowest levels were observed in yeast inoculated batches. Hexanoic acid, which gives unpleasant fatty cheesy and waxy odor, was the other acid detected in

sausages. Highest level was observed in *D.hansenii* batch while lowest level was observed in *Y.lipolytica* batch. During storage, acid contents of sausages mostly came from acetic acid. Highest content was observed in *Y.lipolytica* batch and the lowest content was detected in starter batch at the end of the storage. unpleasant hexanoic acid increased in non-inoculated control batch, starter culture inoculated batch and *D.hansenii* inoculated batch while decresed in sausages inoculated with *Y.lipolytica.*

Ethanol, 1-pentanol, 1-hexanol and bezyl alcohol, which were detected in sausage samples, classify as alcohols that have small effects on the sausage aroma due to their high threshold (Spurvey et al., 1998). Alcohols are the compounds that are formed by degradation of branched chain amino acids (Montel et al. 1998). In some cases, they are are produced as a result of decomposition of hydroperoxides by lipoxygenase enzyme. (Spurvey et al., 1998). Ethanol is an alcohol produced by fermentation of sugar and has a slight odor. Highest level of ethanol was observed in yeast inoculated ripened sausages because yeast has ability to ferment sugar to ethanol. Pentanol has a pleasant sweet odor and yeast inoculated batches had higher pentanol content than control and starter batches. Bezyl alcohol has a pleasant aromatic odor and use of starter cultures and yeast had a positive effect on this compound compared to control batch. Alcohols derive, in addition to from carbohydrate metabolism, from branched amino acids (Olesen and Stahnke, 2000). The enhanced proteolysis of the samples inoculated with the yeast strains result in unequivocal increased alcohol production in contrast to Patrignani et al. (2006). On the other hand, the literature on the relationship between yeast alcohol production and amino acid precursor availability in fermented sausages is scarce (Dura et al., 2004). Moreover, alcohol production is strongly dependent on the alcohol dehydrogenase activity of the microorganisms involved in aroma formation (Olesen and Stahnke, 2000).

Acetone was the ketone detected in suck samples. Ketones are formed by autoxidation and β-oxidation of free fatty acids, which are produced as a result of lipolysis. Starter batch had the lowest percentage (0.54 %) of acetone and control batch had the highest percentage (0.765 %). During storage, acetone content decreased and it was not detected from the 60th day of storage.

Another important chemical group in sausage aroma is aromatic hydrocarbons. Styrene, which has a pleasant sweet odor, was one of the hydrocarbons detected in sausage samples in all stages of ripening. The origin of hydrocarbons shows differences. They are formed by lipid oxidation or they can come from the grass used as animal feed

(Berdague et al., 1993; Meynier et al. 1999). At the end of the ripening, the lowest level of styrene was observed in control batch (0.225 %) and the highest level was observed in *D.hansenii* batch (053 %). However, this volatile group had very low proportions in other volatiles.

Allyl mercaptan, allyl sulfide, allyl methyl sulfide, diallyl disulfide, allyl trisulfide were the detected sulfur compounds, which originated from garlic, in sausage samples. There was a big difference between sulfur compound contents of yeast inoculated batches and other two batches. This difference was due to the alteration of diallyl disulfide compound during ripening. It showed a sharp decrease in yeast inoculated batches at the last day of ripening. During storage, sulfur content of control, starter and *D.hansenii* inoculated batch increased and starter batch had the highest score. Sulfur compound level of *Y.lipolytica* inoculated batch decreased and then increased at the last day of storage.

Terpenes are other volatile groups that have significant effect on sausage aroma. They are mostly comes from the spices added sausage mix (Estevez et al., 2006). 13 terpenes were identified during processing. The greatest terpene percentage was recorded in *Y.lipolytica* inoculated batch (81.56 %) and the lowest terpene contents were detected in control batch (72.68 %).

Table 4.29. Volatile compounds of sausage samples identified during ripening (area %)

	Day 0				**Day 1**			
Identified Compounds	**Control**	**Starter Culture**	***D. hansenii***	***Y. lipolytica***	**Control**	**Starter Culture**	***D. hansenii***	***Y. lipolytica***
Ethanol	0.91±0.64	0.73±0.04	1.04±0.44	1.46±0.62	1.35±0.16	0.71±0.04	0.80±0.02	0.75±0.02
Acetone	0.70±0.09	0.72±0.06	0.96±0.01	0.89±0.33	0.62±0.12	0.67±0.01	0.74±0.01	0.81±0.02
Allyl mercaptan	2.03±0.06	2.02±0.91	3.91±0.41	3.18±0.77	2.30±0.15	2.36±0.03	2.42±0.03	2.56±0.42
Acetic acid	0.75±0.00	0.65±0.09	0.25±0.36	1.08±0.15	1.95±0.06	1.49±0.03	1.57±0.21	1.47±0.13
Allyl sulfide methyl	0.46±0.04	0.50±0.13	0.63±0.03	0.84±0.39	0.64±0.14	0.54±0.02	0.52±0.02	0.49±0.05
Pentanal	0.29±0.007	0.28±0.00	0.25±0.05	0.45±0.28	0.36±0.05	0.32±0.09	0.32±0.04	0.32±0.04
2-Butene	0.36±0.01	0.46±0.16	0.35±0.04	0.43±0.11	0.41±0.07	0.57±0.07	0.35±0.04	0.49±0.09
1-pentanol	0.48±0.28	0.38±0.16	0.34±0.06	0.43±0.07	0.43±0.01	0.41±0.03	0.30±0.01	0.49±0.01
Hexanal	0.95±0.09	0.98±0.05	0.63±0.44	1.17±0.91	0.78±0.04	0.91±0.16	0.28±0.03	0.19±0.05
Allyl sulfide	0.39±0.09	0.36±0.06	0.45±0.04	0.38±0.07	0.37±0.007	0.46±0.03	0.42±0.11	0.42±0.01
Pentanoic acid, 2-methyl	0.00±0.00	0.00±0.00	0.21±0.12	0.45±0.03	0.17±0.02	0.21±0.04	0.19±0.04	0.17±0.05
1-Hexanol	0.30±0.09	0.37±0.08	0.24±0.06	0.48±0.29	0.41±0.04	0.36±0.01	0.26±0.06	0.38±0.01
2-hydroxypropanoic acid	0.00±0.00	0.00±0.00	0.70±0.52	0.42±0.23	0.42±0.01	0.31±0.01	0.34±0.08	0.63±0.05
Styrene	0.55±0.33	0.85±0.04	0.36±0.07	0.35±0.18	0.30±0.03	0.56±0.02	0.24±0.007	0.28±0.07
Dodecanal	0.82±0.11	0.27±0.03	0.27±0.17	0.81±0.07	0.36±0.05	0.43±0.03	0.13±0.01	0.19±0.02
1-propene,3-bromo	0.00±0.00	0.55±0.07	1.59±0.04	1.38±0.48	1.01±0.02	1.39±0.03	1.01±0.05	0.87±0.03
Pinene	10.43±0.24	8.02±1.07	7.89±0.09	11.56±0.23	7.42±0.02	7.85±0.25	8.27±0.22	8.08±0.06
Myrcene	6.01±0.13	6.32±0.49	5.36±0.70	4.87±0.11	6.00±0.02	5.88±0.12	5.92±0.19	5.69±0.14
Delta-3-carene	19.53±0.14	19.45±1.24	18.19±0.66	22.26±0.40	19.38±0.69	18.61±0.04	18.81±0.40	17.09±1.97
Undecanal	0.21±0.03	0.23±0.04	0.16±0.06	0.35±0.21	0.24±0.08	0.185±0.007	0.14±0.03	0.18±0.01

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Table 4.29 (cont.)

	Day 0				Day 1			
Identified Compounds	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***
Cymene	11.15±1.29	12.17±2.19	12.35±4.99	9.04±0.16	9.52±0.13	13.60±0.37	13.12±2.89	14.20±1.48
Limonene	13.96±0.31	14.25±1.76	10.11±3.83	13.53±0.19	13.09±1.61	8.16±0.24	10.39±0.31	13.61±0.81
Sabinene	0.48±0.01	0.53±0.08	0.42±0.02	0.41±0.09	0.47±0.09	0.45±0.03	0.47±0.04	0.44±0.04
Terpinene	3.42±0.07	3.58±0.37	3.46±0.00	2.75±0.75	3.45±0.18	3.37±0.08	3.56±0.17	3.29±0.13
Terpinolen	1.07±0.11	0.92±0.09	0.85±0.05	0.52±0.02	0.95±0.01	1.19±0.59	0.89±0.03	0.87±0.01
Diallyl disulfide	7.5±0.41	8.25±0.57	13.38±2.18	9.42±0.26	10.47±0.13	9.32±0.44	11.72±1.03	10.99±0.89
Linalool	2.72±0.51	2.62±0.57	2.48±0.49	1.46±0.07	2.55±0.05	1.29±0.13	2.55±0.04	2.28±0.27
Nonanal	0.00±0.00	0.35±0.09	0.31±0.03	0.23±0.04	0.46±0.01	1.54±0.16	0.29±0.03	0.31±0.01
Hexanoic acid	0.35±0.05	0.18±0.25	0.42±0.21	0.40±0.02	0.53±0.02	0.30±0.07	0.46±0.11	0.28±0.07
Terpineol	0.40±0.18	0.43±0.03	0.28±0.05	0.28±0.07	0.37±0.01	0.35±0.09	0.39±0.01	0.40±0.02
Benzaldehyde	1.40±0.79	1.49±0.56	1.35±0.87	0.43±0.03	1.12±0.03	0.81±0.04	1.13±0.17	0.86±0.12
2-decenal	0.23±0.05	0.26±0.04	0.12±0.03	0.38±0.03	0.25±0.007	0.69±0.06	0.16±0.04	0.22±0.007
Benzyl alcohol	0.76±0.08	0.77±0.007	0.55±0.04	0.50±0.06	0.75±0.09	0.44±0.03	0.85±0.15	0.96±0.01
Allyl trisulfide	0.29±0.06	0.29±0.09	0.32±0.09	0.34±0.01	0.32±0.04	0.36±0.02	0.28±0.04	0.36±0.03
Elemene	0.44±0.06	0.45±0.007	0.47±0.09	0.33±0.01	0.42±0.07	0.36±0.07	0.42±0.04	0.41±0.01
Copaene	1.47±0.42	1.35±0.33	1.41±0.37	0.92±0.07	1.49±0.22	0.85±0.04	1.28±0.08	1.47±0.22
Caryophyllene	6.74±1.19	6.78±1.48	6.34±0.70	4.05±0.29	6.82±0.10	5.48±0.02	6.58±0.52	7.09±0.55

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Table 4.29 (cont.)

	Day 3				Day 6			
Identified Compounds	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***
Ethanol	1.57±0.03	2.15±0.08	1.19±0.01	0.93±0.05	1.33±0.007	1.36±0.04	1.36±0.00	1.03±0.17
Acetone	0.65±0.007	1.17±0.05	0.77±0.05	0.56±0.02	0.15±0.01	0.53±0.08	0.56±0.04	0.65±0.00
Allyl mercaptan	3.82±0.12	5.92±0.28	3.14±0.11	2.12±0.23	3.70±0.01	3.61±0.08	3.80±0.52	3.18±0.32
Acetic acid	0.51±0.00	1.53±0.04	0.87±0.01	0.75±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Allyl sulfide methyl	0.25±0.08	0.72±0.03	0.36±0.06	0.44±0.11	0.34±0.03	0.31±0.08	0.39±0.02	0.40±0.07
Pentanal	0.38±0.01	0.51±0.007	0.32±0.007	0.26±0.04	0.29±0.007	0.31±0.06	0.36±0.02	0.34±0.09
2-Butene	0.48±0.00	0.83±0.09	0.33±0.01	0.41±0.02	0.16±0.01	0.33±0.05	0.40±0.03	0.44±0.08
1-pentanol	0.31±0.00	0.61±0.06	1.01±0.007	0.21±0.01	0.15±0.007	0.56±0.18	0.40±0.01	0.32±0.15
Hexanal	1.19±0.06	1.42±0.07	0.99±0.01	0.84±0.08	0.87±0.04	1.08±0.01	1.94±0.49	1.46±0.55
Allyl sulfide	0.41±0.09	0.77±0.05	0.45±0.04	0.38±0.14	0.27±0.00	0.39±0.16	0.32±0.06	0.33±0.03
Pentanoic acid, 2-methyl	0.28±0.07	0.55±0.02	0.25±0.05	0.31±0.08	0.19±0.01	0.24±0.06	0.26±0.01	0.35±0.007
1-Hexanol	0.36±0.05	0.90±0.07	0.61±0.007	0.49±0.11	0.17±0.01	0.39±0.16	0.49±0.007	0.33±0.007
2-hydroxypropanoic acid	0.49±0.01	0.74±0.16	0.27±0.007	0.43±0.14	0.26±0.007	0.31±0.11	0.68±0.39	0.51±0.17
Styrene	0.49±0.01	0.78±0.02	0.63±0.01	0.19±0.06	0.14±0.01	0.26±0.06	0.26±0.007	0.19±0.04
Dodecanal	0.86±0.06	0.46±0.00	0.35±0.007	0.21±0.08	0.21±0.01	0.39±0.04	0.54±0.12	0.86±0.08
1-propene,3-bromo	2.03±0.13	2.94±0.09	1.90±0.14	1.34±0.11	1.96±0.02	0.94±0.14	1.53±0.13	1.23±0.14
Pinene	4.31±002	15.67±0.04	5.22±0.02	8.64±0.28	7.50±0.01	7.65±0.19	8.45±0.96	7.91±0.89
Myrcene	6.13±0.39	11.82±0.28	5.14±0.00	6.02±0.22	5.89±0.007	6.42±0.28	6.79±0.11	5.08±1.64
Carene	19.38±0.22	38.10±0.78	15.91±0.01	19.71±0.94	18.57±0.08	20.14±0.22	19.85±0.08	14.96±0.71
Undecanal	0.26±0.05	0.33±0.007	0.21±0.03	0.19±0.06	0.23±0.02	0.20±0.06	0.29±0.06	0.48±0.04

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Table 4.29 (cont.)

	Day 3				Day 6			
Identified Compounds	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***
Cymene	10.0±0.96	20.49±0.007	9.16±0.03	13.15±0.41	10.18±0.04	9.74±0.17	10.37±0.56	10.69±0.19
Limonene	15.52±1.69	27.22±0.71	12.42±0.04	14.09±0.86	12.21±0.03	14.23±0.11	12.78±1.57	10.44±0.39
Sabinene	0.45±0.11	0.95±0.02	0.46±0.02	0.45±0.02	0.42±0.03	0.46±0.04	0.39±0.08	0.33±0.01
Terpinene	3.29±0.04	6.72±0.06	2.73±0.02	3.37±0.34	3.16±0.00	3.56±0.27	3.36±0.06	2.68±0.10
Terpinolen	0.95±0.39	1.94±0.06	0.65±0.007	0.98±0.03	1.34±0.02	1.25±0.23	0.86±0.04	1.34±0.67
Diallyl disulfide	9.19±0.89	23.10±0.58	19.23±0.04	9.53±0.04	11.94±0.03	9.58±1.31	9.66±0.08	10.19±0.19
Linalool	2.29±0.16	4.26±0.11	2.14±0.01	1.78±0.13	2.47±0.01	2.19±0.007	1.96±0.06	2.04±0.39
Nonanal	0.38±0.03	0.65±0.08	0.24±0.02	0.29±0.04	0.42±0.02	0.39±0.05	0.56±0.14	1.07±0.11
Hexanoic acid	0.36±0.04	1.10±0.08	0.63±0.007	0.49±0.05	0.30±0.02	0.39±0.007	0.29±0.04	0.31±0.02
Terpineol	0.39±0.00	0.62±0.03	0.33±0.01	0.28±0.01	0.28±0.01	0.29±0.05	0.24±0.00	0.40±0.14
Benzaldehyde	1.06±0.04	1.96±0.04	0.99±0.007	0.63±0.08	0.95±0.01	0.81±0.04	0.76±0.06	1.35±0.11
2-decenal	0.25±0.06	0.50±0.07	0.39±0.01	0.22±0.06	0.26±0.02	0.29±0.007	0.32±0.04	0.80±0.08
Benzyl alcohol	0.94±0.11	1.56±0.09	0.93±0.04	0.69±0.12	1.11±0.02	0.69±0.04	0.58±0.06	1.84±0.17
Allyl trisulfide	0.24±0.02	0.55±0.007	0.37±0.02	0.26±0.06	0.35±0.02	0.25±0.06	0.20±0.00	0.44±0.02
Elemene	0.43±0.01	0.82±0.01	0.33±0.03	0.38±0.04	0.55±0.007	0.48±0.05	0.43±0.03	0.60±0.39
Copaene	1.61±0.05	2.92±0.09	1.23±0.007	1.36±0.30	1.88±0.02	1.62±0.21	1.33±0.04	2.08±0.14
Caryophyllene	6.93±0.07	12.55±0.36	5.94±0.01	5.71±0.89	7.73±0.01	6.62±1.06	5.64±0.25	7.50±0.32

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Table 4.29 (cont.)

	Day 9			
Identified Compounds	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***
Ethanol	0.89±0.007	0.68±0.00	0.81±0.00	0.98±0.007
Acetone	0.77±0.02	0.54±0.01	0.73±0.007	0.63±0.007
Allyl mercaptan	4.04±0.01	3.87±0.03	4.09±0.007	3.77±0.01
Acetic acid	0.49±0.007	1.06±0.01	0.93±0.00	0.00±0.00
Allyl sulfide methyl	0.24±0.02	0.26±0.01	0.14±0.007	0.41±0.007
Pentanal	0.21±0.01	0.22±0.002	0.31±0.007	0.24±0.01
2-Butene	0.17±0.00	0.22±0.001	0.17±0.001	0.37±0.007
1-pentanol	0.25±0.02	0.32±0.01	0.75±0.007	0.53±0.04
Hexanal	0.77±0.01	0.54±0.007	1.88±0.01	0.51±0.01
Allyl sulfide	0.29±0.007	0.25±0.007	0.44±0.01	0.37±0.00
Pentanoic acid, 2-methyl	0.29±0.007	0.09±0.007	0.28±0.01	0.25±0.05
1-Hexanol	0.09±0.007	0.22±0.007	0.35±0.02	0.32±0.02
2-hydroxypropanoic acid	0.73±0.02	0.74±0.02	0.48±0.03	0.64±0.03
Styrene	0.23±0.007	0.36±0.01	0.53±0.02	0.29±0.007
Dodecanal	0.09±0.007	0.07±0.001	0.93±0.02	0.56±0.01
1-propene,3-bromo	2.03±0.01	1.23±0.001	1.43±0.01	1.36±0.005
Pinene	7.18±0.01	4.39±0.007	4.97±0.02	7.13±0.02
Myrcene	6.11±0.007	6.26±0.01	6.12±0.18	5.89±0.02
Carene	19.63±0.04	19.18±0.01	18.72±0.23	19.99±0.01
Undecanal	0.23±0.007	0.16±0.007	0.23±0.03	0.14±0.02

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Table 4.29 (cont.)

	Day 9			
Identified Compounds	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***
Cymene	9.34±0.05	10.31±0.01	9.05±0.06	9.25±0.99
Limonene	13.92±0.02	13.89±0.007	13.63±0.09	13.48±0.08
Sabinene	0.52±0.03	0.51±0.01	0.39±0.02	0.46±0.02
Terpinene	3.22±0.03	3.64±0.02	2.91±0.07	3.43±0.04
Terpinolen	1.08±0.02	1.05±0.04	13.05±0.04	11.09±0.01
Diallyl disulfide	12.34±0.04	11.17±0.01	1.68±0.03	2.90±0.02
Linalool	2.25±0.007	2.82±0.01	2.83±0.04	2.11±0.06
Nonanal	0.30±0.01	0.31±0.01	0.35±0.04	0.19±0.01
Hexanoic acid	0.19±0.007	0.31±0.03	0.41±0.01	0.14±0.04
Terpineol	0.15±0.001	0.96±0.01	0.27±0.01	0.31±0.01
Benzaldehyde	0.65±0.00	0.13±0.01	0.74±0.04	0.72±0.02
2-decenal	0.05±0.00	1.92±0.02	0.13±0.01	0.18±0.02
Benzyl alcohol	0.49±0.01	0.54±0.04	0.55±0.06	0.54±0.007
Allyl trisulfide	0.22±0.002	0.16±0.01	0.21±0.01	0.46±0.04
Elemene	0.54±0.007	0.43±0.02	0.39±0.02	0.46±0.02
Copaene	1.67±0.01	1.82±0.01	1.32±0.03	1.46±0.05
Caryophyllene	7.10±0.03	7.68±0.04	6.27±0.02	6.53±0.04

Table 4.30. Volatile compounds of sausage samples identified during storage (area %)

	Day 30				Day 60				Day 90			
Identified Compounds	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***
Ethanol	0.96±0.02	0.81±0.01	0.79±0.01	0.71±0.001	1.25±0.01	1.01±0.003	4.30±0.12	0.89±0.006	2.33±0.01	1.55±0.004	1.81±0.04	2.58±0.006
Acetone	0.67±0.02	0.51±0.01	0.41±0.002	1.21±0.001	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.94±0.004	0.00±0.00	0.00±0.00	0.00±0.00
Allyl mercaptan	3.46±0.02	3.83±0.12	2.86±0.003	5.03±0.02	4.65±0.002	5.43±0.16	0.00±0.00	3.02±0.01	0.00±0.00	5.62±0.004	5.36±0.03	0.00±0.00
Acetic acid	0.94±0.01	0.65±0.001	0.58±0.001	1.14±0.004	0.62±0.02	1.15±0.004	5.35±0.26	0.39±0.003	5.53±0.003	2.33±0.01	1.88±0.004	9.21±0.13
Ethyl acetate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.71±0.01	0.36±0.008	2.47±0.14	0.51±0.002	1.25±0.001	0.58±0.03	0.43±0.007	0.62±0.009
Allyl sulfide methyl	0.43±0.01	0.38±0.003	0.37±0.00	0.62±0.001	0.63±0.02	0.54±0.007	0.16±0.003	0.31±0.004	0.54±0.001	0.48±0.04	0.76±0.003	1.25±0.003
Pentanal	0.35±0.007	0.33±0.001	0.27±0.01	0.41±0.001	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2-Butene	0.32±0.02	0.44±0.005	0.29±0.003	0.53±0.001	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1-pentanol	0.62±0.02	0.32±0.001	0.22±0.007	0.42±0.003	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Hexanal	1.19±0.01	0.71±0.002	0.20±0.00	0.62±0.002	0.32±0.006	0.46±0.003	0.64±0.01	2.59±0.23	0.78±0.002	0.62±0.03	0.43±0.06	0.55±0.006
Allyl sulfide	0.39±0.05	0.39±0.001	0.36±0.001	0.58±0.002	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Pentanoic acid, 2-methyl	0.29±0.02	0.28±0.004	0.12±0.006	0.27±0.001	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1-Hexanol	0.42±0.00	0.43±0.007	0.29±0.002	0.43±0.001	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2-hydroxypropanoic acid	0.71±0.01	1.19±0.02	0.69±0.004	1.24±0.06	1.24±0.02	0.00±0.00	0.00±0.00	0.00±0.00	0.42±0.003	0.85±0.03	0.00±0.00	0.00±0.00
Styrene	0.36±0.01	0.49±0.007	0.34±0.00	0.22±0.001	0.00±0.00	0.00±0.00	0.51±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.53±0.003	0.00±0.00
Dodecanal	0.64±0.01	0.61±0.02	0.26±0.007	0.34±0.002	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1-propene, 3-bromo	1.17±0.02	0.69±0.001	1.19±0.002	0.74±0.001	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Pinene	8.60±0.01	8.09±0.001	8.54±0.002	10.6±0.25	8.37±0.001	7.74±0.03	1.50±0.04	13.79±0.14	10.76±0.02	7.37±0.02	7.32±0.003	16.61±0.006

(Cont. on next page)

Table 4.30 (cont.)

	Day 30				**Day 60**				**Day 90**			
Identified Compounds	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***	**Control**	**Starter Culture**	***D.hansenii***	***Y.lipolytica***
Myrcene	6.55±0.03	6.26±0.05	6.97±0.01	8.75±0.04	6.10±0.03	6.47±0.12	10.89±0.19	22.09±1.12	6.28±0.10	4.79±0.13	6.69±0.16	7.05±0.001
Undecanal	0.41±0.04	0.26±0.002	0.20±0.004	0.39±0.002	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Cymene	10.53±0.01	3.37±0.001	3.28±0.004	2.55±0.008	5.94±0.08	4.56±0.01	20.48±0.47	7.42±0.13	14.92±0.81	8.01±0.07	14.39±0.63	8.08±0.56
Limonene	12.59±0.04	7.02±0.01	8.03±0.12	11.04±0.24	6.04±0.16	6.10±0.30	9.48±0.56	3.98±0.12	6.88±0.24	11.99±0.61	8.71±0.47	13.22±0.71
Sabinene	0.40±0.01	0.54±0.002	0.48±0.01	0.69±0.02	0.45±0.007	0.54±0.01	7.79±0.43	3.60±0.03	0.37±0.007	0.44±0.001	0.55±0.01	0.54±0.002
Terpinene	3.52±0.07	3.08±0.01	4.47±0.06	3.40±0.02	3.27±0.02	3.47±0.02	0.46±0.001	0.81±0.001	3.37±0.007	3.05±0.001	3.64±0.01	3.41±0.001
Terpinolen	0.92±0.04	1.27±0.001	1.16±0.008	1.59±0.001	1.65±0.003	1.61±0.02	3.89±0.13	7.14±0.001	5.49±0.01	0.60±0.002	1.09±0.03	0.94±0.001
Diallyl disulfide	10.48±0.62	4.12±0.04	4.96±0.06	4.99±0.03	7.68±0.21	9.63±0.33	8.18±0.17	2.17±0.002	9.85±0.26	12.83±0.53	7.75±0.21	7.75±0.15
Linalool	2.08±0.02	2.32±0.02	2.42±0.02	3.13±0.01	3.86±0.06	5.84±0.24	4.32±0.11	0.24±0.003	2.16±0.01	6.18±0.27	3.93±0.03	2.88±0.10
Nonanal	0.70±0.03	0.43±0.003	0.25±0.006	0.42±0.01	0.00±0.00	0.00±0.00	0.32±0.004	0.00±0.00	0.00±0.00	0.00±0.00	1.13±0.01	0.00±0.00
Hexanoic acid	0.58±0.03	0.59±0.01	0.45±0.002	0.41±0.01	0.51±0.006	0.49±0.01	0.00±0.00	0.00±0.00	0.75±0.00	0.77±0.001	0.53±0.007	0.35±0.00
Terpineol	0.28±0.06	0.29±0.00	0.18±0.003	0.42±0.00	0.39±0.003	0.34±0.01	0.37±0.003	0.63±0.00	0.33±0.003	0.35±0.002	0.34±0.004	0.26±0.006
Benzaldehyde	0.77±0.07	0.99±0.02	0.58±0.005	0.77±0.004	0.99±0.004	1.03±0.001	1.12±0.004	0.64±0.04	1.14±0.001	1.03±0.003	0.77±0.004	0.58±0.004
2-decenal	0.33±0.04	0.24±0.02	0.14±0.001	1.15±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Benzyl alcohol	0.47±0.03	0.69±0.03	0.58±0.00	0.74±0.004	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Allyl trisulfide	0.23±0.02	0.19±0.004	0.19±0.001	0.36±0.006	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Elemene	0.46±0.05	0.43±0.007	0.38±0.003	0.49±0.002	0.61±0.001	0.72±0.007	0.76±0.02	2.07±0.005	0.67±0.003	1.94±0.02	0.65±0.003	0.30±0.00
Copaene	1.44±0.01	1.68±0.04	1.12±0.00	1.59±0.001	2.05±0.02	2.51±0.005	2.42±0.003	7.87±0.23	2.05±0.001	2.23±0.03	2.25±0.02	1.05±0.20
Caryophyllene	5.97±0.65	7.09±0.10	4.70±0.14	8.41±0.20	8.49±0.41	8.64±0.12	7.50±0.01	1.44±0.006	6.96±0.001	8.40±0.16	7.54±0.42	4.51±0.02

4.12. Sensory Analysis of Sausage Samples

Figure 4.3, Figure 4.4, Table 4.31, Table 4.32, Table 4.33 show the sensory analysis results of sausage samples that were ready to consumption. Surface color, cut surface color, outer surface appearance, cut surface appearance, texture, flavor and overall acceptance were evaluated on a 5 point scale.

Among the ripened sausages yeast inoculated batches took the greatest scores by panelists. The difference between treatments was not significant ($p>0.05$) at the 0th day. As seen in the figure, interaction of type of batch and storage time did not have a significant ($p>0.05$) effect on the surface color of ripened sausages.

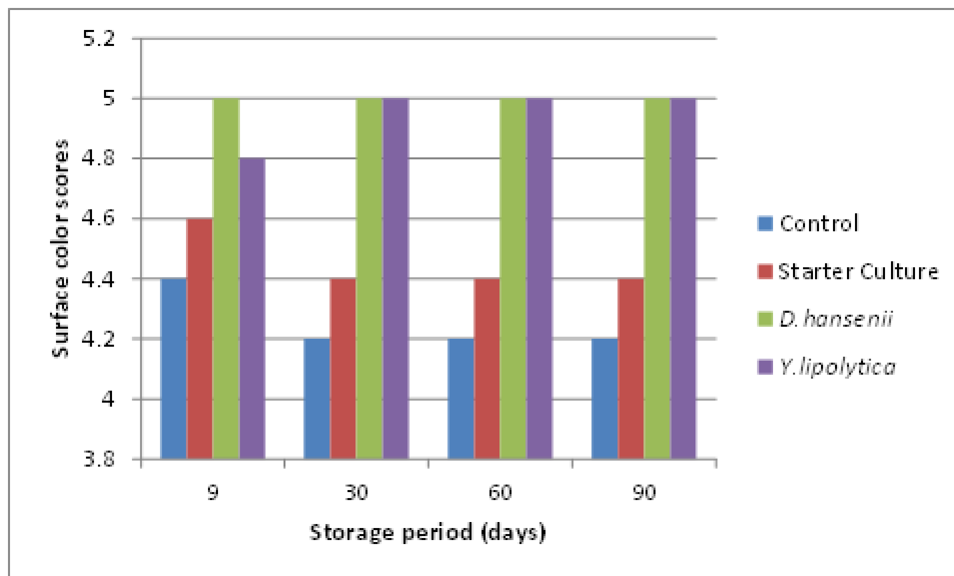

Figure 4.3. Surface color scores of sausage samples during storage

Among the ripened sausages, control group had the lowest surface color score with 4.4. Scores of other treatments ranged between 4.6 and 5. Surface color scores showed a decrease in control and starter batches while indicated an increase in *Y.lipolytica* inoculated batch and leveled off in *D.hansenii* batch after the ripening period. During storage period, yeast inoculated batches had significantly ($p<0.01$) highest scores in terms of surface color.

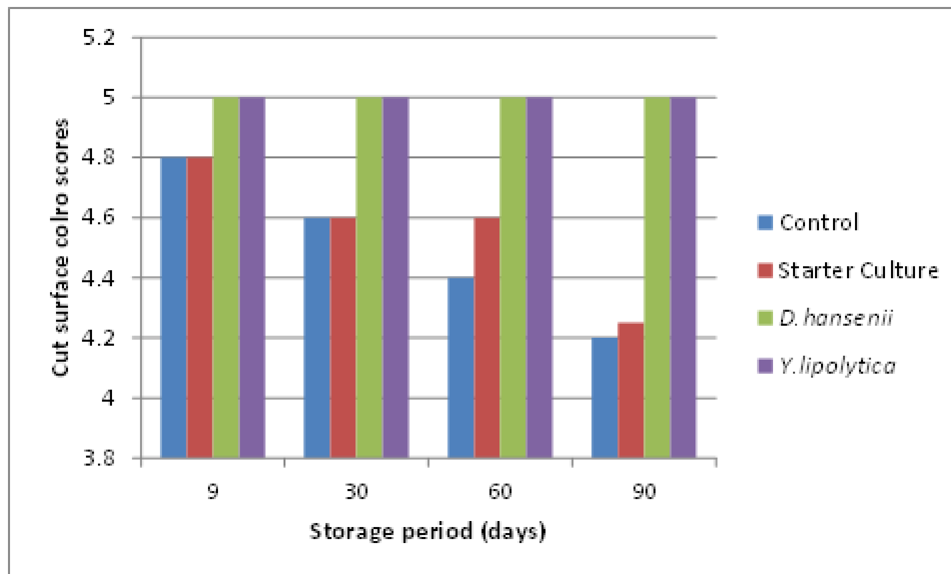

Figure 4.4. Cut surface color scores of sausage samples during storage

Among the ripened sausages yeast inoculated batches got the highest score and control and starter batches had the lowest score (Figure 4.4). The difference between treatments was not important at the last day of ripening (day 0) and at the first two months of storage. At the end of 30 days of storage, cut surface color scores decreased in control and starter batches while leveled off in yeast inoculated batches. At the end of the 60 days of storage, surface color score of control batch decreased to 4.4 and scores of other groups remained constant. At the last month of storage period, yeast inoculated batches significantly greatest points in terms of color surface color and control batch had the lowest point with 4.2. This result was supported by *a** values of sausage samples which were determined objectively. The interaction of type of treatment and storage time did not have a significant effect on the cut surface color of ripened sausages.

Table 4.31. Outer surface appearance scores of sausage samples during storage

Groups	Day 9	Day 30	Day 60	Day 90
Control	4.2^{b}	4.2^{b}	4.2^{b}	4.2^{b}
Starter culture	4.8^{a}	4.6^{ab}	4.6^{ab}	4.6^{ab}
D. hansenii	5^{a}	5^{a}	5^{a}	5^{a}
Y. lipolytica	5^{a}	5^{a}	5^{a}	5^{a}

a-b : Means having different letters within each coloumn denote significant difference at $p<0.05$.

Outer surface appearance scores did not change in control and yeast inoculated batches during the storage period while decreased in starter batch at the first month of the storage and remained constant in the rest of the storage period (Table 4.31). Control batch had the significantly ($p<0.01$) lowest score after ripening and in all stages of the storage period. The highest outer surface appearance scores were observed in yeast inoculated batches with 5 which means these groups had smooth surface appearance and dispersion of fat particles were homogeneous in these batches. Interaction of type of batch and storage time did not have an important ($p>0.05$) effect on the outer surface appearance of sausage samples.

Table 4.32. Cut surface appearance of sausage samples during storage

Groups	**Day 9**	**Day 30**	**Day 60**	**Day 90**
Control	4.6^{a}	4.4^{a}	4^{b}	4^{b}
Starter culture	4.8^{a}	4.8^{a}	4.8^{a}	4.6^{ab}
D. hansenii	5^{a}	4.8^{a}	4.8^{a}	4.8^{a}
Y. lipolytica	5^{a}	5^{a}	5^{a}	5^{a}

a-b : Means having different letters within each coloumn denote significant difference at $p<0.05$.

Panelists determined the meat:fat dispersion (mosaic structure) of sausage samples by evaluating cut surface appearance and results were given in Table 4.32. Cut surface appearance of yeast inoculated samples was scored as very good and the lowest score were given to control samples by panelists among the ripened sausages. At the 30th day of storage, scores of cut surface appearance changed between 4.4 and 5. Panelists gave the significantly ($p<0.01$) low scores for control batch at the 60th day of storage. At the end of the storage, control had the significantly ($p<0.01$) lowest score while yeast inoculated batches got the highest scores. *Y.lipolytica* inoculated batch was given the greatest scores during all the stages of the storage period.

Table 4.33. Texture scores of sausage samples during storage

Groups	Day	Day 30	Day 60	Day 90
Control	3.4^{c}	3^{a}	3.2^{b}	3.2^{c}
Starter culture	4^{b}	4^{b}	4^{c}	4^{b}
D.hansenii	5^{a}	5^{c}	4.8^{a}	4.8^{a}
Y.lipolytica	5^{a}	5^{c}	4.8^{a}	5^{a}

a-c : Means having different letters within each coloumn denote significant difference at $p<0.05$.

Another sensory evaluation criterion is texture. Texture was evaluated in cooked sausage samples by panelists and scores were shown in Table 4.33. Difference between treatments was significant ($p<0.001$) in ripened sausages (day 0) and during storage while interaction of type of batch and storage period did not have a significant ($p>0.05$) effect on texture. Among the sausages that were ready to consumption (day 0) control group had significantly ($p<0.001$) lowest score while yeast inoculated batches had the greatest values that means these sausages were easily chewable. At the 60th day of storage, texture scores of all groups showed a decrease because texture hardened depending on dehydration. At the end of the storage period, yeast inoculated batches were given higher scores by panelists. Control and starter batches were given lower scores.

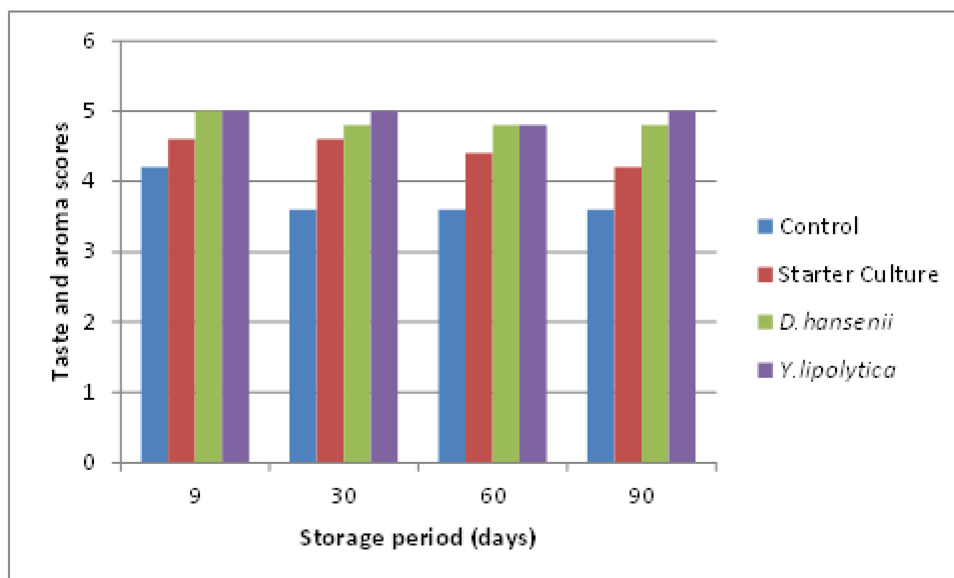

Figure 4.5. Taste and aroma scores of sausage samples during storage

Panelists evaluated taste and aroma in cooked sausages and results were given in Figure 4.5. Taste and aroma of sausage samples showed difference during storage and this difference was significant ($p<0.01$) and interaction of type of treatment and storage period did not have an important ($p>0.05$) effect on texture of sausage samples. Taste and aroma scores of control batch decreased during storage and at the end of the storage had significantly lowest score. Sausages inoculated with yeasts had higher scores in all stages of the storage period. Concerning the volatile compounds, the highest level of terpenes and the lowest level of lipid oxidation derived compounds were detected in sausages inoculated with *Y. lipolytica*.

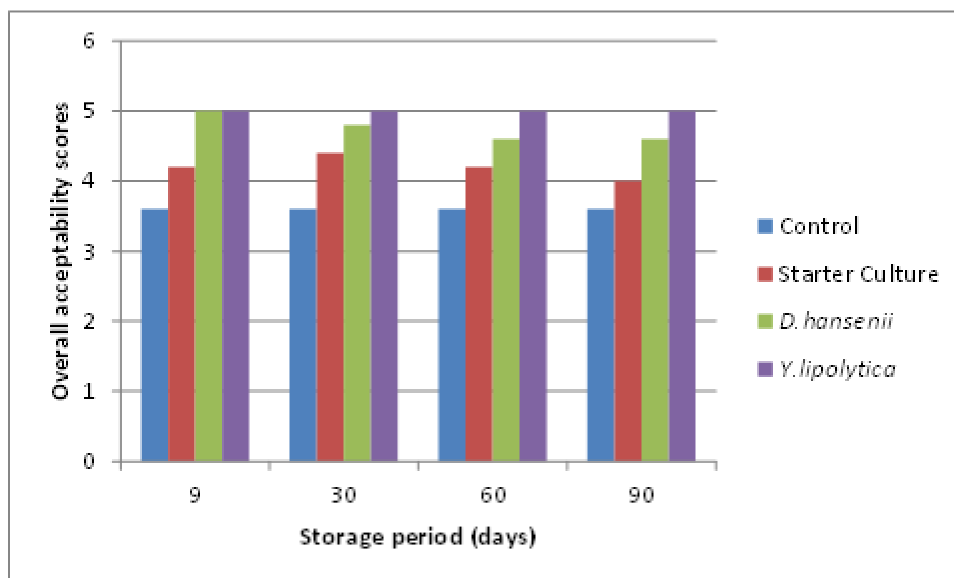

Figure 4.6. Overall acceptability of sausage samples during storage

During the 90 days of storage, overall acceptability score (Figure 4.6) did not change in non-inoculated control batch and *Y.lipolytica* inoculated batch. Difference between treatments were significant ($p<0.01$) statistically, whereas type of treatment and ripening period interaction was not significant ($p>0.05$). The lowest scores were given to control batch and the best scores were given to *Y.lipolytica* batch in all period of storage.

4.13. Microbiological Analysis Results

4.13.1. Total Aerobic Mesophilic Bacteria (TAMB) Counts

Total mesophilic aerobic bacteria counts of sausage samples during ripening were shown in Figure 4.7 while TAMB counts of sausages during storage were shown in Figure 4.8.

Before stuffing in sausage mix TAMB counts were between 6.66-6.98 log cfu/g and increased until the third day of ripening. The effect of treatment type on TAMB counts was not important ($p>0.05$) statistically. From the third day to the last day of the ripening, TAMB counts decreased and at the end of the ripening changed between 8.89-9.20 log cfu/g. The interaction of type of batch and time did not significantly ($p>0.05$) affect TAMB counts of sausage during ripening. Only at sixth day of ripening yeast inoculated batches showed significantly ($p<0.05$) higher TAMB counts than control and starter batches. Patrignani et al. (2006) reviewed that TAMB counts changed between 8.1-8.3 log cfu/g for control batches, 8.3-8.5 log cfu/g for *D.hansenii* inoculated batches and 8.2-8.7 log cfu/g for *Y.lipolytica* inoculated batches at the end of the ten days of ripening. Our results were just above these results.

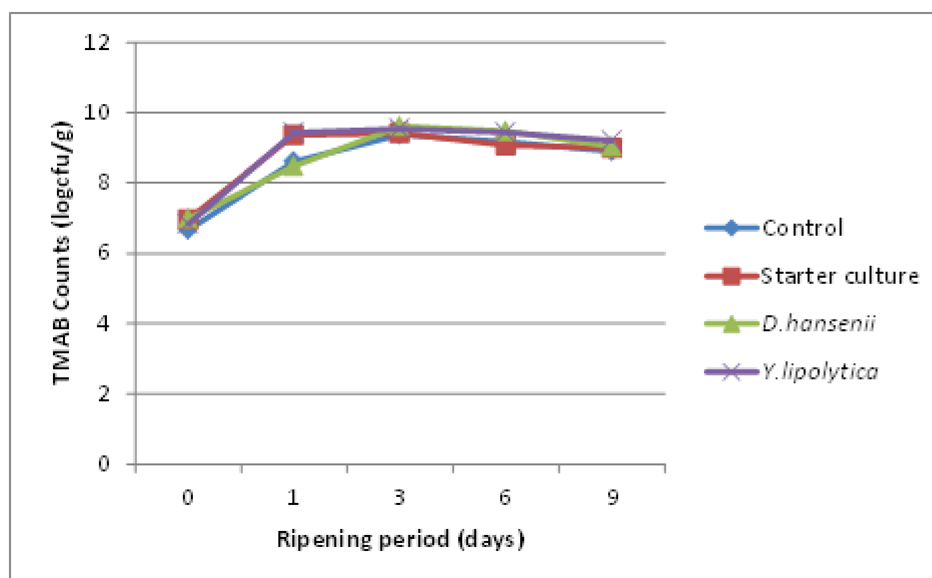

Figure 4.7. Total aerobic mesophilic bacteria counts of sausage samples during ripening

During storage, TAMB counts indicated a decreasing trend. At the end of the storage, TAMB counts ranged between 7.72-7.98 and yeast inoculated batches had the significantly ($p<0.001$) greatest TAMB counts. Type of batch and time interaction did not significantly ($p>0.05$) affect the TAMB counts of sausage samples. Mesophilic bacteria followed the same pattern of lactic acid bacteria as stated by Patrignani et al. (2006). Bozkurt (2002) stated that TAMB counts decreased during storage and reached 5.0 log cfu/g at the end of 60 days storage. Ockerman and Gökalp (1987) reported that total bacterial counts of Turkish sausages with 10% fat, produced at different ripening temperatures, showed a desirable increase (probably growth of lactic acid producing bacteria) during the ripening period by day 9 and then decreased at all ripening temperatures (at 12–14, 16–18 and 20–22 ˚C).

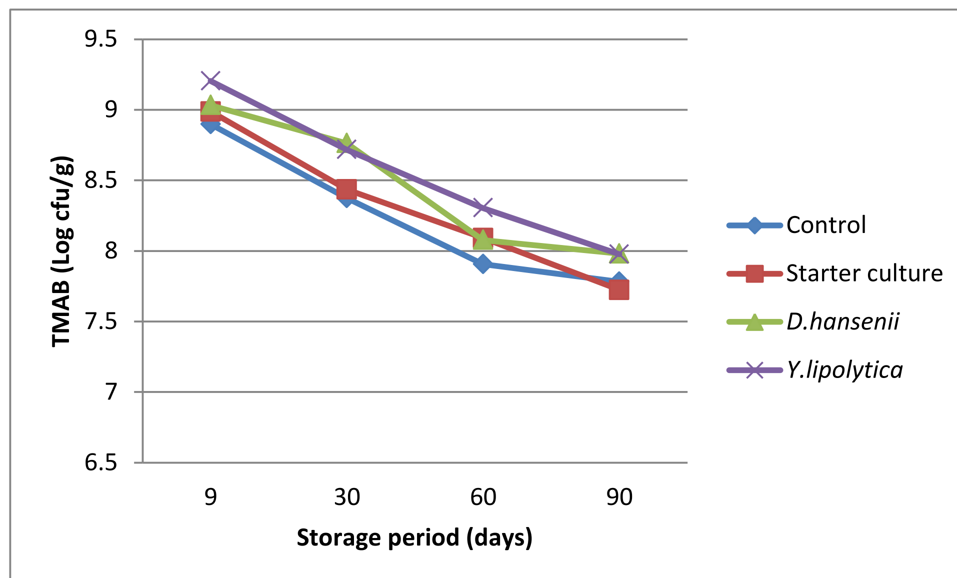

Figure 4.8. Total aerobic mesophilic bacteria counts of sausage samples during storage

4.13.2. Lactic Acid Bacteria (LAB) Counts

Changes in Lactic Acid Bacteria counts of all sausage batches were shown in Figure 4.9 and changes in LAB counts during storage were indicated in Figure 4.10.

Before stuffing, the initial LAB concentrations of sausage mixes were between 5.46-6.17 log cfu/g, showed a sharp increase during fermentation (day 1) and reached the maximum level at the sixth day of the ripening. Control batch showed significantly lowest LAB concentration before stuffing ($p<0.01$) and at the first day of fermentation

(p<0.05). Type of batch and time interactions did not significantly (p>0.05) affect the LAB concentration during ripening.

At the last day of the ripening LAB counts decreased to 8.95-9.22. There was no significant (p>0.05) difference among batches. Interaction of batch type and time did not have an important (p>0.05) effect on LAB counts during ripening. During the fermentation lactobacilli established dominance in all the batches. Dalmış and Soyer (2007) reported that LAB counts of Turkish fermented sausages, produced with different methods, increased from 4.0 and 5.4 log cfu/g to 7.4 and 7.8 log cfu/g after 4 days of fermentation in control and starter inoculated sausages, respectively. Kaban (2007) reviewed that LAB counts of traditionally produced sausages were 6.26 log cfu/g for control and 7.98 log cfu/g for starter inoculated batches at the end of the ripening period. Patrignani et al. (2006) reviewed that LAB had reached levels of about 8.0 log cfu/g in sausages produced by the addition of yeast species after 24 h fermentation. However, they also reported that LAB remained higher than 8.0 log cfu/g during ripening and reached to 9.0-9.7 log cfu/g at the end of the 30 days of storage.

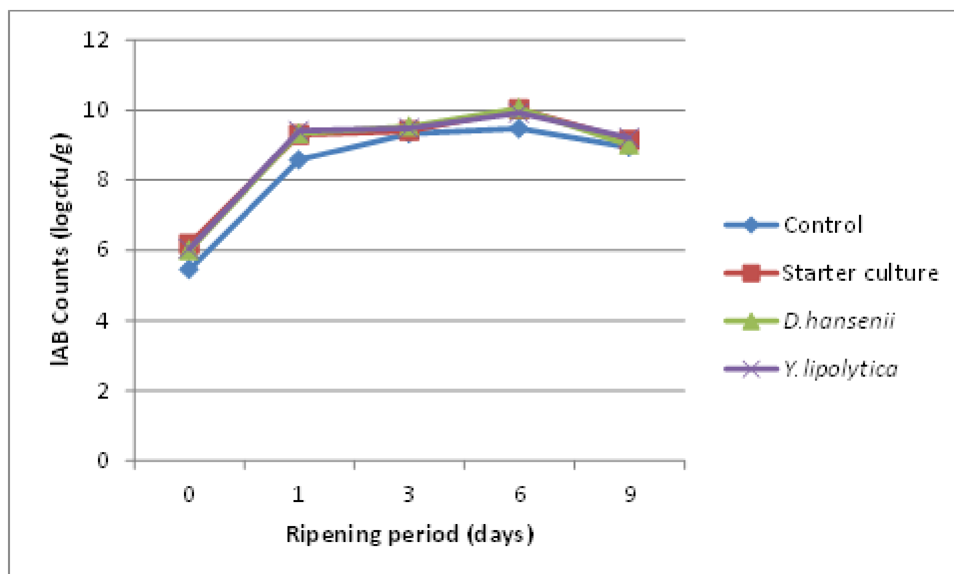

Figure 4.9. Lactic acid bacteria counts of sausage samples during ripening

LAB counts of control and starter batches decreased gradually during storage period. Yeast inoculated batches first showed a decline then a small increase at the end of the storage and they had significantly (p<0.001) higher LAB counts than control and starter batches. Interaction of treatment type and time did not significantly affect LAB counts during storage.

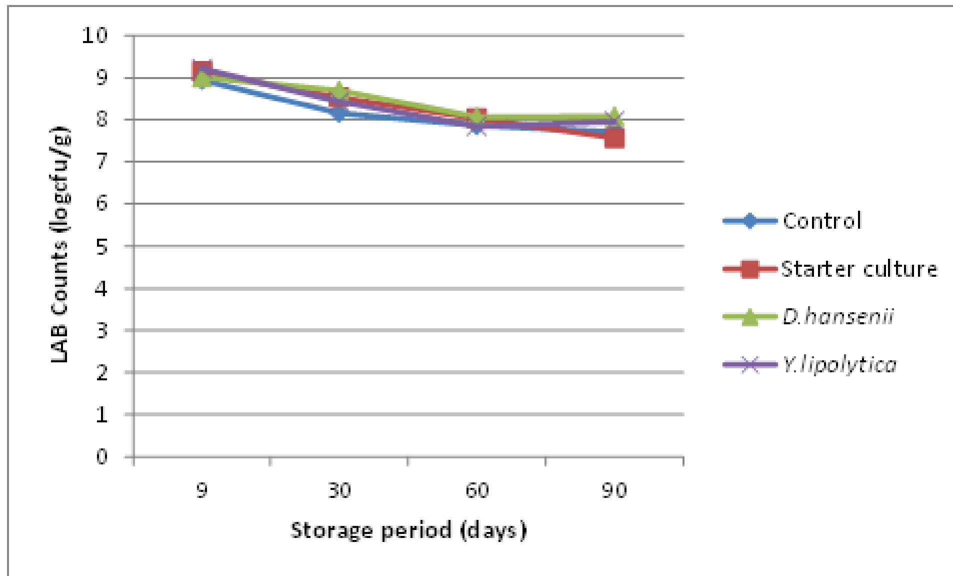

Figure 4.10. Lactic acid bacteria counts of sausage samples during storage

4.13.3. *Enterobacteriaceae* Counts

Changes in *Enterobacteriaceae* count of all treatments of sausage were shown in Figure 4.11.

Before stuffing, *Enterobacteriaceae* were present at levels between 4.21 and 4.83 log cfu/g. During fermentation, *Enterobacteriaceae* counts of starter and yeast inoculated batches showed a decline whereas control batch indicated an increase which was significant ($p<0.01$). They decreased markedly during the ripening period and, in the 9 days ripened sausages, they were under the detection limit (1 log cfu/g) in all the samples. This reduction can be mainly attributed to decreases in water activity (Hierro, de la Hoz, and Ordonez, 1999).

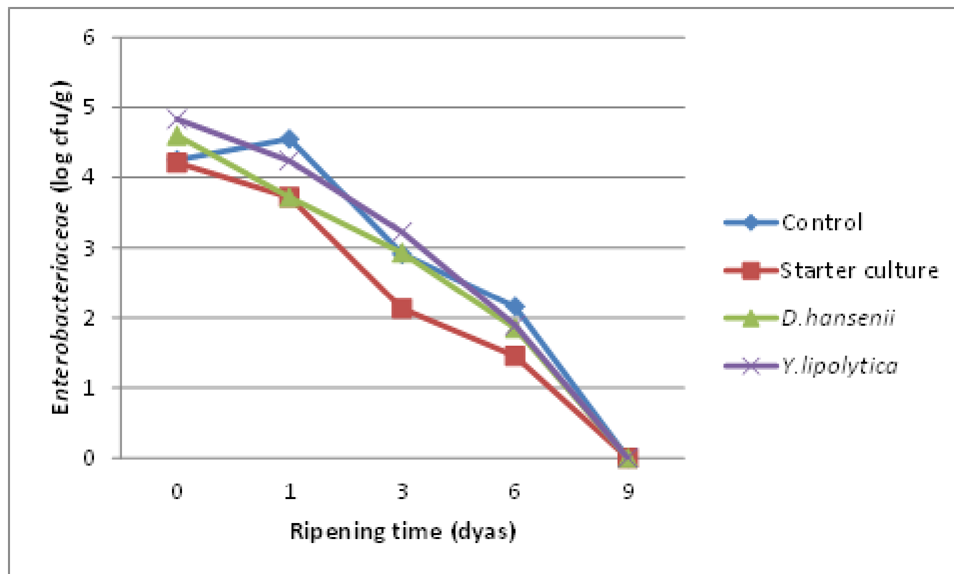

Figure 4.11. *Enterobacteriaceae* counts of sausage samples during ripening

Patrignani et al. (2006) stated that Enterobacteriaceae counts were between 3.6 and 5.3 log cfu/g at the beginning of ripening and fall below detectable limits in the sausages ripened for 30 days. Gençcelep et al. (2007) reviewed that the initial concentration of *Enterobactericeae* in sausage samples was about 2.70 log cfu/g and decrease under detection level on the third day of ripening. Generally, *Enterobacteriaceae* are outcompeted and suppressed by the fermentation microflora (Bauer, 2004). Ayhan et al. (1999) showed that *Enterobacteriaceae* counts of sausage made with starter culture decreased from an initial value of 3.20 log cfu/g to 2.25 log cfu/g on the 8th day of fermentation. Another study (Samelis, Metaxopoulos, Vlassi and Pappa, 1998) showed that *Enterobacteriaceae* were progressively eliminated from the ripened dry sausages after 3 or 7 days of fermentation for different batches of sausages. *Enterobacteriaceae* agreed with literature data on dried fermented sausages.

4.13.4. *Micrococcus/Staphylococcus* (MS) Counts

Changes in *Micrococcus/Staphylococcus* counts of all groups during ripening were shown in Figure 4.12 while changes in *Micrococcus/Staphylococcus* counts during storage were indicated in Figure 4.13.

The initial counts of *Micrococcus/Staphylococcus* was between 5.58 and 5.77 log cfu/g, decreased gradually to the level of 3.93-4.49 log cfu/g at the end of the ripening period. Differences between treatments were significant ($p<0.001$) only during

fermentation. Type of treatment and time interactions did not have a significant ($p>0.05$) effect on *Micrococcus/Staphylococcus* counts during ripening and storage.

Generally, this microbial group presents great variability in fermented sausages (Coppola et al., 1997; Gonzalez & Diez, 2002). Although Micrococcaceae levels between 5 and 6log cfu/g are the most common, cell loads higher than 8 log cfu/g are reported in naturally fermented or artisanal sausages (Cocolin, Manzano, Cantoni, & Comi, 2001; Fontana, Cocconcelli, & Vignolo, 2005a). The inhibition of the growth of staphylococci in the presence of yeast has been reported by other authors (Dura et al., 2004; Gehlen, Meisel, Fischer and Hammes, 1991).

Ercoşkun et al. (2009) reported that MS counts of traditionally fermented sausages decreased from the initial value of 7.09 log cfu/g to the level of 5.09 log cfu/g at the end of nine days of ripening.

The MS counts were in the range reported by other authors (Dalmıs and Soyer, 2008; Nazlı, 1998) showing a decrease after the beginning of fermentation.

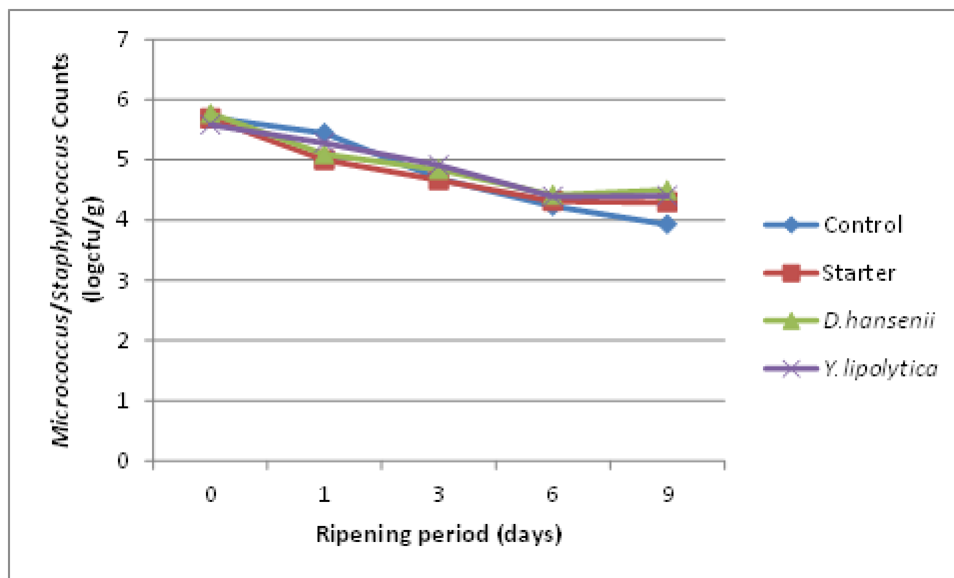

Figure 4.12. *Micrococcus/Staphylococcus* counts of sausage samples during ripening

During storage MS counts decreased to the level of 2.41-2.91 log cfu/g. at the last day of storage control batch showed significantly ($p<0.01$) lowest count while other groups had similar MS counts.

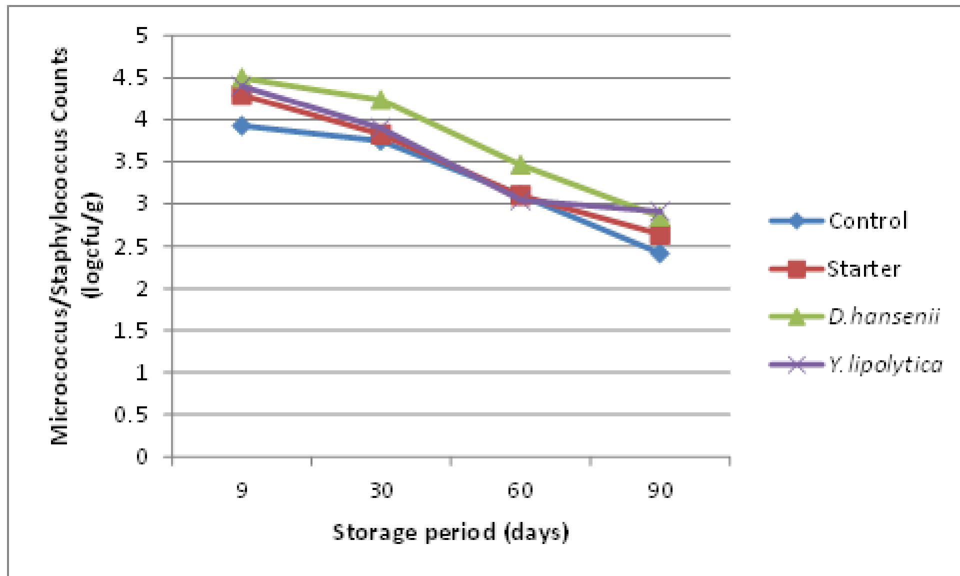

Figure 4.13. *Micrococcus/Staphylococcus* counts of sausage samples during storage

4.13.5. Yeast and Mold Counts

The changes in yeast counts in log cfu/g of sausages were indicated in Figure 4.14 and changes during storage were shown in Figure 4.15.

Debaryomyces hansenii and *Yarrowia lipolytica*, which were resuspended in sterile distilled water, was added to sausage mixture at about 6 log cfu/g and 6.11 log cfu/g, respectively. Before stuffing in the sausage mix, their concentration decreased to 4.64 log cfu/g for the former and 5.18 log cfu/g for the latter. Yeasts existed in control and starter batches were probably due to the natural microbial flora of meat. Great variability is reported for yeast counts in fermented sausages (Gardini et al., 2001). Other reports show that this microbial group attained in traditional products level between 5.0 and 7.0 log cfu/g (Comi and Cantoni, 1980; Coppola et al., 2000; Fontana, Vignolo and Cocconcelli, 2005; Gardini et al., 2001).

The initial yeast counts of treatments were between 4.21 and 5.18 log cfu/g, decreased during ripening and ranged between 2.62 and 4.21 at the end of the ripening. Difference between treatments was important ($p<0.05$) statistically during all stages of the ripening period. Interaction of type of treatment and time had significant effect on the yeast counts of sausage samples. In all stages of the ripening *Y.lipolytica* concentration was higher than other batches.

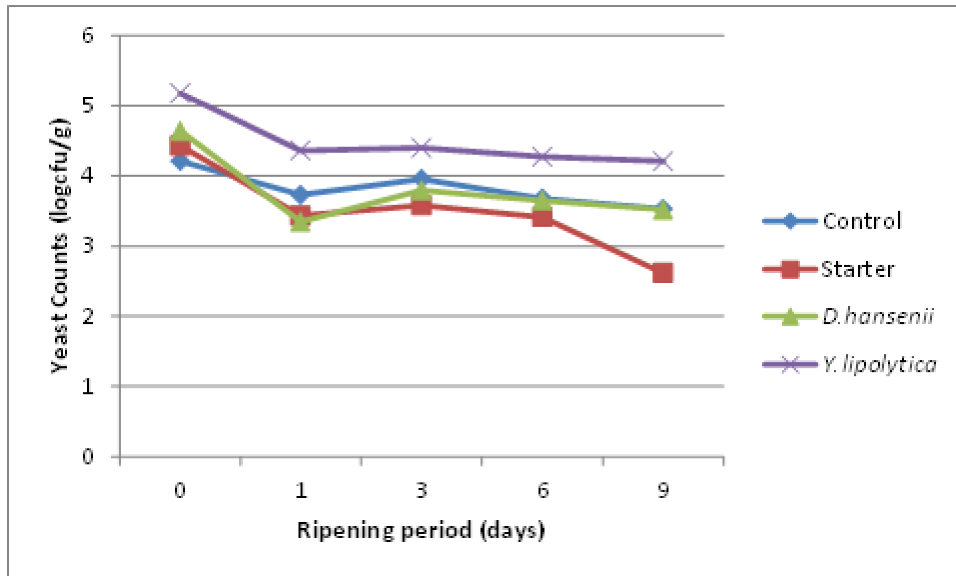

Figure 4.14. Yeast counts of sausage samples during ripening

Dura et al. (2004) and Encinas et al. (2000) stated that a reduction of the yeast population was observed in yeast inoculated batches during the whole process and this reduction was most pronounced during the first 6 days of processing.

D.hansenii surprisingly showed a weak survival compared to other batches, although it is very tolerant to low water activities and high salt content. This result was in agrrement with Patrignani et al. (2006). According to Conner and Beuchat (1984) essential oils of garlic are a potent inhibitor of yeast growth, especially for *D.hansenii*. However, fresh garlic used in this study is a less concentrated compared to the dried garlic powder.

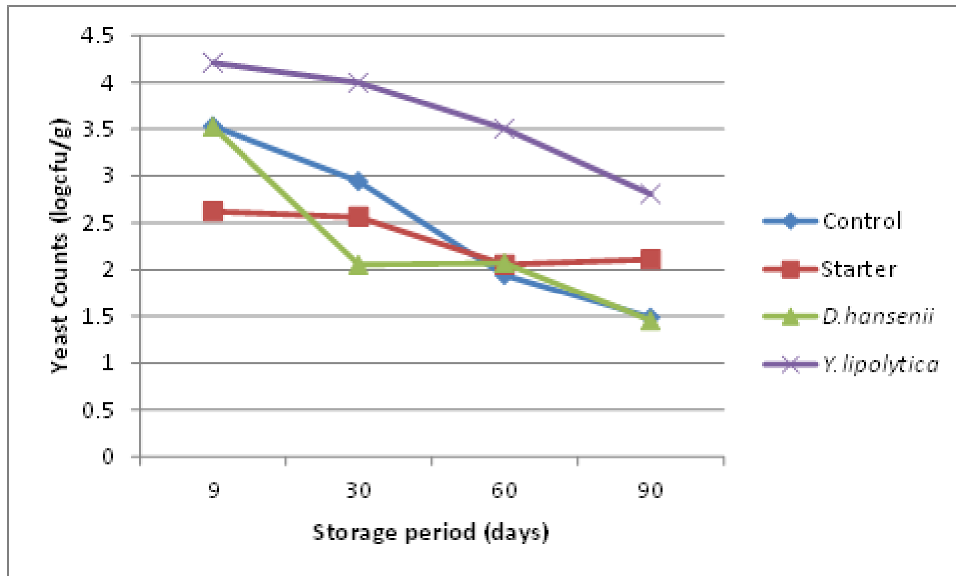

Figure 4.15. Yeast counts of sausage samples during storage

During storage, yeast population of all groups indicated a reduction. Difference between treatments was statistically significant ($p<0.001$).

CHAPTER 5

CONCLUSION

In this study, the effect of *Debaryomyces hansenii* and *Yarrowia lipolytica* used in combination with starter cultures on the physical, chemical, microbiological, organoleptic characteristics and volatile compounds of fermented Turkish sausages during fermentation, ripening and storage were determined.

Moisture content of all treatments decreased during ripening and storage. At the end of the ripening period, yeast inoculated batches had higher moisture content than control and starter batches. In contrast, protein and fat content showed an increase throughout the ripening. During storage, protein content of control and starter batches decreased while a small increase was observed in yeast inoculated batches. Salt content of sausage samples was also increased in all batches depending on drying. Water activity values of all groups decreased depending on reduction in water holding capacity of proteins due to the low pH values. An increase was observed in the weight loss values of sausage samples during ripening.

The pH values of sausage samples decreased during fermentation as a result of microbial activity. At the end of the ripening a small increase was observed in the pH values of starter and *D. hansenii* batches as a result of protein denaturation. Control and *Y. lipolytica* inoculated batches had similar pH values at the end of the ripening. During storage, the pH value of control group remained constant while the pH values of other batches showed an increase and yeast inoculated batches had the highest pH values. Titratable acidity of all groups increased during ripening depending on the formation of lactic acid as a result of carbohydrate degradation.

TBA values of sausage samples increased during ripening and control batch had the highest value, while *Y. lipolytica* inoculated batch had the lowest TBA value. In addition at the last stage of the storage period, a decline was observed in TBA value of *Y. lipolytica* inoculated batch. As a result, yeast inoculation had positive effect on preventing lipid oxidation.

NPN content is one of the proteolytic indices. During ripening, NPN content of sausage samples showed an increase depending on the enzymatic and microbial activity.

The use of yeasts in sausage production caused NPN content to increase. During storage, a decrease was observed in NPN contents of sausages depending on the protein solubility. Protein solubility of all sausage treatments decreased during both processing and storage. Yeast inoculation did not produce an important difference.

In the SDS-PAGE gel of sarcoplasmic fractions, there were three major bands having molecular weight in the ranges 35-70 kDa. These bands kept their intensities during processing and storage. There was a considerable degradation in myofibrillar proteins. However, all sausage treatments showed similar protein patterns in terms of both sarcoplasmic and myofibrillar proteins. In conclusion, yeast inoculation did not produce a relevant difference in proteolytic behavior.

*L** values of sausage samples decreased during ripening while increased during storage. At the end of the ripening, the greatest L* value was observed in control batch. *a** values of sausage samples increased partially during fermentation and decreased during ripening. Starter inoculated batch had the highest a* value at the end of the ripening. During storage, a value of *D.hansenii* inoculated batch increased, whereas a values of other batches decreased. Yeast inoculated batches had higher *a** values and *D. hansenii* was more effective to protect red color of sausage samples at the end of the storage period. *b** values indicated a decline in all batches during ripening and storage. *D.hansenii* inoculated batch had the lowest *b** value at the end of the ripening.

Thirty seven volatile compounds were identified during ripening and storage. Volatile fractions of sausage samples were mostly composed of terpenes. Other identified compounds were aldehydes, alcohols, acids, sulfur compounds, ketones and aromatic hydrocarbons. The highest level of terpenes and the lowest level of lipid oxidation derived volatile compounds were detected in sausages inoculated with *Y. lipolytica*.

In the sensory analysis, yeast inoculated batches had the highest scores in all sensory attributes.

During fermentation, TAMB and LAB counts showed an increase and then indicated a decrease throughout ripening periods depending on the increase of metabolites in acid characteristics produced during drying. *Enterobacteriaceae* counts decreased markedly during the ripening period and at the end of ripening, they were under the detection limit in all the samples. *Micrococcus*/S*taphylococcus* counts decreased during ripening and storage.

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