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THE EFFECT OF NATURAL ANTIOXIDANTS ON THE BIOGENIC AMINES DURING THE RIPENING OF SUCUK

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The Effect of Natural Antioxidants on the Biogenic Amines during the Ripening of Sucuk

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ABSTRACT

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In this study, the effect of natural (*Salvia officinalis L., Urtica dioica, Rosmarinus officinalis L.* and *Hibiscus sabdariffa*) and synthetic (buthylatedhydroxytoluene (BHT) and nitrite/nitrate) antioxidants on the pH, 2-thiobarbituric acid reactive substances (TBARS) value, biogenic amine, Hunter L, a- and b- value and sensory attributes of sucuk were investigated during the ripening periods.

The highest ($P<0.05$) pH value was observed in *Salvia officinalis* (600 ppm) extract added recipe while the lowest in control recipe. About 42 % of retailed sucuk had higher pH value than 5.4 which is maximum level declared in Turkish Food Codex while 26 % of retailed sucuks were lower than 5.2 that is the minimum level for Turkish Food Codex. At the end of the ripening period, TBARS values were ranged between 0.35 (*Salvia officinalis*) and 0.81 mg/kg (in control). Sucuks containing natural antioxidant extract had significantly lower ($P<0.05$) TBARS value when compared to the control recipe. *Salvia officinalis* extracts reduced ($P<0.05$) TBARS values more than the others. Also, the natural antioxidant extracts appeared to be more effective ($P<0.05$) than BHT and nitrite/nitrate. During the first 2 days of ripening, histamine concentration increased significantly ($P<0.05$). The highest ($P<0.05$) histamine concentration was determined in the control recipe. *Urtica dioica* and *Salvia officinalis* were more effective ($P<0.05$) on decreasing histamine concentration than the other antioxidants. Histamine concentration of retailed sucuks was found to be ranged of 0.682 to 210.993. Putrescine concentration in recipes increased ($P<0.05$) from 1.13 to about 1.53 mg/kg during the first 2 days. The lowest

($P<0.05$) putrescine concentration was observed in nitrite/nitrate added recipe. In 3 of 19 retailed sucuk putrescine was not detected. Tyramine concentration increased significantly ($P<0.05$) during the ripening period. *Rosmarinus officinalis* (300 ppm) and nitrite/nitrate added recipe had the lower ($P<0.05$) tyramine concentration than the other recipes. The control recipe had the highest $P<0.05$) tyramine concentration. Hunter L-values were not affected ($P>0.05$) from ripening time and addition of antioxidants into recipe except control and *Salvia officinalis* added recipe. Control sucuk had the lowest ($P<0.05$) and *Urtica dioica* added sucuk had highest ($P<0.05$) L-values at the end of the ripening period. The Hunter a-value increased ($P<0.05$) during the ripening periods. The a-value of control recipe was lower ($P<0.05$) than the other recipes. Hunter b-values decreased ($P<0.05$) from 12.58 to about 10.53. Overall sensory quality scores increased ($P<0.05$) from 3.25 to about 9.00. In all sucuk recipes same trends ($P>0,05$) in overall sensory quality scores were observed during ripening period.

These results indicated that the natural antioxidants are the most effective than synthetic ones. So, they could be used in sucuk to enhance quality and provide safer products.

Key Words: Sucuk, natural antioxidants, biogenic amines, safety, quality

ÖZET

SUCUĞUN OLGUNLAŞMA SÜRECİNDE DOĞAL ANTİOKSİDANLARIN BİOJENİK AMİNLER ÜZERİNE ETKİSİ

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Bu çalışmada, olgunlaşma sürecinde doğal [*Salvia officinalis L.* (adaçayı)*, Urtica dioica* (ısırgan)*, Rosmarinus officinalis L.* (biberiye) ve *Hibiscus sabdariffa* (Japon gülü)] ve sentetik [butilleşmiş hidroksi toluen (BHT) ve nitrit/nitratın] antioksidanların sucuğun pH, TBARS değeri, biyojen amin, Hunter L, a and b-değerleri ve duyusal özellikleri üzerine etkileri araştırılmıştır.

En yüksek (P<0,05) pH değeri *Salvia officinalis* eklenen numunede iken en düşük kontrol (P<0,05) numunesinde gözlenmiştir. Piyasadan temin edilen sucukların % 42'sinin pH değeri Türk Gıda Kodeksinin deklere ettiği maksimum değer olan 5,4'den büyük olduğu ve % 26'sı ise kodekste belirtilen minimum değer 5,2'den küçüktür. Olgunlaşma süreci sonunda TBARS değerlerinin 0,35 (*Salvia officinalis*) ile 0,81 mg/kg (kontrol) arasında değiştiği bulunmuştur. Doğal antioksidan ekstraktları içeren sucuklar ile kontrol numunesi karşılaştırıldığında daha düşük (P<0,05) TBARS değerlerine sahip oldukları görülmüştür. *Salvia officinalis* TBARS değerlerini diğer antioksidanlardan daha fazla (P<0,05) düşürmüştür. Aynı zamanda, doğal antioksidan ekstraktlarının BHT ve nitrit/nitrat'tan daha etkili (P<0,05) oldukları görülmüştür. Olgunlaşma sürecinin ilk 2 gününde, histamin konsantrasyonu artmıştır (P<0,05). En yüksek histamin konsantrasyonu kontrol numunesinde (P<0,05) tespit edilmiştir. *Urtica dioica* ve *Salvia officinalis* histamin konsantrasyonunu düşürmede diğer antioksidanlardan daha etkili olduğu bulunmuştur. Piyasa sucuklarında histamin konsantrasyonu 0,682 ile 210,993 mg/kg arasında değiştiği bulunmuştur. Putresin konsantrasyonu ilk 2 günde 1,13'ten 1,53 mg/kg seviyesine yükselmiştir. En düşük (P<0,05) putresin konsantrasyonu nitrit/nitrat eklenen numunede gözlenmiştir. Piyasadan temin edilen 19 sucuktan 3 tanesinde putresin tespit edilmemiştir. Tiramin konsantrasyonu olgunlaşma sürecinde artmıştır (P<0,05). *Rosmarinus officinalis* (300 ppm) ve nitrit/nitrat eklenmiş numuneler diğerlerinden düşük (P<0,05) tiramin konsantrasyonuna sahiptir.En yüksek (P<0,05) konsantrasyon ise kontrol numunesinde gözlenmiştir. Kontrol ve *Salvia officinalis* eklenmiş numuneler haricindeki numunelerin Hunter L-değerleri olgunlaşma süresi ve antioksidanlardan etkilenmemiştir (P>0,05). Olgunlaşma süreci sonunda kontrol numunesi en düşük (P<0,05) ve *Urtica dioica* en yüksek (P<0,05) L-değerine sahiptir. Hunter a-değeri olgunlaşma süreci boyunca artmıştır (P<0,05). Kontrol numunesinin a-değerinin diğer numunelere göre daha düşük (P<0,05) olduğu tespit edilmiştir. Hunter b-değeri 12,58'den yaklaşık 10,53 değerine düşmüştür (P0,05). Toplam duyusal kalite puanları 3,25'ten yaklaşık 9,00'a yükselmiştir (P0,05). Tüm sucuk numunelerinin toplam duyusal kalite puanlarının olgunlaşma süreci boyunca aynı trendde (P>0,05) değiştiği gözlenmiştir.

Tüm bu sonuçlar doğal antioksidanların sentetik antioksidanlardan daha etkili olduklarını göstermektedir. Böylelikle, sucuğun kalitesini arttırmak ve daha güvenilir ürünler için doğal antioksidanlar kullanılabilir.

Anahtar Kelimeler: Sucuk, doğal antioksidanlar, biyojen aminler, güvenirlilik, kalite

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CHAPTER I

INTRODUCTION

Sucuk (Turkish-style sausage) is one of the most popular fermented meat products consumed in Turkey. Sucuk is produced mostly in small-scale enterprises using traditional fermented sausage type methods involving mixing of ground (3 mm diameter orifice plates) meat (beef, mutton or goat) and sheep tail fat with salt, sugar, spices, dry garlic and other ingredients. This mixture is stuffed into natural or artificial casing. Traditionally, they are hung for fermentation and dried at ambient temperature for 7-14 days. The fermentation of sausage is accomplished by microorganisms of the natural meat micro flora or by added starter cultures (Gürakan et al., 1995)

Traditionally, sausage casing was made of the intestines of animals. Today, however, natural casing are often replaced by collagen, cellulose or even plastic casings especially in the case of industrially manufactured sausages (Gökalp, 1995).

The appearance of food is one of the major determinants of its appeal to consumers and consequently, sales of the product. Lipid oxidation and bacterial contamination are the main factors that determine food quality loss and shelf-life reduction. Therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly relevant to food processors. The growth of microorganisms in meat products may cause spoilage or food borne diseases. Oxidative processes in meat lead to the degradation of lipids and proteins which, in turn, contribute to the deterioration in flavour, texture and colour of displayed meat products (Lopez *et al.*, 2005).

Although synthetic additives have been widely used in the meat industry to inhibit both, the process of lipid oxidation and microbial growth, the trend is to decrease their use because of the growing concern among consumers about such chemical additives. Consequently, search for natural additives, especially of plant origin, has notably increased in recent years. Compounds obtained from natural sources such as grains, oilseeds, spices, fruit and vegetables have been investigated. Therefore, the development and application of natural products with both antioxidants and antibacterial activities in meat products may be necessary and useful to prolong their storage shelf life and potential for preventing food diseases.

Meat products typically spoil due to one of the two major causes; microbial growth or chemical deterioration. The most common form of chemical deterioration is oxidative rancidity (Kanner, 1994). Oxidative rancidity in meat can vary greatly, ranging from extensive flavor changes, color losses and structural damage to proteins (Xiong, 1996) to a more subtle ‘‘loss of freshness’’ that discourages repeat purchases by consumers. The acceptability of a sucuk depends on the extent to which this deterioration has occurred. Lipid oxidation may have significant problems on the quality e.g. color, flavor, texture and nutritional value changed due to the lipid oxidation in sucuks (Chizzolini *et al.,* 1998).

Determination of some intermediates, which occurs as the result of lipids oxidation, is important according to the designation of the quality of the product. As a result of oxidative bitterness malonaldehyde is formed. The amount of malonaldehyde limit is not stated in meat and meat product. Formation of malonaldeyhde was followed by 2-thiobarbituric acid reactive substances (TBARS) value. Hoyland and Taylor (1991) reported that the TBA (2-Thiobarbituric acid) reaction measures the total manolaldehyde present in free form under the conditions of the TBA reaction. The latter is probably the most important for food processors because it is not obvious, yet results in consumer dissatisfaction. Several processed meat products are particularly susceptible to oxidative rancidity because of exposure to oxygen and/or elevated temperatures during processing. All processed meats utilize antioxidants to control oxidative changes. Cured meats include a very effective antioxidant in sodium nitrite (Kanner *et al.,* 1984), whereas uncured meats typically depend on the synthetic phenolic antioxidants, butylated hydroxy anisole (BHA) and butylated hydroxyl toluene (BHT). USDA regulations permit up to 0.01 % (based on fat content) each of BHA and BHT in fresh sausage and up to 0.003 % (based on total weight) each in dry sausage (USDA, 2000).

Sources of natural antioxidants are spices, herbs, teas, oils, seeds, cereals, cocoa shell, grains, fruits, vegetables. Researchers concentrate on ascorbic acid, tocopherols and carotenoids as well as on plant extracts containing various individual antioxidants such as flavonoids (quercetin, kaemferol, myricetin), catechins, or phenols (carnosol, rosmanol, rosamaridiphenol). Phenolic is regarded as a "natural" antioxidant, because its body is hydrolyzed to ascorbic and palmitic acids. (Ordonez *et al*., 1999; Lopez *et al.*, 2005).

However, because of concerns for the synthetic antioxidants by some human health professionals and consumers, (Sebranek *et al*, 2005) many meat processors have been seeking alternative natural antioxidants.

For centuries, meat has been preserved with salt. At certain levels, salt prevents growth of some types of bacteria that are responsible for meat spoilage by either inhibiting the growth of those bacteria directly or removing enough water from the meat that they cannot survive.

The use of salt as a meat preservative spread, a preference developed for certain salts that produced a pink color and special flavor in meat. This is the effect we see in cured meats today (Gökalp, 1995). Near the turn of the century it was determined that nitrate, present in some salt, was responsible for this special color and flavor. Still later it was determined that nitrate actually is changed nitrite by bacterial action during processing and storage and that nitrate itself has no effect on meat color. Today the nitrite used in meat curing is produced commercially as sodium nitrite.

Nitrite in meat greatly delays development of botunial toxin (botulism), develops cured meat flavor and color, retards development of rancidity and off-odors and off-flavors during storage, inhibits development of warmed-over flavor, and preserves flavors of spices, smoke, etc.

Adding nitrite to meat is only part of the curing process. Ordinary table salt (sodium chloride) is added because of its effect on flavor. Sugar is added to reduce the harshness of salt. Spices and other flavorings are often added to achieve a characteristic "brand" flavor. Most, but not all, cured meat products are smoked after curing process to impart a smoked meat flavor.

Sodium nitrite, rather than sodium nitrate, is most commonly used for curing (although in some products, such as country ham, sodium nitrate is used because of the long aging period). In a series of normal reactions, nitrite is converted to nitric oxide. Nitric oxide combines with myoglobin, the pigment is responsible for the natural red color of uncured meat. They form nitric oxide myoglobin, which is a deep red color (as in uncooked dry sausage) that changes to the characteristic bright pink normally associated with cured and smoked meat when heated during the smoking process.

Biogenic amines are compounds commonly present in living organisms in which they are responsible for many essential functions. They can be naturally present in many food such as fruit and vegetables, meat, fish, chocolate and milk but they can also be produced in high amounts by microorganisms through the activity of amino acid decarboxylases. Excessive consumption of these amines can be of health concern because their not equilibrate assumption in human organism can generate different degrees of diseases determined by their action on nervous, gastric and intestinal systems and blood pressure. High microbial counts, which characterize fermented food, often unavoidably lead to considerable accumulation of biogenic amines, especially tyramine, 2-phenylethylamine, tryptamine, cadaverin, putrescine and histamine.

1.1. Aim of the Research

Major quality and safety changes take place during the ripening periods of sucuk (Bozkurt, 2002). The use of natural antioxidants to produce high quality and safe sucuk is important. The effects of *Salvia officinalis L., Urtica dioica L., Rosemarinus officinalis L.*, and *Hibiscus sabdariffa* flowers extract on the lipid oxidation have been studied previously; however, the relationship between *Salvia officinalis L., Urtica dioica L., Rosemarinus officinalis L.*, and *Hibiscus sabdariffa* flowers and biogenic amines has not been studied previously.

Thus the aim of this research was to;

1. Follow the quality and safety parameters of sucuk during the ripening period,

2. Utilize the natural antioxidants *Salvia officinalis L., Urtica dioica L., Rosemarinus officinalis L.*, and *Hibiscus sabdariffa* flowers in sucuk production,

3. Determine their effects on chemical (pH, TBARS value, biogenic amine formation and colour) and sensory properties (overall sensory quality score) of sucuks during the ripening,

4. Determine the effect of concentration of these natural antioxidants on quality and safety of sucuks during the ripening periods,

5. Determine the effect of nitrite/nitrate and BHT which are synthetic antioxidants on chemical and sensory properties of sucuk,

6. Compare the effects of natural antioxidants with nitrite/nitrate and BHT,

7. Compare the prepared sucuks with retailed ones in Gaziantep region,

8. Find the natural equivalent of synthetic antioxidants (because some synthetic antioxidants have carcinogenic activity and their usage in the food industry is more than the natural antioxidants).

CHAPTER II

LITERATURE REVIEW

2.1. Meat

Meat is defined as the flesh of animals consumed for food and, as is the case with most biological material, it is rather variable in composition. The lean or muscle tissue which comprises the bulk of all carcasses is the most important meat constituent. Its value lies in its palatability and nutritive value. Consequently, it is the part of the meat in which the consumer must be first interested. Fat is the most variable constituent of meat, the amount depending upon the manner in which the animal was fed. Fat has a high energy value and contributes materially to the flavor and tenderness of the lean (Oliphant, 1998).

2.2. Sausage and Sucuk

The word “sausage” is derived from the Latin word “salsus” which means salted or preserved by salting. Sausage may be classified in any number of ways, such as by the type of meat, type of preparation and ingredients.

Historically, it appears that the first fermented sausages were made in certain parts of the Mediterranean. This may be due to the fact that a proper climate (temperature and relative humidity) is crucial for the drying process, and fairly stable wet and cool conditions prevail there during the winter.

Fermented (has been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification) sausages are defined as ground meat mixed with salt and curing agents, stuffed into casings and subjected to a fermentation process in which microorganisms play a crucial role. Most fermented sausages are dried and can be stored with little or no refrigeration. In Europe, the manufacturing process does not include a final heat treatment although some varieties (especially fresh raw sausages) are consumed after cooking.

Salting and drying mainly preserve dry cured, ungrounded raw meats, and excellent raw hams can be prepared without significant microbial activity. Rather, the activity of meat enzymes is important for the development of the aroma and tenderness of these products. Bacteria are needed mainly for the reduction of nitrate which is still frequently used as a curing agent, and bacteria have also been reported to improve the flavor. In addition, injection of lactic acid bacteria along with sugar has been suggested in order to lower the pH of hams and facilitate water removal. Accordingly, some bacterial strains are available or have been suggested as starter cultures (Leistner and Lücke, 1989; Lücke *et al.*, 1990). Some perishable meat products may also be preserved by addition of selected strains of lactic acid bacteria antagonistic to pathogens and spoilage flora (Lücke and Earnshaw, 1991).

Sucuk, spicy-Turkish dry fermented sausage, is one of the meat products in Turkey. This fermented meat product has a taste quite distinctive from that of similar products produced in central Europe (Gürakan *et al*, 1999). It is divided into two groups according to their moisture content as dry and semi-dry sucuk. Dry sucuk contains moisture content 35% and below. That is approximately 50% for semi-dry ones. Sucuk is prepared generally from minced meat that may include beef or sheep, fat rendered from the tail of sheep, fermentable sugar, curing agents such as salt, spices, garlic and nitrite. These ingredients are thoroughly mixed and filled into collagen skins. The sucuks are then suspended in a fermentation chamber and subjected to natural fermentation by indigenous organisms and ripening processes. The fermentation temperature changes from 15ºC to 30ºC and the time required varies between 20 and 60 hours depending on the type of the product or the type of process whether it is produced by using starter cultures or not (Ordonez *et al.*, 1999).

Sucuk has traditionally been produced by chance inoculation. This method is undesirable because of uncontrollable quality and standards. Also, the product can undergo spoilage. To make this product acceptable, safe and standardized, both in the national and international markets, it is essential to use starter cultures. Food technologists have developed different types of starter cultures to be used for sucuk products (Gökalp and Ockerman, 1985).

The manufacturing of sucuk made in modern factories by obtaining commercial starter cultures from western countries. The use of commercial starter cultures in sucuk manufacture and the color, slicing properties, odor, flavor, and some other quality characteristics of these products were studied by different researchers (Gökalp and Ockerman, 1985; Turantaş *et al.* 1993). Although the use of these cultures ensures the safety of the product, it may lack the distinctive taste and flavor of a sausage product produced successfully through natural fermentation. Hammes *et al.* (1990) have reported that only meat-borne lactobacilli strains exhibit the activities essential to the production of fermented meat products. Thus, it would be beneficial to select starter culture originating from sucuk (Gürakan *et al.* 1995).

The fermentable sugar generally glucose is used as substrate for lactic acid bacteria which plays a main role in the fermentation. The mixture of glucose and nitrite and/or nitrate is called as curing salt used for stabilization of color and microbial reliability. In the case of addition of nitrate to mixture, nitrate is reduced into nitrite by *Micrococcus varians* and *Staphylococcus carnosus* or some other lactic acid bacteria. However, in the case of addition of nitrite there is no necessity for these bacteria. The amount of lactic acid available in the mixture during reduction of nitrate, is important for microorganisms especially Micrococcaceae species responsible for this reduction. Since, it may cause the prevention of growth of these microorganisms. So, the pH value must be higher than 5.4 until sufficient amount of nitrite is obtained.

One of the main important points in the production of sucuk is to get acid, flavor and aroma at desired level without forming sourish taste. The main common commercial starter cultures are *Lactobacillus plantarum, Pediococcus acidilactici,* and *P. pentosaceus*. The researches about sucuk, produced by traditional technique, show that psychrophilic facultative, heterofermentative Lactobacillus species, mainly *L.sake* and *L.curvatus,* whose tolerance to acidic environment is relatively small, are dominant. At the initial stages of fermentation, homofermentative lactic acid bacteria such as *Lactobacillus plantarum* grow vigorously. Then, in the first 6 days of fermentation, heterofermentative lactic acid bacteria like *Lactobacillus brevis* and *Lactobacillus buchneri* grow dominantly by the means of changes in pH and oxidation-reduction potential results from activities of these bacteria.

The pH value of the product drops from 5.8-5.9 to below 5.2. It is observed for dry sausages that the pH value drops from 5.8 to 4.8 and then remains constant at this value in the first 15 days of maturation. Generally the pH value is 4-4.5 in the case of using starter culture, and 4.6-5.0 in the other case for sausages produced commercially. During drying, pH of the product increases 0.1-0.2 unit. Decreasing of the pH below 5.2 due to acid production causes the coagulation of meat proteins, by this way it contributes the improvement of flavor and aroma with removing moisture during fermentation. So microbial stability and reliability is increased.

Spices, used in the production of sucuk, show antimicrobial effect to microflora that can cause spoilage of product. At the same time, they stimulate the growth of lactic acid bacteria due to their manganese ions content (Ordonez *et al.*, 1999)

Limits for high quality fermented sucuks are 40 % humidity, 5 % NaCl, 150 mg/kg nitrite, 22 % protein, 35 % fat and pH value is between 4.7-5.4 according to the Turkish Standard Institute (TSI 1070).

2.2.1. Selection and Preparation of Raw Material for Sucuk Production

Properties of meat and tail fat are the most important parameters in the quality of end product. Meat should obtain middle aged animal and complete rigor motris stage. Microbial load of meat must be low. Temperature of meat must be between (-5 ºC) and (0 ºC) during production. Using very old animal meat is not preferred because of excessive connective tissue. If very young animal meat are used, water proportion is very high so yield decreases. Excessive fat of meat and snew tissue must also be removed to obtain high quality sucuks (Gökalp, 1986).

pH of meat should be between 5.4-5.8. If pH value is higher than this range because of high water holding capacity, some problems occur during ripening and drying process.

In the production of sucuk spices and additives are also important as well as the quality of meat and fat. These ingredients stabilize the mixture and add specific characteristics, flavor, odor and color to the final product. Generally spices are used in the natural form (as leaves, seeds, powder etc.). Paprica is a spice that is considered as both a flavoring and a coloring agent because of its strong red color. Garlic (*Allium sativum*) is another important ingredient used in the manufacture of Turkish style fermented sausage (Ordonez *et al*., 1999).

The most important change during the conversion of muscle to meat is post-mortem glycolysis where glycogen is converted to lactic acid (Hamm, 1986; Oliphant, 1998; Öztan, 1999). As the accumulation of lactic acid in the muscle increased pH decreases to 5.6-5.8. If an animal is young, pH drops fast, but in old animal pH drops slowly. Also, the decrease in glycogen level and increase in lactic acid, post-mortem glycolysis give rise to many changes. Inorganic phosphate (Pi) and adenosine diphosphate (ADP) are formed during the post-mortem glycolysis. Creatine phosphate (CP) is interacted with ADP to produce adenosine triphosphate (ATP) in muscle. As ATP disappears, the muscle ceases to be elastic and tends to stiffen, i.e, rigor mortis occur. Meat used for sucuk production should be completed rigor-mortis, if not, sucuk could be tough and may be unacceptable (Hamm, 1986; Oliphant, 1998; Öztan, 1999).

In sucuk production dark-firm-dry (DFD) meat must not be used. DFD meat has high water holding capacity and suitable environment for bacteria. Also the pale-soft-exudative (PSE) meat is not preferred for sucuk manufacturing, because PSE meat has low water holding capacity and it contains less amount of pigment (myoglobin). Thus, rate of water removal from sucuk will be too high during the drying period (Gökalp *et al.,* 1999). High pH value of meat has high water holding capacity, so it causes some problems during the ripening. Excessive amount of microorganisms must not present in meats. Meat storage temperature must not exceed 2°C. Meat must be high quality and fresh (Hamm, 1986).

2.2.2. Casings

Two types of casings are used in the production of sausage: natural and artificial (Oğan, 1996).

Natural casings used for cooked and smoked sausages are derived from the stomach and intestines of hogs the intestines of bung, bladder of cattle and the intestines of sheep. They are edible, they allow smoke and moisture to permeate the sausage during processing (Oliphant, 1998; Piette and Postec, 1993).

Synthetic and artificial casings are made from special papers impregnated with cellulose, saran casings are made from synthetic plastics and hydro-cellulose casings are made from regenerated cellulose. Cellulose casings are created from dissolved fibers extracted from cotton seeds or paper pulp. Each of these types of casings are available in a wide range of size and characteristics and are easy to handle. However these types of casings are not edible and must be removed from sausage prior to consumption. Artificial casings provide high strength and are available with excellent permeability to moisture and smoke or as impermeable casings for use in producing water cooked products such as braunschweiger.Artificial casing is more hygienic than natural one so, using of artificial casing is recommended (Oliphant, 1998).

2.2.3. Fermentation and Ripening

Meat fermentation is solid substrate type fermentation with bacteria growing in microcolonies (Incze, 1998). In contrast to many other fermentation substrates, meat cannot be pasteurized in sucuk production.

Fermentation is the metabolic process in which carbohydrates and related compounds are oxidized with the release of energy in the absence of any external electron acceptors. Meat contains considerable amount of peptides and amino acids and small amount of sugar (glucose and glucose-6-phosphate). The content of these fermentable sugar depends on the glycogen content of the muscle. As a rule, meat with a pH above 5.9 contains too little lactate and sugar for safe fermentation; it holds water tightly and provides better condition for growth of acid-labile bacteria. Such muscles should be sorted out and used for other purposes (Lücke, 1994). The final electron acceptors are organic compound produced directly from the breakdown of carbohydrates. Consequently, only partial oxidation of the parent compound occurs and a small amount of energy is released during the process. The lactic acid bacteria lack functional heme-linked electron transport systems or cytochromes and they obtain their energy by substrate-level phosphorylation while oxidizing carbohydrates, they do not have a functional Krebs cycle (Jay, 1978).

In sucuk, fermentation is started naturally by lactic acid producing bacteria. These bacteria break down carbohydrates and the amount of L (+) lactic acid, L (+) lactates or other organic acids increase (Öztan, 1999).

Fermentation of sucuks with little or no catalase-positive cocci may result with product of acceptable color and sufficient shelf-life. But those sucuks may have high residual nitrate content, high rancidity and aroma. This type of sucuk may not be acceptable because of high lactic acid formation (Lücke, 1994).

In Turkey, sucuks which are produced traditionally have poor color stability, high amounts of residual nitrate, biogenic amine (such as histamine) and pathogenic bacteria (Gökalp *et al.*, 1988). Although companies have used starter cultures to improve the quality of sucuks in recent years, they observed that their products do not have good flavor as it has been in the traditionally produced sucuks (Özdemir *et al.*, 1984; Gökalp *et al.*, 1999).

2.2.4. Starter Cultures and Their Effects

In Turkey, starter culture isn't used in traditionally produced sucuks. Therefore fermentation occurs in uncontrolled conditions and the quality of sucuks varies. That's why starter culture needs to be used for safe and quality productions (Gürakan et al, 1995; Gökalp, 1986).

Starter cultures are pure or mixed harmless microorganisms used in fermented meat products. It is designed that; the starter restricts the fermentation time in sucuks, their participating the standard formation of products, helping the development of color in sucuk, providing the inhibition of pathogen microorganisms that can be found in nature of the foods during the fermentation (Ordonez *et al.*, 1999).

The primary genera of bacteria, which have been successfully utilized as fermented sucuk starter cultures are *Lactobacillus, Micrococcus* and *Pediococcus, Staphylococcus* and yeasts *Debaryomyces,* and *Penicillium* (Erkmen and Fadıloğlu, 2001). Among the micrococcus, the widely used one is *Micrococcus aurantiacus,* and it is used for nitrate reducing and catalase activities. *L. plantarum* are the predominantly used lactobacilli in sucuks for fermentation purpose. Sucuk starter cultures consist of single bacterial strains and/or mixed cultures. Mixed cultures of lactobacilli and micrococci have given much better results for the ripening period, development of flavor, color, texture and firmness and prolonging the shelf life (Ordonez *et al.*, 1999).

2.3. Ripening Period and Chemical Changes in Sucuk

2.3.1. Fermentation

Once the casing has been filled, the sausages are kept in ripening cabinets under the conditions of controlled temperature, relative humidity and airflow. Here, the sausages are stored for 1 to 2 days in a controlled temperature of 18 ºC to 26 ºC and a relative humidity of 90 %.

During fermentation, several critical microbiological changes take place. Before fermentation, the microbial load in the sausages ranges from 10^5 to 10^6 cfu/g (Ordonez *et al.*, 1999). The initial microbial population is always varied and is similar to that found in the fresh meat, that is, it comprises lactobacilli, micrococci, enterobacteria, *Pseudomonas* spp., *Achromobacter* spp., *Flavobacterium* spp., *Bacillus* spp., etc. and also molds and yeasts. However, the conditions that inhibit the bacteria responsible for the spoilage of fresh meat, especially the Gram-negative bacteria (predominantly consisting of pseudomonas) have already been established**.** The a_w is reduced from 0.99 to 0.96 by adding curing salts and other solutes, and specific inhibitory effects of nitrates and low oxygen tension also come into play. Therefore, in naturally produced dry-fermented sausages (without starters), the typical microbiota (LAB and *Micrococcaceae*) of these products are soon established and boosts the growth of starters when these are added (Ordonez *et al.*, 1999).

Meats contain considerable amount of peptides and amino acids, and small amount of sugar (glucose and glucose 6-phosphate). The content of these fermentable sugar depend on glycogen content of the muscle. As a rule, meat with a pH above 5.9 contains too little amount of lactate and sugar for safe fermentation; it holds water tightly and provides better condition for the growth of acid-labile bacteria. Such muscles should be sorted out and used for other purposes (Lücke, 1994).

2.3.2. Acid Formation (pH Reduction)

In fermented products lactic acid bacteria, especially lactobacilli have a great importance. Acids formed (mainly lactic acid) from the sugar by lactic acid bacteria following glycolitic pathway. The rate and extent of acid formation are critical in the manufacture of fermented sucuks. They must be adjusted carefully to achieve both favorable sensory quality and safety from pathogens (prevent the multiplication of undesired microorganisms in fermented products). Also, acids are effective on the taste in fermented, dry and semi-dry sausages by rapid ripening and cause the product to be more acidic. These kinds of products contain little aromatic substances that are formed by microbial activity (Lücke, 1994).

2.3.3. Lipid Oxidation (Malonaldeyhde Formation)

One of the most important causes of sucuk deterioration is the lipid oxidation that affects fatty acids and polyunsaturated fatty acids (Fernandez *et al.*, 1997). The acceptability of a sucuk depends on the extent to which this deterioration has occurred. Lipid oxidation may have significant problems on the quality e.g. color, flavor, texture and nutritional value changed due to the lipid oxidation in sucuks (Chizzolini *et al.,* 1998).

Usually, lipids are the major component of dry-fermented sausages. The fat content of these products ranges from 25 to 55%, and their sensory characteristics are closely related with lipid breakdown and transformation during ripening (Demeyer *et al.,* 1974).

During lipid oxidation many volatile and nonvolatile substances are formed that change the taste and odor of the food products. In many products, these modifications are not desirable because they produce a rancid flavor. Nevertheless, a certain degree of oxidation can make an important contribution to the characteristic taste and odor of some products such as dry-fermented sausages and dry hams. The substrates of these reactions are mainly unsaturated fatty acids. In free form these are generally oxidated more rapidly than when they form the part of triglycerides or phospholipids.

Lipid peroxides, also called hydroperoxides, formed during the propagation phase are the primary products of oxidation. These compounds have no odor or taste and therefore do not participate in the flavor or odor of the food products. However, being unstable they are rapidly broken down into by-products that are responsible for the organoleptic changes induced by phenomena of autoxidation (De Man, 1992). Each unsaturated fatty acid produces specific hydroperoxides that also decompose to form specific aldehydes.

The final products of lipid oxidation (aldehydes, alcohols, ketones, furans, etc.) are highly volatile and have a low olfactory threshold, and therefore acquire an important role in the development of the flavor and odor of the food products in which they are present. Their spectra and the amounts in which they are present determine whether they generate desirable or undesirable flavors and, in consequence, the degree of acceptance of the product by consumers.

2.3.4 Color Formation (Nitrosomyoglobin Conversion)

The characteristic color of dry-fermented sucuk is produced by interaction between the meat pigments (myoglobin) and nitrite/nitrate which are added to sucuk dough. Nitrite is the only toxic substance used in the foodstuff and/or allowed for consumption. It has been forbidden to add it to the meat products directly in many countries, and it is used as their sodium or potassium salts.

The color of the meat products depends on the reaction of meat pigments such as myoglobin and hemoglobin (Brewer *et al.*, 1991; Han *et al.*, 1994) with curing components. Nitrosomyoglobin which is termed by myoglobin and nitrite is the main component in the color formation of meat products as a stable color substance.

The production of nitric oxide from nitrite and the reaction with muscle or blood pigment is influenced by factors such as temperature, pH, oxygen, and reducing substances.

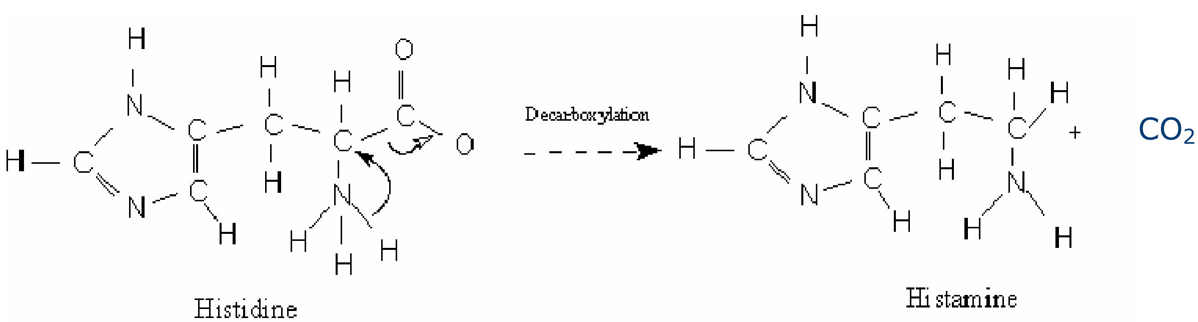
$$\underset{\text{nitrate}}{NO_3} \xrightarrow[\text{Bacterial count pH, temp/time}]{\text{Bacterial conversion}} \underset{\text{nitrite}}{NO_2}$$

$$\underset{\text{nitrite}}{NO_2} \xrightarrow[\text{pH, reduction agent temp/time}]{\text{Chemical conversion}} \underset{\text{nitric oxide}}{NO}$$

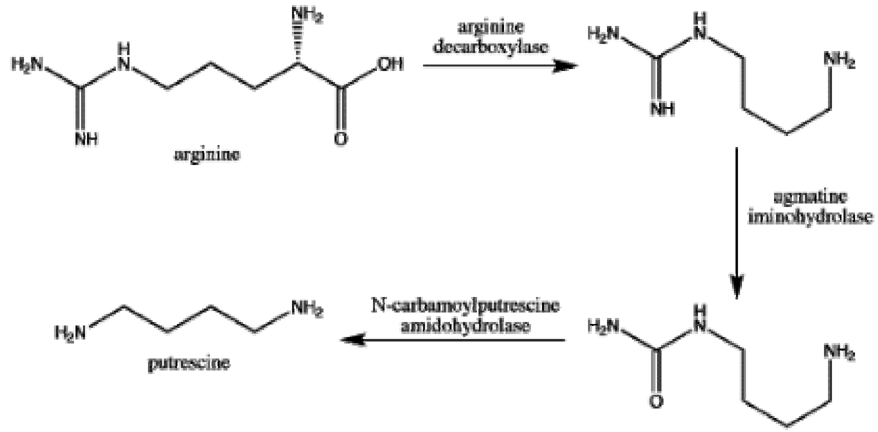
$$\text{NO + myoglobin} \longrightarrow \text{Nitric oxide myoglobin (“cured red”)}$$

2.3.5. Biogenic Amine Formation

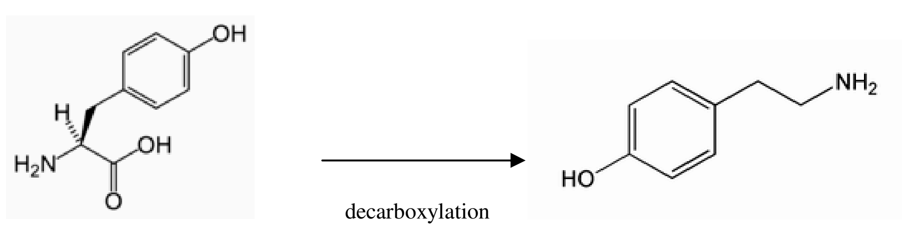
Biogenic amines are natural anti-nutrition factors and are important from a hygienic point of view as they have been implicated as the causative agents in a number of food poisoning episodes, and they are able to initiate various pharmacological reactions. Histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine, and spermidine are considered to be the most important biogenic amines occurring in food (Bover-Cid *et al.*, 2001). These amines are designated as biogenic because they are formed by the action of living organisms. Histamine has been implicated as the causative agent in several outbreaks of food poisoning, while tyramine and β-phenylethylamine have been proposed as the initiators of hypertensive crisis. The toxicity of biogenic amines to chicks in terms of loss of weight and mortality was also reported. The toxicity of histamine appeared to tyramine. Biogenic amines may also be considered as carcinogens because of their ability to react with nitrites to form potentially carcinogenic nitrosamines. The biogenic amine content of various food and feed have been widely studied and found in cheese, fish and meat products, eggs and mushrooms. Food substances that have been prepared by a fermentative process, or have been exposed to microbial contamination during aging or storage, are likely to contain amines. Alcoholic beverages such as beer can contain biogenic amines, as do some other fermented food such as sauerkraut and soy bean products. Amines were also considered as endogenous to plant substance that is commonly used for food, where some fruits and vegetables were found to contain high concentrations of various amines (Shalaby, 1996).



Meat and products have been reported to contain tyramine, cadaverine, putrescine, spermine, and spermidine (Koehler and Eitenmiller, 1978; Nakamura *et al.*, 1979; Edwards *et al.*, 1983; Santos-Buelga *et al.*, 1986; Stratton *et al.*, 1991; Shalaby, 1993, 1996). The highest amount of biogenic amines recorded in available literature for dry sausage is 654 mg/100g for histamine, 1506 mg/100g for putrescine, 535 mg/100 g for cadaverine and 151 mg/100g for tyramine.



Fermentation may be important in the formation of histamine in certain types of sausages. Semi-dry sausages are fermented for short periods often with lactic acid cultures added, while dry sausages are allowed to ferment from the action of natural microflora for a longer period. In general, quite variable quantities of biogenic amines were reported for sausages. The variable concentration could be due to the variation of the ripening process time, the variation and difference of decarboxylase activity of the natural microflora responsible for fermentation, and the biosynthesis and metabolism of such amines (Shalaby and El-Rahman, 1995) in addition to variations in the manufacturing process, the great variation in the type and quality of the meat used, the proportion of meat content included, and the length of maturation (Shalaby, 1996).



Tyrosine Tyramine

Table 2.1. Biogenic amines in foods and their pharmacological effects

Amine	Precursor	Pharmacological effects
Histamine	Histidine	Liberates adrenaline and noradrenaline Excites the smooth muscles of the uterus, the intestine and the respiratory tract Stimulates both sensory and motor neurons Controls gastric acid secretion
Tyramine	Tyrosine	Peripheral vasoconstriction Increases the cardiac output Causes lacrimation and salvation Increases blood sugar level Releases noradrenaline from the sympathetic nervous system Causes migraine
Putrescine and cadaverine	Ornithine and lysine	Hypotension Bradycardia Lockjaw Paresis of the extremities Potentiate the toxicity of other amines
β – Phenylethylamine	Phenylalanine	Releases noradrenaline from the sympathetic nervous system Increases the blood pressure Causes migraine
Tryptamine	Tryptophane	Increases the blood pressure

2.4. Natural Antioxidant

Since ancient times the crude herbal extracts of aromatic plants have been used for different purposes, such as food, drugs and cosmetics. The essential oils are considered among the most important agents present in these plants and may also have antioxidant and antiinflammatory activities. Volatile oils are a complex mixture of compounds, mainly monoterpenes, sesquiterpenes and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides). Other volatile compounds include phenylpropenes and specific sulphur- or nitrogen-containing substances. Generally, the oil composition is a balance of various compounds, although in many species one constituent may prevail over all others (Delamare *et al.*, 2005).

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of food. Spices used in different types of food to improve flavours, since ancient times, are well known for their antioxidant properties (Lu and Foo, 2001).

Natural antioxidants can be found in almost every vegetable oil, mostly as tocopherols. Ascorbic acid , iso-ascorbic acid and their esters derivatives, such as ascorbyl palmitate, are used in many food as antioxidants. A great number of spices are known for their excellent antioxidant properties, e.g., rosemary and sage. Rosemary (*Rosemarinus officinals L.*) and sage (*Salvia officinals L.*) leaves are commonly used as spices and flavouring agents (Wei and Ho, 2006).

Sausage acceptance by the consumer is often based on color, texture and flavor. The flavor results from the meat and spice combinations and the processing conditions. Spices and herbs are generally used in sausage for enhancing the flavour and/or colour attributes. Recently, antimicrobial and antioxidant activities of this natural material have been discovered (Bozkurt, 2006). Moreover, natural antioxidants have been reported as being more powerful and more potent than synthetic antioxidants, especially; rosemary, sage and green tea extracts. However, common antioxidants and antimicrobials used in sausage, as in most foodstuffs, are synthetic. The most common synthetic antioxidants used in food industry are butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), tert-butylhdyroquinone (TBHQ) and prophyl gallate (PG). Because of the possible toxicities of the synthetic antioxidants and antimicrobials are prohibited in several countries. Nowadays, natural antioxidants are consumed to decrease, cancer and tumours and also diabetes (Bozkurt, 2006).

Antioxidants can act by the following mechanisms in lipid peroxidation (Dormen *et al.*,2003).

i) decreasing localized oxygen concentrations
ii) preventing chain initiation by scavening initiating radicals
iii) binding catalysts, such as metal ions to prevent initiating radical generation
iv) decomposing peroxides so they can not be reconverted to initiating radicals
v) chain-breaking to prevent continued hydrogen abstraction by active radicals.

2.4.1. Rosemary (*Rosmarinus officinalis L.*)

Rosemary (*Rosmarinus officinalis L.*) is popular herbs belonging to the Lamiaceae family with a verified potent antioxidant activity. Rosemary has been reported to contain certain compounds including, rosmanol, rosmariquinone, rosmaridiphenol and carnosol, which may be up to four times as effective as buthylated hydroxyanisole (BHA) and equal to buthylated hydroxytoluene (BHT) as antioxidants (Lopez *et al.*, 2005). Rosemary extracts have been found to be effective in meat systems. Yu *et al.* (2002) reported that in addition to inhibition of lipid oxidation; rosemary extracts improved the color stability of turkey rolls.

2.4.2. *Urtica dioica L.*

Urtica dioica L. has become a very important plant, with beneficial effects on human health all over the world. The seeds and leaves of Urtica dioica L. contain vitamins, minerals and amino acids. The leaves of the plant have 14.4 mg/100 g α-tocopherol, 0.23 mg/100 g riboflavin, 13 mg/100 g iron, 0.95 mg/100 g zinc, 873 mg/100 g calcium, 75 mg/100 g phosphorus, and 532 mg/100 g potassium (Aksu and Kaya, 2004).

Various researchers have stated that the use of *Urtica dioica L.* (seed and leaves) with or without other plants have useful effects on curing some illnesses such as diabetes, eczema, hemorrhoid, liver inflammation, anemia, rheumatism, and prostate cancer. The use of *Urtica dioica L.* in sucuk manufacturing as a new meat ingredient might improve human health and nutrition (Uzun *et al.*, 2004).

2.4.3. *Hibiscus sabdariffa L.* flowers (Roselle)

Hibiscus sabdariffa L. (Roselle) an annual shrub, is commonly used to make jellies, jams and beverages. Recently, it has gained importance as a soft drink material in local regions. However, little information is available about the pharmacological and biological effects of *Hibiscus sabdariffa*, even though the polysaccharide constituents have been asseyed for their possible immuno modulating effects (Müler and Franz, 1992).

The brilliant red color and unique flavor make it a valuable food product. The anthocyanin pigments in *Hibiscus sabdariffa*, that create the color are responsible for the wide range of coloring in many food. Recently, the biological activities of anthocyanin, such as antioxidant activity, protection from atherosclerosis and anticarcinogenic activity have been investigated and shown to have some beneficial effects in the treatment of diseases. There have been reports on the antioxidant activity of anthocyanin (Igarashi *et al.,* 1989; Tamura and Yamagami, 1994) and the biological effect of anthocyanin in low density lipoprotein and lecithin-liposome systems (Meyer *et al.,* 1997). Anthocyanins were also found to have many times more activity than common antioxidants such as ascorbate (Wang *et al.,* 1997). There is now increasing evidence that antioxidants in the human diet are of major benefit for health and well-being. Being high in anthocyanin, Roselle petal is both a good colorant and potentially a good source of antioxidants. This activity is relatively stable over a storage period, although provided by different compounds (Tsai *et al.*, 2002).

The dried flowers of *Hibiscus sabdarrifa* have been used effectively in folk medicines against hypertension, pyrexia, liver disorders and also to prevent cardiovascular and hepatic disease (Prenesti *et al*., 2005).

2.4.4. Salvia officinalis (Sage)

Salvia officinalis (sage) is a common aromatic and medicinal plant native from mediterranean countries that is in widespread use. Sage, a popular herb in the mint family (Labiatae), has been a subject of intensive study in the past decades for its antioxidative components. Sage has been used in folk medicines for the treatment of all kinds of ailments, but to most people it is better known as an additive used in the preparation of different types of food (Tepe *et al*, 2006). Several studies have shown sage to be one of the sources of some potent antioxidants (Chipault *et al.*, 1952; Cuvelier *et al.*, 1996). The antioxidant properties were found to be related the presence of rosmarinic acid and carnosic acid (Chang *et al.*, 1977; Cuvelier *et al.*, 1994). More recent studies on sage have revealed the presence of a large number of diterpenoids (Gonzalez *et al.*, 1992; Tang and Eisenbrand, 1992) and phenolic acids

(Tanaka *et al.*, 1996, 1997; Li, 1998; Tezuka *et al.*, 1998) including a number of novel caffeic acid metabolites such as sagerinic acid (Lu and Foo, 1999) and sagecoumarin (Lu *et al.*, 1999), but comparably few flavonoids and phenolic glycosides (Ulubelen and Miski, 1981; Gökdil *et al.*, 1997; Wang *et al.*, 1998, 1999).

Major phenolic compounds of sage are rosmarinic acid, carnosic acid, salvianolic acid and its derivatives carnosol, rosmanol, epirosmanol, rosmadial and methyl carnosate. These compounds may contribute to the health property of sage used as a popular folk medicine for the treatment of various ailments (Keller, 1978). There is increasing evidence to suggest that many degenerative diseases, such as brain dysfunction, cancer, heart diseases and immune system decline, could be the result of cellular damage caused by free radicals, and antioxidants present in human diet may play an important role in disease prevention (Aruoma, 1998; Nees and Powles, 1997; Steinmetz and Potter, 1996). Carnosol and carnosic acid, which have orthodihydroxyl groups on the aromatic ring, possess good peroxyl and hydroxyl radicals scavening activities. They inhibit the formation of hydroxyl radicals and chelate metals while only carnosic acid appears to scavenge H_2O_2.

CHAPTER III

MATERIALS AND METHODS

3.1. Materials

Potassium nitrite and potassium nitrate were obtained from Merck (Darmstadt, Germany); histamine dihydrochloride, putrescine dihydrochloride and tyramine hydrochloride were obtained from Sigma (St. Louis, MO), were used as biogenic amine standards; sodium hydroxide, sodium bicarbonate (Merck, Darmstadt, Germany), ammonia (25 %) and acetone (Reidel De Haen, Germany), dansyl chloride (Sigma Co, St. Louis, MO), ammonium acetate (Merck, Darmstadt, Germany) and perchloric acid (JT Baker, Holland) , 2-thiobarbituric acid (Sigma Co, St.Louis, MO), glacial acetic acid (Merck, Darmstadt, Germany) were used in analysis. All chemicals except acetonitrile were of analytical grade (extra pure), and HPLC grade acetonitrile was used.

Starter culture mixture of *Pediococcus pentosaceus, Lactobacillus plantarum, Staphylococcus carnosus,* and *Staphylococcus xylosus* was obtained from Biocarna (Wiesby, Germany).

Meat (lamb), tail fat, salt, sugar, commercial virgin olive oil was obtained from a local supermarket in Gaziantep. Spices; *Salvia officinalis L., Urtica diocia, Rosmarinus officinalis L.* and *Hibiscus sabdariffa* flowers were obtained from a local market in Mersin.

3.2. Sample Preparation

3.2.1. Extraction of *Salvia officinalis L., Urtica dioica, Rosmarinus officinalis L.* and *Hibiscus sabdariffa*

Aqueous extracts were prepared from *Salvia officinalis, Urtica dioica, Rosmarinus officinalis* and *Hibiscus sabdariffa* by maceration. Then extracts were filtered with filter paper. Each extract brix value was measured by Index Instrument PTR 46X Refractometer (Index Instrument LTD, Cambridgeshire, England).

3.2.2. Sucuk Preparation

Sucuk dough was prepared from meat, tail fat, salt, sugar, garlic, spices, olive oil and starter culture. 420 g meat was minced within a meat grinder (Tefal Prep'Line 1600, France) and then 2.75 g black pepper, 5.5 g cumin, 0.55 g cinnamon, 5.71 g allspice, 0.20 g clove, 10.38 g garlic, 5.5 g red pepper, 2.2 g sugar, 9 g salt and 1.05 g olive oil were added and mixed with minced meat about 15 min. While the mixing 0.084 g starter culture mixture was added. Sausage dough was rested for 12 h at 0-4 ^{0}C, and 93 g refrigerated minced tail fat was mixed in meat grinder. Then nitrite, nitrate, BHT, *Salvia officinalis, Urtica Dioica, Rosmarinus officinalis* and *Hibiscus sabdariffa* were added into sausage dough according to the following table.

Table 3.1. Sucuk samples prepared with natural antioxidants

Additives	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
Control	+	-	-	-	-	-	-	-	-	-	-
Nitrite/Nitrate (150/300 ppm)	-	+	-	-	-	-	-	-	-	-	-
BHT (300 ppm)	-	-	+	-	-	-	-	-	-	-	-
Urtica dioica extract (300 ppm)	-	-	-	+	-	-	-	-	-	-	-
Urtica dioica extract (600 ppm)	-	-	-	-	+	-	-	-	-	-	-
Rosmarinus officinalis extract (300 ppm)	-	-	-	-	-	+	-	-	-	-	-
Rosmarinus officinalis extract (600 ppm)	-	-	-	-	-	-	+	-	-	-	-
Hibiscus sabdariffa extract (300 ppm)	-	-	-	-	-	-	-	+	-	-	-
Hibiscus sabdariffa extract (600 ppm)	-	-	-	-	-	-	-	-	+	-	-
Salvia officinalis extract (300 ppm)	-	-	-	-	-	-	-	-	-	+	-
Salvia officinalis extract (600 ppm)	-	-	-	-	-	-	-	-	-	-	+

After that each dough was filled into artificial collagen casings with filling machine (Tefal, Prep'Line 1600, France). Eleven sucuks were prepared as 50 g .

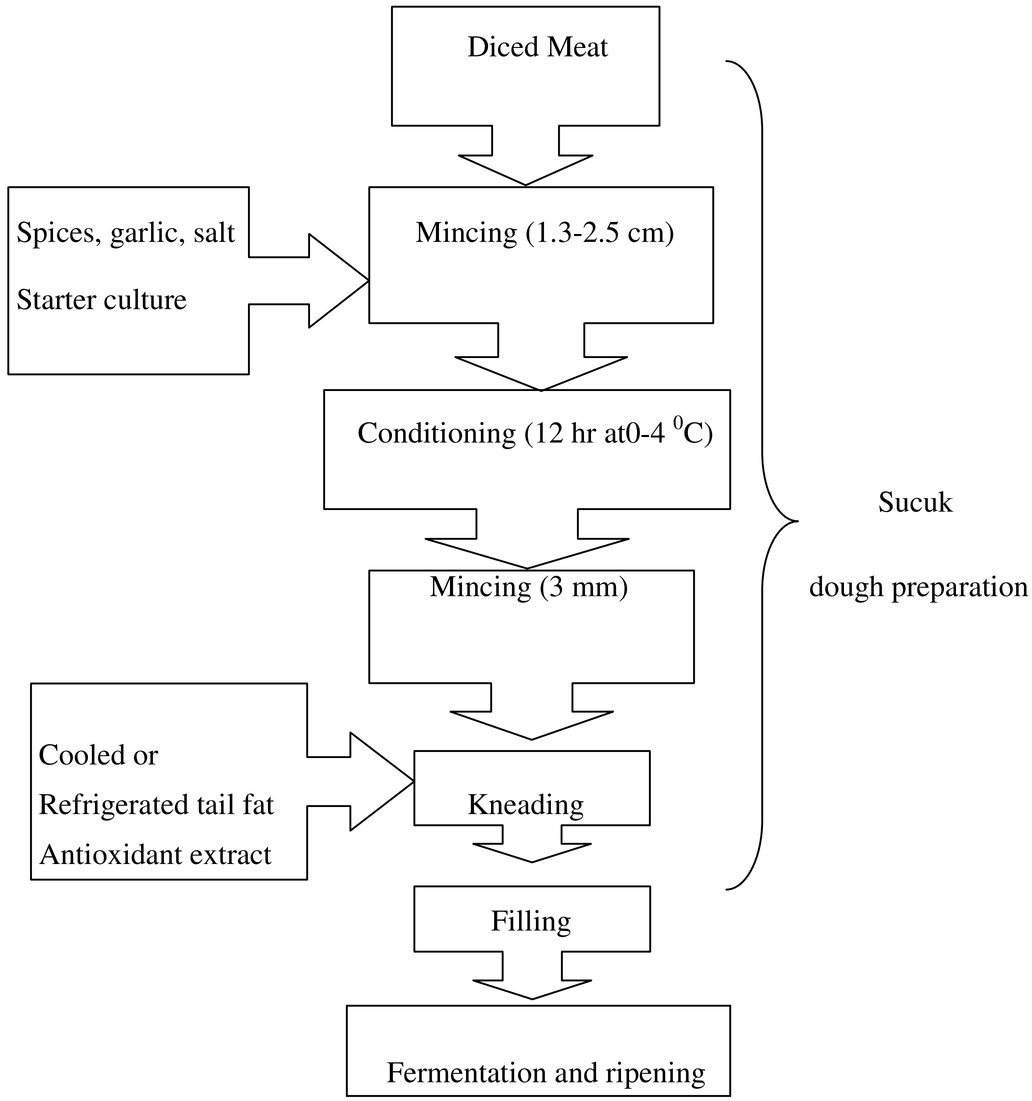


Figure 3.1 Production flow-chart of sucuk (Bozkurt and Erkmen, 2002a, b)

The sausages were kept in ripening cabinets under the conditions of controlled temperature and relative humidity as below:

Time (days)	Temperature (ºC)	Relative Humidity (%)
0-2	24	90
2-4	22	85
4-6	20	80
6-8	18	75
8-10	18	70
10-13	18	65
13-15	18	60

During the ripening period chemical and sensory analyses were determined.

3.3. Sampling and Sample Preparation

Each 50 g of unit sucuk types was removed to be analyzed at sampling time. Sucuks were cut into small pieces and stored at –20°C for chemical analysis.

3.4. Chemical Analysis

3.4.1. Determination of pH

Ten g of ground sucuk was homogenized in 90 ml distilled water and pH value of sucuk was determined using a pH meter (Jenway 3010; Jenway LTD, Essex, UK) equipped with an electrode (J95, 924001, Jenway LTD, Essex, UK) (AOAC, 1990).

3.4.2. Determination of 2-Thiobarbituric Acid Reactive Substances (TBARS) Value

Formation of malonaldeyhde was followed by TBARS value. It was determined by Spectrophotometric method at 538 nm by using TBA reagent as described by Bozkurt (2006).

A 2 g sample was homogenized and TBARS was extracted with 10 ml of 0.4 M perchloric acid twice. Extracts were collected in a test tube and diluted to 25 ml with 0.4 M perchloric acid. Then extracted solution was centrifuged for 5 min. at 1790xg. 1 ml of extract was taken into test tube and 5 ml of TBA reagent was added and stopped with the stopper. And it was heated in a boiling water bath for 35 min. At the end of this time solution was cooled and the absorbance of sample was measured at 538 nm against blank with spectrophotometry.

3.4.3. Determination of Biogenic Amine

Biogenic amines were analysed using HPLC (Shimadzu Solvent Delivery Module, LC-10Advp, Kyoto, Japan) equipped with a Hewlett Packard UV detector in the wavelength 254 nm.

Mobile Phase: Two solvents were used as mobile phase. These are ; Solvent A: acetonitrile and Solvent B: 0.1 M amonium acetate which was prepared by dissolving 3.8 g of amonıum asetate in a 500 ml of distilled water. They were filtered through a 0.45 µm Millipore filter (Bedford, USA).

Sample preparation and extraction: Two g of sample was homogenized in 10 ml of 0.4 M perchloric acid using a Waring blender. The sample was centrifuged for 10 min at 1790 x g and filtered. The extraction was repeated with in a further 10 ml of 0.4 M perchloric acid solution and the supernatants were combined and made up to 25 ml with 0.4 M perchloric acid.

Derivatization of sample extracts and mixed standards: One ml of extract was pipetted into glass stoppered test tube and 200 µl of 2 N NaOH and 300 µl of saturated sodium bicarbonate solutions were added. Two ml of dansyl chloride (10 mg/ml) solution was added to each sample and incubated for 45 min at 40°C. Residual dansyl chloride was removed by adding 100 µl of 25 % ammonia. After 30 min, the solution was adjusted to 5 ml acetonitrile, centrifuged for 5 min at 1790xg, the supernatant filtered (0.45 µm) and 20 µl then injected onto the HPLC.

Chromatographic separation: The gradient profile was 0 - 20 min , 50 % solvent A and 50 % solvent B , 20 – 45 min , 90 % solvent A and 10 % solvent B. Analyses were done at a flow rate of 1 ml/min.

Standardization and Calibration curve: The standard solutions (0, 12.5, 25, 50, 100, 200, 500 mg/kg) were taken for each biogenic amine and diluted to 2 ml with 0.4 M perchloric acid. The same procedure was applied as it was in the determination of biogenic amine of sucuk.

3.4.4. Determination of Color

Color measurements ((L=0 white, L=100 black, +a=red, -a=green and +b=yellow, -b=blue) were made by using a HunterLab ColorFlex (A60-1010-615 Model Colorimeter, Hunter Lab, Reston, VA). The instrument was standardized each time with a white and black ceramic plate (L_0 = 93.01, a_0 = -1.11, and b_0 = 1.30). The Hunter L, a, and b- values correspond to lightness, greenness (-a) or redness (+a), and blueness (-b) or yellowness (+b), respectively. The color measurements were performed on sucuks (0.5 cm thickness of cutted surface) at room temperature (20±2°C) in triplicate.

3.5. Sensory Analysis

Sensory attributes (flavour, color and ease of cutting) of 25 grams of sucuk samples were evaluated during the ripening period, twice for each sample, by a panel of trained panellists. Panellist gave scores for each of sample with respect to their perceptions of flavour and color as 1 (worst) to 10 (best). Cutting score was evaluated by panellists by assessing whether the sucuk was easily cut or stuck to the knife, on a scale of 10 (best) to 1 (worst). The overall sensory quality scores of sucuks was determined from Eq. (1) (Bozkurt and Erkmen, 2004a) as:

$$\textit{Overall Sensory Quality} = (\textit{flavour} \times 0.50) + (\textit{color} \times 0.25) + (\textit{cutting} \times 0.25) \quad (1)$$

3.6. Determination of Quality Attributes of Retailed Sucuks

Nineteen different types of sucuks were collected from local markets in February 2006. All the analyses were also applied to retailed sucuks.

3.7. Statistical Analysis

Statistical analysis was performed by using the SPSS 11.0 (SPSS Inc, USA) software for Windows. The one-way analysis of variance (ANOVA test) was performed for pH, TBARS value, putrescine, histamine and tyramine content, Hunter L, a, and b- values and overall sensory quality scores to determine any significant differences within the 95% confidence interval. If there was significant difference among the factors of recipe and time Duncans multiple range tests were carried out for determination of the differences between these factors, also for finding the highest and lowest of these factors during the ripening period.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. pH Value

The changes of pH values are given in Figures 4.1 - 4.3. Statistical analysis was applied in order to find differences between recipe and time. Their results are shown in Table 4.1.

Statistical analysis indicate that the pH values of sucuk were affected ($P<0.05$) from the ripening time but not ($P>0.05$) from the addition of antioxidants. During the first 2 days of ripening all sucuks pH value decreased ($P<0.05$) rapidly from 5.78 to about 4.52 which could be due to production of organic acids by microorganisms. There was an initial acidification, basically attributable to the production of lactic acid bacteria that use sugar as substrate (Lücke, 1994). The decline in the pH value during the first days of ripening, when fermentation occurs, is very important due to the inhibition of undesired bacteria, rate of conversion of color, and formation of desired flavour in dry-fermented sausages. After this period, pH values of all sucuks did not change ($P<0.05$) during the ripening period. The highest ($P<0.05$) pH value 5.04 was observed in *Salvia officinalis* (600 ppm) extract added recipe at the end of the ripening while the lowest pH value in control recipe about 4.89. Öztan (1999) reported that at the end of the ripening period pH is usually in the range of 5.6-6.2. These results were in agreement with literature that pH values of sucuk decreased sharply during the first 3 days of ripening due to the production of acid and then its value increased during the further ripening period (Gökalp, 1986; Vural, 1998; Bozkurt and Erkmen, 2004b; Kayaardı and Gök, 2003).

The similar trend in pH values of all recipes was observed during the ripening period. This observation was correlated with statistical analysis (both one-way and two-way anova) that the pH of recipes were not significantly ($P>0.05$) affected by the addition of nitrite/nitrate, BHT, *Urtica dioica, Rosmarinus officinalis, Hibiscus sabdariffa and Salvia officinalis*. There were small differences between them, but not significant ($P<0.05$).

The Turkish Food Codex (Anonymous, 2000) states that high quality ripened sucuks should have pH values between 5.2 - 5.4. According to the Turkish Standard Institute (Anonymous, TS 1070, 2002) pH for high quality sucuk should be in the range of 4.7 – 5.4. The sucuks prepared without additive (control; no antioxidants) and with natural antioxidants, nitrite/nitrate, and BHT were found to be close to range 4.89-5.04. So, it can be concluded that pH results were in the range of the standard value.

The results of pH values of retailed nineteen sucuk samples taken from local market in Gaziantep region are shown in Table 4.2. Their values were found to be in the range of 4.89 – 6.89. According to statistical analysis of retailed sucuks there was difference (P<0.05) on pH values between them.

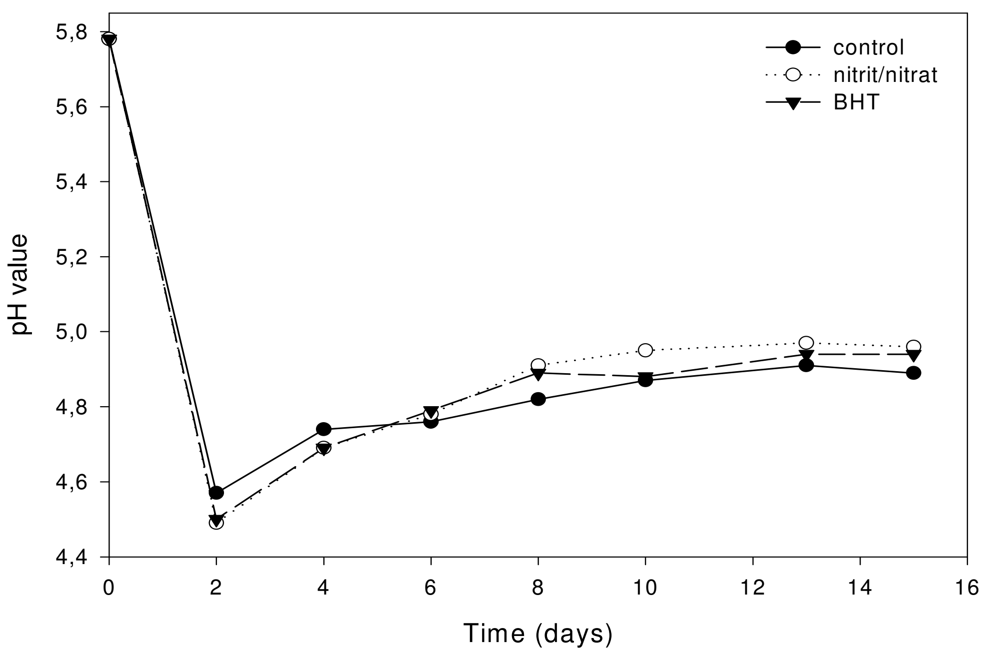


Figure 4.1. Changing the pH values of sucuks prepared with non, nitrite/nitrate, and BHT

Table 4.1. Statistical results for changes of pH values of sucuk recipes during the ripening period

Time (days)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	5.78aA	5.78aA	5.78aA	5.78aA	5.78aA	5.78aA	5.78aA	5.78aA	5.78aA	5.78aA	5.78aA
2	4.57bA	4.49baA	4.50ba	4.52bA	4.52bA	4.53bA	4.52bA	4.52bA	4.47bA	4.52bA	4.58bA
4	4.74bA	4.69bA	4.69bA	4.67bA	4.70bA	4.68bA	4.67bA	4.67bA	4.66bA	4.68bA	4.71bA
6	4.76bA	4.78bA	4.79bA	4.79bA	4.79bA	4.80bA	4.77bA	4.78bA	4.73bA	4.78bA	4.78bA
8	4.82bA	4.91abA	4.89bA	4.88bA	4.93abA	4.91abA	4.86bA	4.87bA	4.83bA	4.88bA	4.90bA
10	4.87bA	4.95abA	4.88bA	4.92abA	4.96abA	4.92abA	4.92abA	4.90bA	4.90bA	4.95abA	4.96abA
13	4.91bA	4.97abA	4.94bA	4.98abA	4.95abA	4.97abA	4.99abA	4.97abA	4.93bA	4.98abA	5.00abA
15	4.89bA	4.96abA	4.94bA	5.00abA	4.99abA	4.99abA	5.00abA	4.99abA	4.93bA	5.04abA	5.02abA

S1: Control, no antioxidant added recipe; S2: Nitrite/Nitrate (150/300 ppm) added recipe; S3: 300 ppm BHTadded recipe; S4: 300 mg soluble solid/kg of *Urtica dioica* extract added recipe; S5: 600 mg soluble solid/kg of *Urtica dioica* extract added recipe; S6: 300 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S7: 600 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S8: 300 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S9: 600 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe and S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe.

Different small letters indicate statistical difference at α=0.05 level in each column.

Different capital letters indicate statistical difference at α=0.05 level among products at each time.

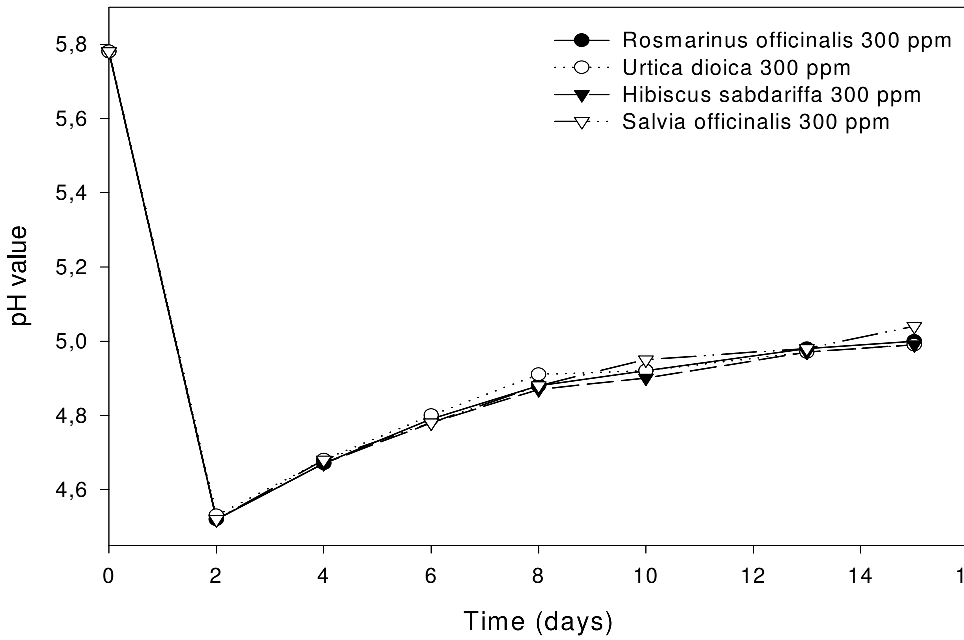


Figure 4.2. Changing the pH values of sucuks prepared with 300 ppm natural antioxidants extracts

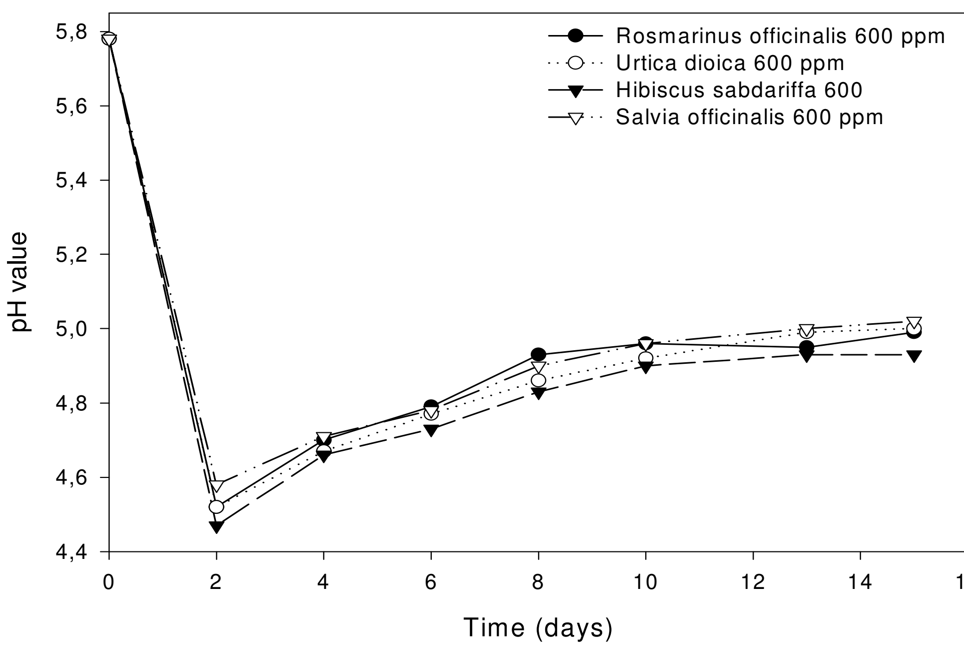


Figure 4.3. Changing the pH values of sucuks prepared with 600 ppm natural antioxidants extracts

About eight retailed sucuks (about 42 %) had higher pH value than 5.4 which is maximum level declared in Turkish Food Codex (Anonymous, 2000) while pH value of five retailed sucuks (26%) were lower than 5.2 that is the minimum level Turkish Food Codex (Anonymous, 2000). Only six sucuks (32%) were found to be in the range of Codex limits. Comparing retailed sucuks with prepared sucuks in this study; all of the prepared sucuks were in the range of allowable limits however, 68 % of retailed sucuks were not in the allowable range.

Table 4.2. pH values of retailed sucuks

Sample	pH value	Sample	pH value
R1	5.18	R11	5.13
R2	5.25	R12	5.21
R3	6.89	R13	5.05
R4	6.90	R14	5.35
R5	4.89	R15	5.35
R6	5.00	R16	6.20
R7	5.43	R17	5.80
R8	5.45	R18	5.35
R9	6.42	R19	5.36
R10	6.34		

4.2. Thiobarbituric Acid Reactive Substance (TBARS) Value

The results of deterioration of lipids (TBARS value) for sucuks are shown in Figure 4.4 - 4.6. Statistical analysis was carried out in order to find differences between recipe and time and their results are shown in Table 4.3.

Statistical analysis indicate that TBARS values were affected significantly ($P<0.05$) from ripening time and the addition of antioxidants (recipe). TBARS values increased from 0.52 to about 0.72 mg/kg significantly ($P<0.05$) during the first 4 days except *Urtica dioica* (300 ppm), *Rosmarinus officinalis* (300 ppm) and *Hibiscus sabdariffa* added recipes (Table 4.3). Lopez *et al.* (2005) reported that rosmanol, rosmariquinone, carnosol and rosmaridiphenol were responsible antioxidant effect of *Rosmarinus officinalis*. At the end of the ripening period, TBARS values were ranged between 0.35 (*Salvia officianalis*) and 0.81 mg/kg (in control). Wu *et al*. (1991) reported that if TBARS value is higher than 1 mg/kg generally off-odors formed and it is considered as the begining of lipid oxidation. TBARS values of sucuk were lower than this limit so it can be concluded that off-odour formation were not observed in the sucuks.

Statistical anaysis indicate that TBARS values of sucuk were affected ($P<0.05$) from the addition of natural antioxidants. Sucuks containing natural antioxidant extract had significantly lower ($P<0.05$) TBARS value when compared to the control recipe. It was observed that the natural antioxidants extracts appeared to be more effective ($P<0.05$) than BHT and nitrite/nitrate (Table 4.3). The addition of *Hibiscus sabdariffa* was less effective ($P<0.05$) on the reduction of TBARS value than the other antioxidants. Addition of *Salvia officinalis* (300 and 600 ppm) extracts reduced ($P<0.05$) TBARS values more than that of the other synthetic and natural antioxidants. Thus, it can be used as an alternative to synthetic antioxidants nitrite/nitrate and BHT for extending the shelf life of sucuks with benefits to human health. The practical significance of these finding is that the natural extracts were capable of maintaining TBARS values below 0.45 mg/kg throughout the 15 day study, whereas the TBARS values of control and nitrite/nitrate treated sausage exceeded 0.60 mg/kg. It is well known that phenolic compounds in herbs and spices, BHT, α-tocopherol, nitrite and nitrate have antioxidative properties (Faustman *et al.,* 1989; Ordonez *et al.*, 1999; Ockerman and Sun, 2002) and the presence of these compounds may retard ($P<0.05$) the lipid oxidation during the ripening periods of sucuks. The addition of antioxidants might have reduced lipid deterioration (TBA) through the inhibition of some lipid derived volatiles generation. A lot of studies (Chipault *et al.*, 1952; Cuvelier *et al.*, 1996; Gonzalez *et al.*, 1992; Tang and Eisenbrand, 1992; Lu and Foo, 2000) reported that the antioxidant effect of *Salvia officinalis* was due to the presence of rosmarinic acid and carnosic acid.

Changing the concentration of natural antioxidants did not change ($P>0.05$) the TBARS values (from two-way anova). Although the results of one-way anova (Table 4.3) indicates opposite results, when the overall data was considered (two-way anova) this result was observed.

Table 4.3. Statistical results for changes of TBARS values of sucuk recipes during the ripening period

Time (days)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0.52aA	0.52abA	0.52abA	0.52aA	0.52aA	0.52aA	0.52aA	0.52aA	0.52aA	0.52abA	0.52aA
2	0.55aA	0.44acBC	0.41cdB	0.57abA	0.50abABC	0.71bD	0.60bA	0.91bE	0.85bE	0.56bA	0.53aAC
4	0.82bAB	0.53bC	0.57bC	0.61bCD	0.63cCD	0.71bAD	0.73cAD	0.74cAD	0.92bB	0.73cAD	0.87bB
6	0.58aA	0.48abB	0.45cB	0.43cB	0.33deC	0.43cB	0.33dC	0.33dC	0.45abB	0.31dC	0.30cdC
8	0.58aA	0.35dBC	0.48acD	0.33dBC	0.38dfCE	0.35cdBC	0.38deCE	0.49aD	0.31dB	0.42eDE	0.23cF
10	0.51aA	0.32dBC	0.33dBC	0.38cdC	0.30eBD	0.28dBD	0.33dBC	0.30dBD	0.38cdC	0.30dBD	0.26cD
13	0.56aA	0.38cdBC	0.35dBCD	0.33dCD	0.42bfB	0.35cdBCD	0.40deB	0.38dBC	0.33dCD	0.30dD	0.37dBC
15	0.81bA	0.45acBC	0.50abC	0.38cdBD	0.49abC	0.43cBCD	0.42eBD	0.51aC	0.62eE	0.46aeBC	0.35dD

S1: Control, no antioxidant added recipe; S2: Nitrite/Nitrate (150/300 ppm) added recipe; S3: 300 ppm BHTadded recipe; S4: 300 mg soluble solid/kg of *Urtica dioica* extract added recipe; S5: 600 mg soluble solid/kg of *Urtica dioica* extract added recipe; S6: 300 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S7: 600 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S8: 300 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S9: 600 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe and S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe.

Different small letters indicate statistical difference at α=0.05 level in each column.

Different capital letters indicate statistical difference at α=0.05 level among products at each time.

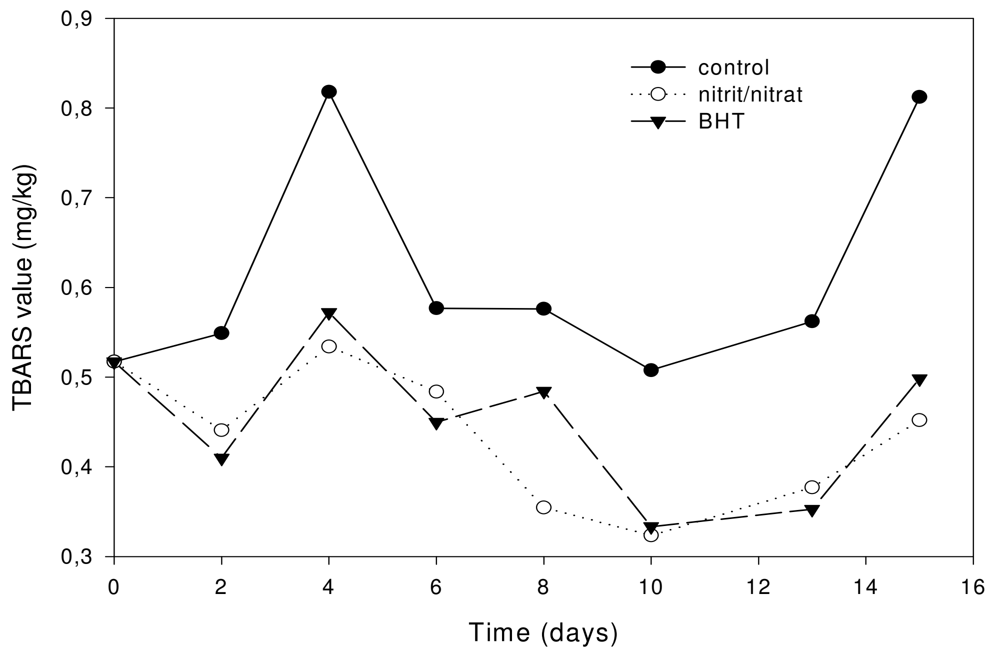


Figure 4.4. Changing the TBARS values of sucuks prepared with non, nitrite/nitrate, and BHT

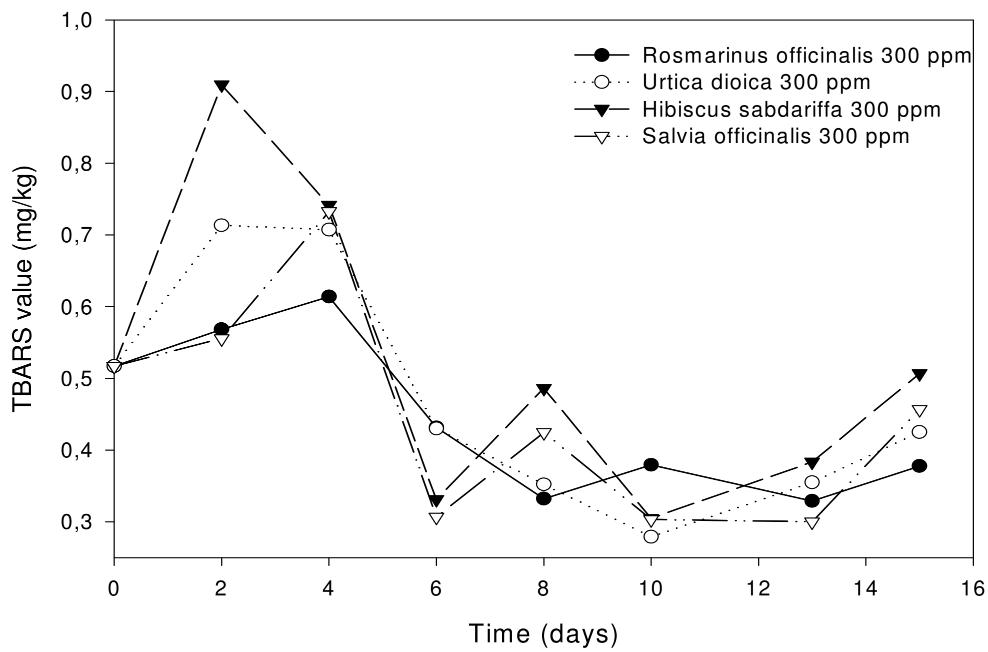


Figure 4.5. Changing the TBARS values of sucuks prepared with 300 ppm natural antioxidants extracts

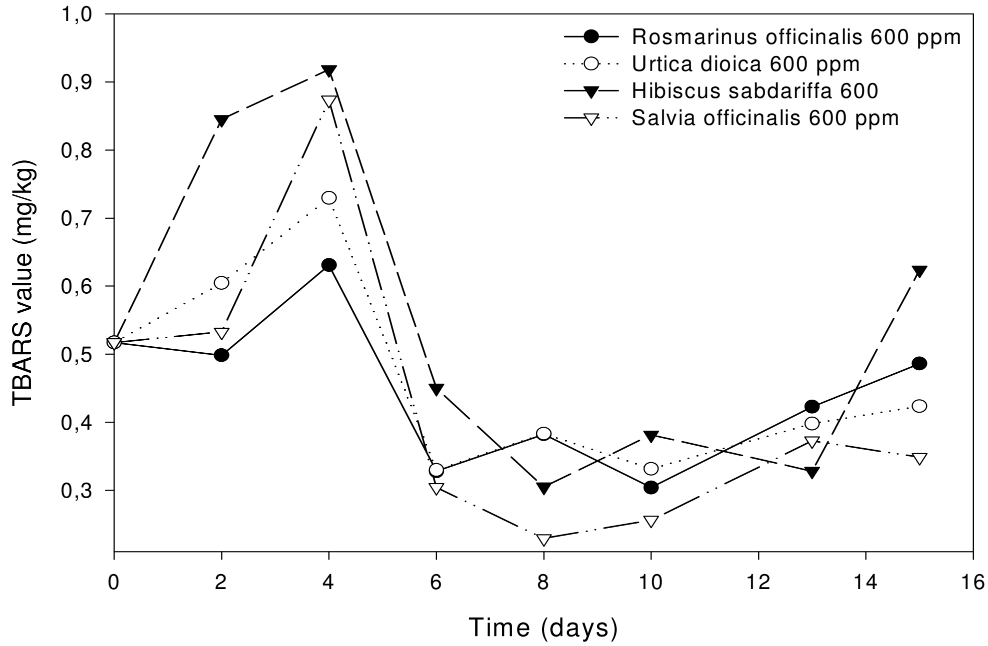


Figure 4.6. Changing the TBARS values of sucuks prepared with 600 ppm natural antioxidants extracts

The results of TBARS analyses of retailed sucuks are shown in Table 4.4. The TBARS values of retailed sucuks were found to be in the range of 0.155-0.459. As a result of statistical analysis of retailed sucuks, TBARS values were significantly ($P<0.05$) different. The average of TBARS values was 0.295. Sucuks prepared in this study had higher TBARS values than this value. This could be due to the differences in production conditions (such as high ripening temperature) and recipe (retailed sucuk may have contained high amount of synthetic antioxidants). Wu *et al*. (1991) reported that if TBARS value is higher than 1 mg/kg generally off-odors formed and it is considered as the begining of lipid oxidation. From that it can be concluded that off-odour formation were not observed in the retailed sucuks. Bozkurt and Erkmen (2005) reported that TBARS values of traditional and firm-made retailed sucuks which were collected from Gaziantep region were in the range of 0.44 – 2.87 mg/kg and 0.03 – 2.35 mg/kg, respectively. Ertaş (1982) found that the TBARS values of sucuks consumed in Ankara varied between 0.05 and 0.91 mg/kg.

Table 4.4. TBARS values of retailed sucuk

Sample	TBA value	Sample	TBA value
R1	0.376	R11	0.254
R2	0.376	R12	0.496
R3	0.485	R13	0.347
R4	0.481	R14	0.152
R5	0.148	R15	0.250
R6	0.179	R16	0.309
R7	0.177	R17	0.277
R8	0.125	R18	0.403
R9	0.200	R19	0.428
R10	0.152		

4.3. Biogenic Amines Formation

Biogenic amines are toxic substances. The intake of food with high concentration of biogenic amines can cause migraine, headaches, gastric and intestinal problems, and pseudo-allergic poisoning by the toxic action of histamine and tyramine (Ruiz et all, 2004). Therefore, they should be controlled during the ripening periods.

Amino acid decarboxylation is the most common mode of synthesis of amines in food and the aromatic amines may render a food toxic when these amines formed by the action of living organisms, they are destinated biogenic. (Shalaby, 1996)

Biogenic amines have been used as quality indexes and indicators of unwanted microbial activity in meat and meat products.

4.3.1. Changes of Histamine concentration

Changes of histamine concentration are given in Figures 4.7 – 4.9. Statistical analysis was applied to find statistical differences between recipe and ripening time. Their results are shown in Table 4.5.

It was observed from the statistical analysis that histamine concentration was affected ($P<0.05$) from ripening time. During the first 2 days of ripening process, histamine concentration increased significantly ($P<0.05$) from 8.80 to 10.79 mg/kg (Table 4.5). After that time, its concentration decreased significiantly ($P<0.05$) until 6^{th} days as 1.81 mg/kg and then again increased up, at the end of ripening time to about 5.36 mg/kg level. Bozkurt (2002) found similar result that histamine concentration in fermentation period increased and then decreased during the further ripening period. Parente *et al.* (2001) reported that histamine intake of 8-40 mg, 40-100 mg and higher than 100 mg may cause slight, intermediate and intensive poisoning, respectively. Shalaby (1996) pointed out that histamine concentration should be lower than in the range of 50-100 mg/kg in sausages. It was found that histamine concentration in sucuk were too lower than this maximum level after the ripening period. So it can be concluded that sucuks prepared in this study were safe with respect to their histamine levels.

Statistical analysis (one and two way anova) indicate that histamine formation was affected significiantly ($P<0.05$) from addition of antioxidant. The highest ($P<0.05$) histamine concentration (10.77 mg/kg at the end of the ripening) was determined in the control recipe. The addition of *Hibiscus sabdariffa* (600 ppm) was less effective ($P<0.05$), *Urtica dioica* (600 ppm) and *Salvia officinalis* (600 ppm) were more ($P<0.05$) effective ($P<0.05$) on decreasing histamine concentration than the other antioxidants. At the end of the ripening period. Their concentration was 3.21 and 3.59 mg/kg, respectively (Table 4.5). The addition of nitrite/nitrate and BHT, however, were not as efficient as the addition of Salvia *officinalis* and *Urtica dioica*. Several studies (Gülçin *et al.*, 2004; Tepe *et al.*, 2006) reported that *Salvia officinalis* contains strong antioxidative substances.

Table 4.5. Statistical results for histamine content of sucuk recipes during the ripening period

Time (days)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	8.80abA	8.80aA	8.80aA	8.80aA	8.80aA	8.80aA	8.80aA	8.80aA	8.80aA	8.80aA	8.80aA
2	13.32cA	10.10bBC	11.08bB	8.96aC	10.28bBC	10.57bBC	9.90bBC	10.72bBC	8.90aC	9.01aC	15.82bD
4	9.79bdA	1.24cB	1.00cBC	3.18bD	1.47cdB	0.53cC	0.50cE	0.99cBC	1.09bB	1.09bB	1.15cB
6	5.43eA	1.00cB	1.00cB	1.08cBC	1.00cB	2.28dD	1.51dE	1.23cBCE	1.55bcE	2.45cdD	1.39cCE
8	4.04eA	1.90cBC	3.38dD	4.70dE	2.00dBC	1.70dBFG	2.09dBC	1.40cFG	2.18cC	1.75bcBCF	1.30cG
10	5.03eA	3.89dBC	3.74deBD	3.79bdBCD	3.27eDE	4.36eC	3.18eDE	2.57dF	3.27dDE	3.24dDE	3.11dEF
13	8.16aA	2.95eB	5.94fC	4.73dD	4.36fD	4.49eD	5.80fC	4.39eD	6.31eC	6.53eC	3.99dD
15	10.77dA	4.21dBC	4.48eBC	4.00bdBD	3.21eD	5.55fDE	5.06fCD	5.77fDE	6.37eE	5.97eDE	3.59dBD

S1: Control, no antioxidant added recipe; S2: Nitrite/Nitrate (150/300 ppm) added recipe; S3: 300 ppm BHTadded recipe; S4: 300 mg soluble solid/kg of *Urtica dioica* extract added recipe; S5: 600 mg soluble solid/kg of *Urtica dioica* extract added recipe; S6: 300 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S7: 600 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S8: 300 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S9: 600 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe and S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe.

Different small letters indicate statistical difference at α=0.05 level in each column.

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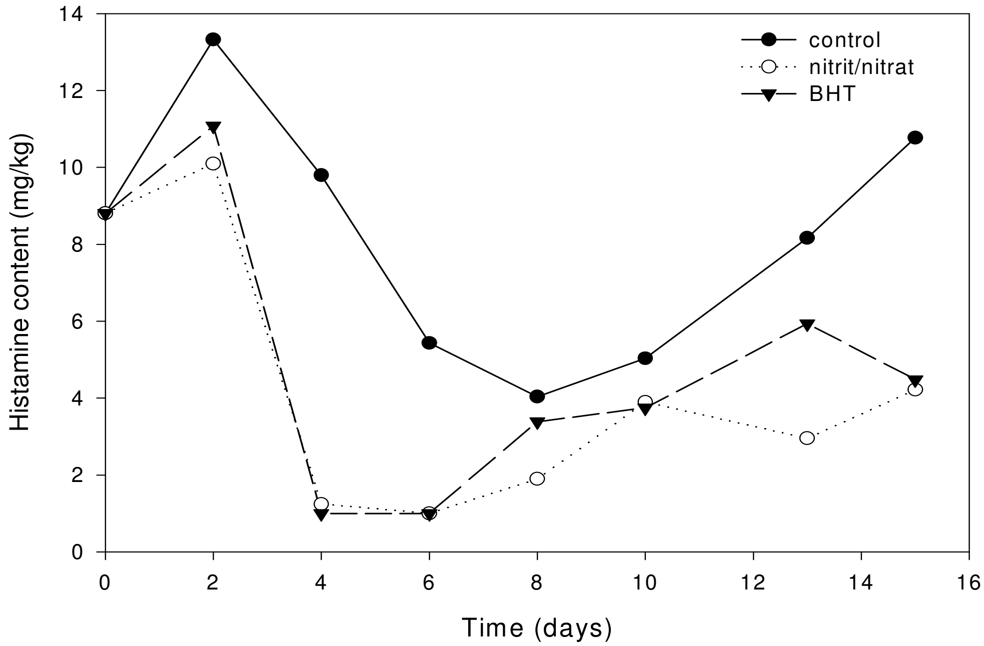

Figure 4.7. Histamine concentration of sucuks prepared with non, nitrite/nitrate, and BHT

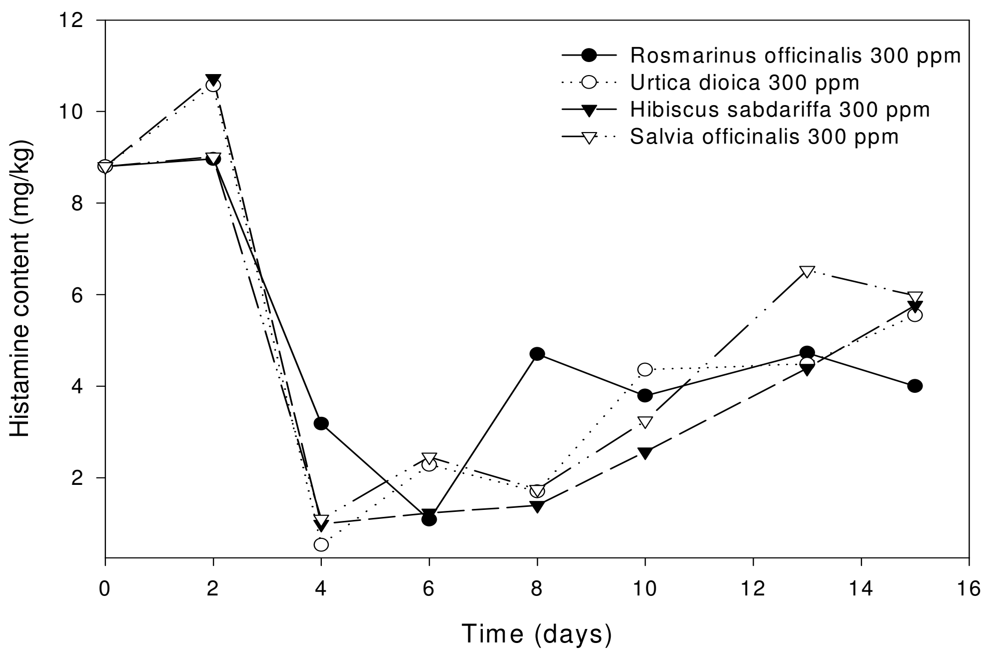

Figure 4.8. Histamine concentration of sucuks prepared with 300 ppm natural antioxidants extracts

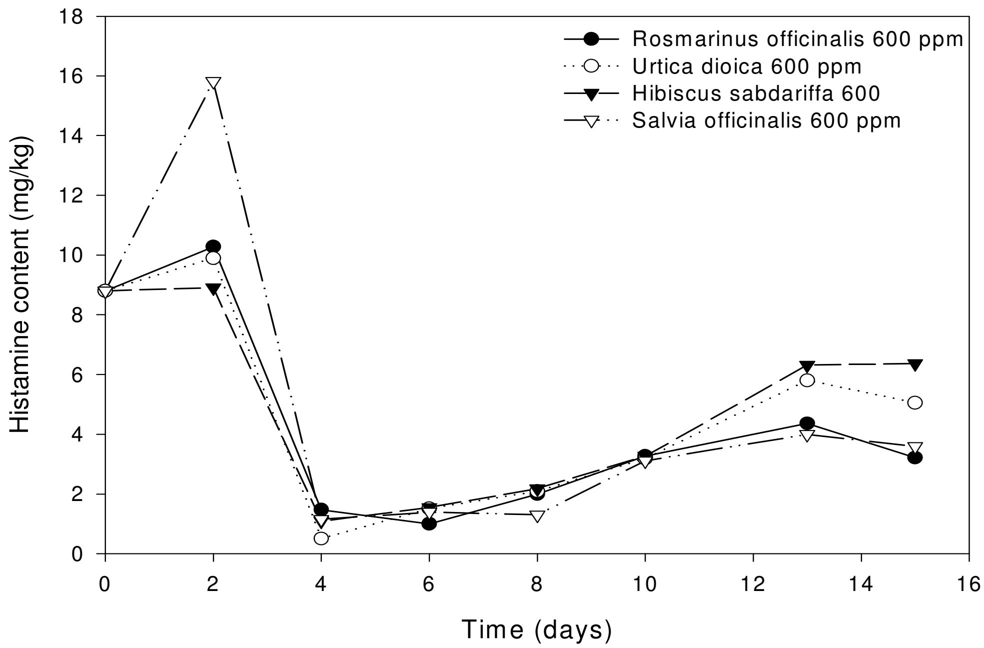

Figure 4.9. Histamine concentration of sucuks prepared with natural antioxidants at 600 ppm

Histamine concentration of retailed sucuks was analyzed and their results are given in Table 4.6. Their concentrations were found to be ranged from 0.682 to 210.993 mg/kg (Table 4.6.) and statistical analysis indicate that histamine concentration of retailed sucuks were different ($P<0.05$) from each other. This difference could be due to the variation of the production conditions and recipe. Shalaby (1996) reported that the tolerance level of histamine was 50 – 100 mg /kg. Only 1 of 19 retailed sucuks histamine concentration was higher than this level. Histamine concentration in sucuks that was collected from local markets in Ankara varied in the range of 6.72 to 362.22 mg/kg (Şenöz *et al.,* 2000). Bozkurt and Erkmen (2005) reported that histamine concentration of firm-made and traditional sucuks sold in Gaziantep was ranged between 2.75 – 8.80 and 3.03 – 8.00 mg/kg, respectively. Biogenic amines are formed by micro flora that's why the prevention of bacterial growth or the inhibition of such amine production would be very important for food safety.

Table 4.6. Histamine concentration of retailed sucuk

Sample	Histamine conc.	Sample	Histamine conc.
R1	0.697	R11	16.913
R2	0.842	R12	9.710
R3	2.867	R13	210.993
R4	2.810	R14	63.059
R5	2.830	R15	35.077
R6	2.274	R16	0.747
R7	6.082	R17	0.682
R8	6.238	R18	7.311
R9	12.275	R19	8.348
R10	16.780		

4.3.2. Changes of Putrescine concentration

Putrescine is proposed as an indicator of sausage spoilage (Hernandez-Jover *et al.,* 1997b; Eerola *et al.*, 1997). There is no specific standard for putrescine amount in meat products.

Changes of putrescine concentration that were followed during the ripening period are given in Figures 4.10 – 4.12. Statistical analysis was carried out to find statistical differences between recipe and ripening time. Their results are shown in Table 4.7.

It was observed from the statistical analysis that putrescine concentration was affected ($P<0.05$) from ripening time. Putrescine concentration in recipes increased ($P<0.05$) from 1.13 to about 1.53 mg/kg during the first 2 days except nitrite/nitrate, BHT and *Rosmarinus officinalis* added recipe. After that time, putrescine concentration decreased sharply ($P<0.05$) in all recipes. Then, their values became nearly constant ($P>0.05$) till the end of the ripening period as 1.37 mg/kg level.

It was found that putrescine concentration was affected ($P<0.05$) from the addition of antioxidants in sucuk recipe. According to statistical analysis, control sucuk had the highest ($P<0.05$) putrescine concentration about 5 times greater than its initial value

(1.13 mg/kg) at the end of ripening (5.63 mg/kg). Two-way anova indicate that the lowest ($P<0.05$) putrescine concentration was observed in nitrite/nitrate added recipe. In nitrite/nitrate, BHT, *Urtica dioica* and *Hibiscus sabdariffa (300 ppm)* added recipes, putrescine concentration was lower than initial putrescine concentration. Putrescine formation depends on the activity of bacteria. Nitrite and nitrate are known as one of the most effective on decreasing the activity of putrescine producing bacteria, therefore the reduction of putrescine could be seen in nitrite/nitrate added recipe (Bozkurt, 2002). This result is in agreement with Aksu and Kaya (2004).

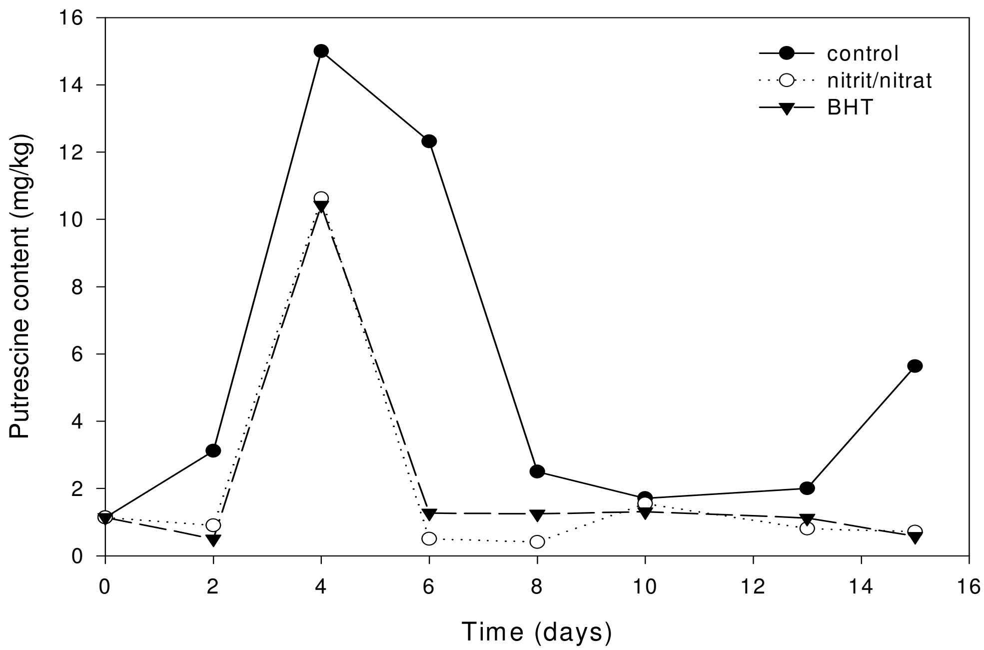

Figure 4.10. Putrescine concentration of sucuks prepared with non, nitrite/nitrate, and BHT

Table 4.7. Statistical results for putrescine content of sucuk recipes during the ripening period

Time (days)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	1.13aA	1.13abA	1.13abA	1.13aA	1.13abA	1.13aA	1.13aA	1.13aA	1.13aA	1.13aA	1.13aA
2	3.12bA	0.90abcB	0.50cC	2.33bD	1.81bE	0.56aF	0.50bC	2.23bDG	2.14bDG	2.00bcE	1.75abE
4	15.00cA	10.62dBC	10.42dB	13.19cADE	13.20cADE	13.64bAE	11.07cBCD	12.71cCDE	15.34cA	11.10dBCD	11.85cBCDE
6	12.32dA	0.50eB	1.27bCD	0.99aC	6.00dE	1.14aC	1.00aC	0.98aC	1.12aC	1.45abCD	1.89abD
8	2.50eA	0.41ceB	1.24bC	0.55adBD	1.48bE	1.01aF	0.93aF	0.71adD	0.96aF	1.29abCE	1.39abCE
10	1.71aeAB	1.55bAC	1.31bC	2.12bDE	1.97bBD	1.37aC	2.00dBD	2.13bDE	1.54abAC	2.32cE	1.96bBD
13	2.00abeA	0.81acB	1.12abC	0.95aBC	1.08abC	1.12aC	1.12aC	0.50dD	1.42abE	1.64abcF	1.49abF
15	5.63fA	0.71acBC	0.58acB	0.50dD	0.54aB	1.16aDE	1.42adE	0.98aCD	1.30abDE	1.49abE	1.28abDE

S1: Control, no antioxidant added recipe; S2: Nitrite/Nitrate (150/300 ppm) added recipe; S3: 300 ppm BHTadded recipe; S4: 300 mg soluble solid/kg of *Urtica dioica* extract added recipe; S5: 600 mg soluble solid/kg of *Urtica dioica* extract added recipe; S6: 300 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S7: 600 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S8: 300 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S9: 600 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe and S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe.

Different small letters indicate statistical difference at α=0.05 level in each column.

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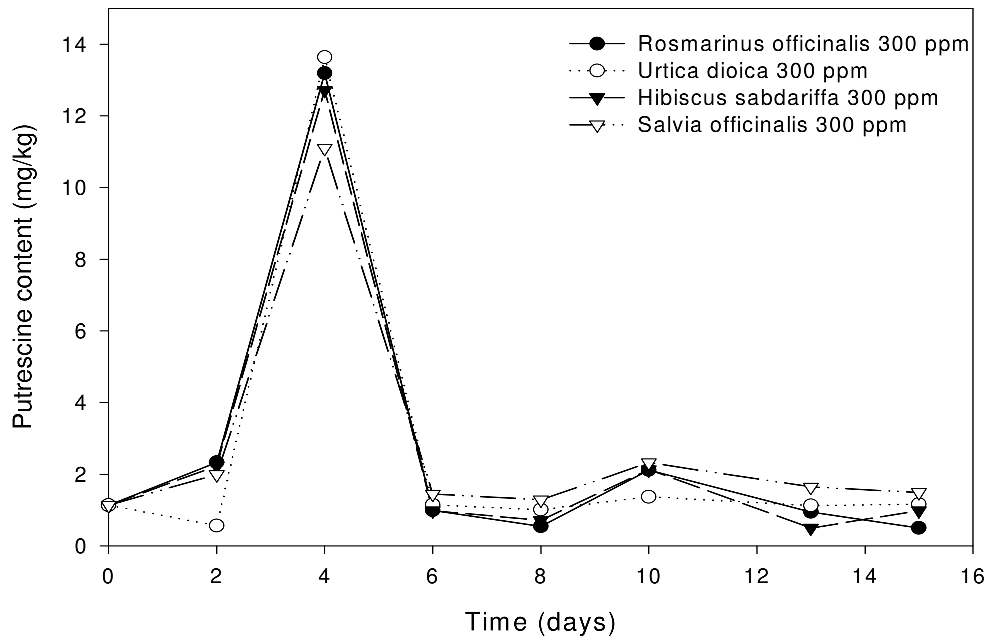

Figure 4.11. Putrescine concentration of sucuks prepared with 300 ppm natural antioxidants extracts

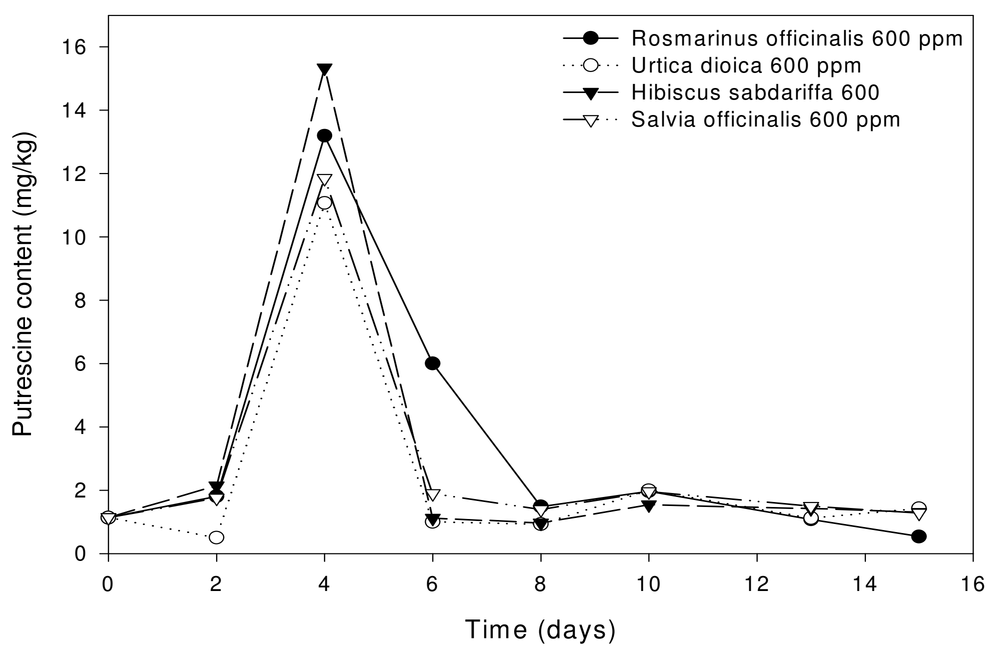

Figure 4.12. Putrescine concentration of sucuks prepared with 600 ppm natural antioxidants extracts

Retailed putrescine concentration of sucuks are shown in Table 4.8. Putrescine concentration was ranged from 0.00 to 9.332 and in 3 of 19 retailed sucuks putrescine was not detected. Putrescine concentration of retailed sucuks was lower than their histamine concentration. In this study putrescine concentration of prepared sucuks was in the range of 0.50 - 5.63 mg/kg at the end of the ripening which was as low as retailed sucuks. Ayhan *et al.* (1999) detected putrescine formation increased about to 412 mg/kg in sucuks that stored at +4°C with 90 % RH.

Table 4.8. Putrescine concentration of retailed sucuks

Sample	Putrescine conc.	Sample	Putrescine conc.
R1	2.988	R11	1.120
R2	2.999	R12	0.000
R3	1.406	R13	0.000
R4	0.981	R14	0.000
R5	0.900	R15	1.297
R6	1.441	R16	1.172
R7	1.054	R17	0.838
R8	1.047	R18	3.346
R9	0.993	R19	9.332
R10	1.139		

4.3.3. Changes of Tyramine concentration

The allowable maximum level of tyramine in food is 100-800 mg/kg, 1080 mg/kg of tyramine is toxic for human (Shalaby, 1996). Ansorena *et al.* (2002) indicated that tyramine is one of the main biogenic amines in ripened northern and southern European sausages.

Changes of tyramine concentration in sucuk during the ripening period are given in Figures 4.13-4.15 and statistical analysis results are shown in Table 4.9.

Statistical analysis showed that tyramine concentration increased significantly ($P<0.05$) during the ripening period from 5.55 to 83.21 mg/kg. Its concentration increased sharply during first 4 days and reached about 5 times of its initial value from 5.55 to 24.16 mg/kg. After that time, increment in tyramine concentration was not as high as in this period, however it was significant (P<0.05) till end of the ripening period.

Statistical analysis showed that tyramine formation in sucuks was affected (P<0.05) from the addition of antioxidants. *Rosmarinus officinalis* (300 ppm) and nitrite/nitrate added recipe had lower (P<0.05) tyramine concentration than the other recipes as 36.27 and 37.49 mg/kg. The control recipe had the highest (P<0.05) tyramine value which was 20 times higher than initial value. Sebranek *et al* (2004) reported that *Rosmarinus officinalis* is effective in meat system. Lopez *et al.* (2004) concluded that rosmanol, rosmariquinone, carnosol and rosmaridiphenol are the effective compounds in *Rosmarinus officinalis*. The decrease in tyramine formation by natural antioxidants is important with respect to human health.

Tyramine concentrations of nineteen retailed sucuks are given in Table 4.10. Tyramine concentration of retailed sucuks was ranged from 2.596 to 96.514 (Table 4.10). Tyramine concentrations in 13 of 19 retailed sucuks were lower (P<0.05) than 20 mg/kg and only one of them had about 100 mg/kg tyramine. Shalaby (1996) reported that the tolerance level of tyramine is 100 – 800 mg /kg. According to these results, tyramine levels were lower than this tolerance level so they were acceptable. Bozkurt and Erkmen (2005) reported that tyramine concenrations in firm-made and traditional sucuks were ranged from 3.23 to 547.51 and from 0.00 to 244.41 mg/kg, respectively.

Table 4.9. Statistical results for tyramine content of sucuk during the ripening period

Time (days)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	5.55aA	5.55aA	5.55abA	5.55aA	5.55aA	5.55aA	5.55aA	5.55aA	5.55aA	5.55aA	5.55aA
2	14.60aA	10.93abBC	4.00aD	11.91aBCE	12.96aAE	11.96aCE	12.41aCE	13.25bAE	7.55aF	11.35aBCE	10.04aB
4	14.37aA	13.60bA	11.79bA	33.72bB	32.50bB	34.43bB	28.35bC	26.32cCD	23.20bDE	27.26bCD	20.23bE
6	44.04bA	33.17cB	31.81cBC	36.18bB	42.89cA	44.62cA	30.66bBC	33.83dB	33.15cB	36.10cB	26.43bC
8	46.68bA	35.87cBC	46.19dA	62.51cD	43.52cAC	37.32bdBC	49.93cA	37.44dBC	35.18cB	61.81dD	46.89cA
10	75.46cA	47.50dB	64.86eACD	64.92cACD	71.19dACD	46.81cB	64.23dCD	62.33eC	74.18dAD	64.60dCD	51.72cB
13	97.11dA	70.05eBC	77.77fDE	84.54dD	74.81dCDE	43.27cdF	65.02dBC	61.89eBG	77.41dDE	51.92eFG	85.70dD
15	103.93dA	83.24fBC	97.52gD	88.94dCD	83.67eBC	66.20eE	81.78eBC	75.51fBCE	81.58dBC	79.41fBCE	73.48eBE

S1: Control, no antioxidant added recipe; S2: Nitrite/Nitrate (150/300 ppm) added recipe; S3: 300 ppm BHTadded recipe; S4: 300 mg soluble solid/kg of *Urtica dioica* extract added recipe; S5: 600 mg soluble solid/kg of *Urtica dioica* extract added recipe; S6: 300 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S7: 600 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S8: 300 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S9: 600 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe and S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe.

Different small letters indicate statistical difference at α=0.05 level in each column.

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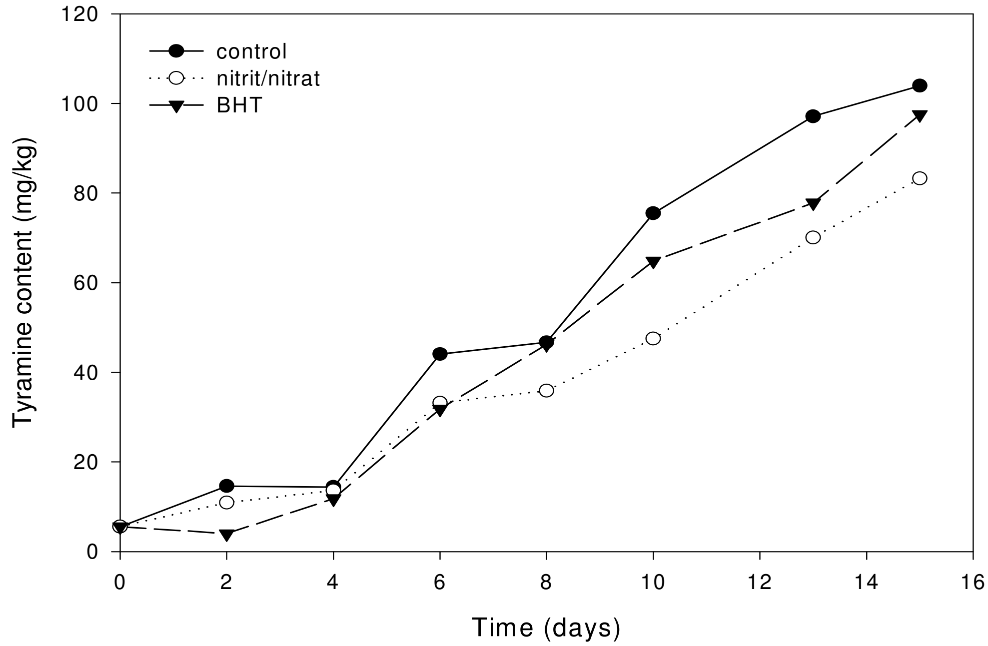

Figure 4.13. Tyramine concentration of sucuks prepared with non, nitrite/nitrate, and BHT

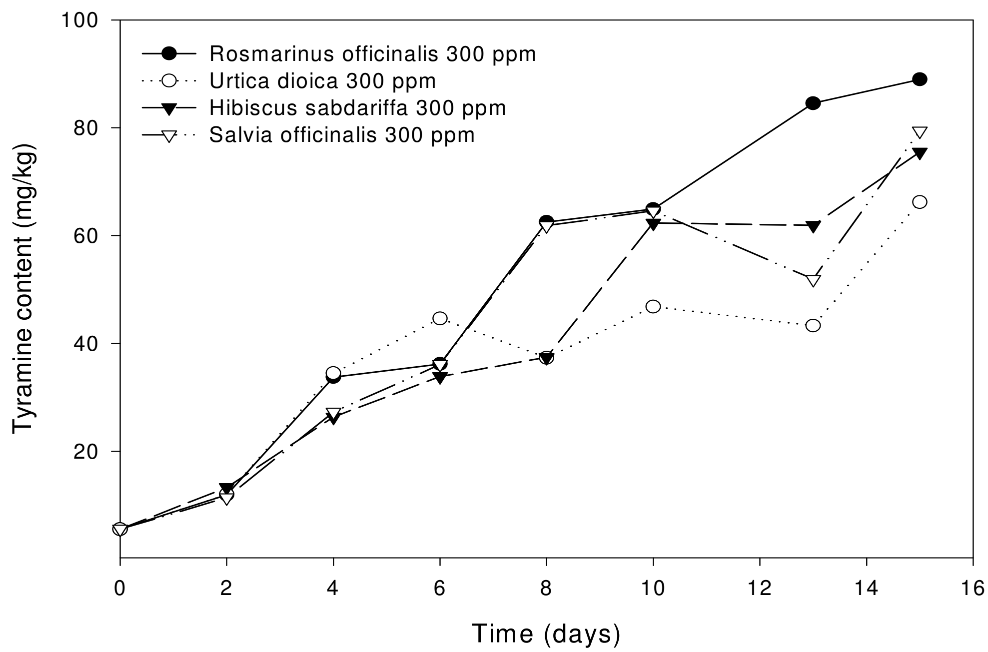

Figure 4.14. Tyramine concentration of sucuks prepared with 300 ppm natural antioxidants extracts

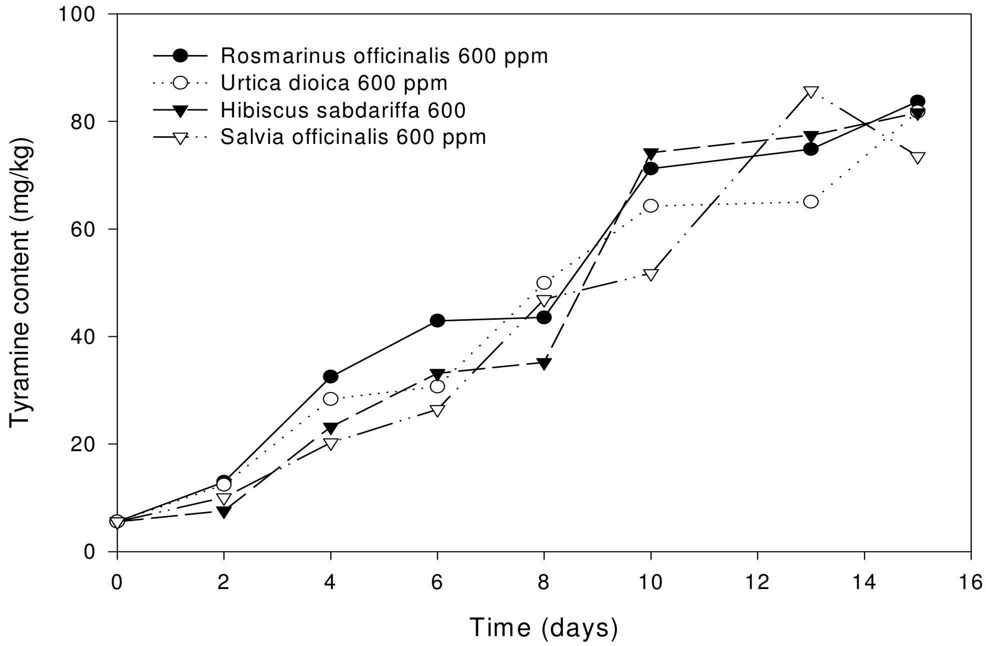

Figure 4.15. Tyramine concentration of sucuks prepared with 600 ppm natural antioxidants extracts

When tyramine concentration was compared with histamine and putrescine, tyramine was about 20 times higher than the others. As Bover *et al*. (2000) pointed out tyramine is usually the major amine found in fermented meat products; its production is mainly associated with tyrosine decarboxylase activity of lactic acid bacteria. Tyramine production has also been correlated with the observed decrease in pH.

Table 4.10. Tyramine concentration of retailed sucuk

Sample	Tyramine conc.	Sample	Tyramine conc.
R1	2.596	R11	16.506
R2	2.989	R12	10.021
R3	3.727	R13	56.564
R4	3.402	R14	25.887
R5	5.368	R15	26.413
R6	4.787	R16	23.027
R7	5.660	R17	28.096
R8	5.236	R18	73.653
R9	3.934	R19	96.514
R10	4.559		

4.4. Changes in Hunter L, a, b values

The results of lightness (L) values are given in Figure 4.16-4.18 and their statistical results are given in Table 4.11. It was found from the statistical analysis that Hunter L-values were not affected (P>0.05) from ripening time and the addition of antioxidants into recipe except control and *Salvia officinalis* added recipe. The initial L value was 40.41 and increased to 42.36 which was the highest value (in *Salvia officinalis*) after 2nd day. At the end of the ripening period L- values were decreased but not significiantly (P>0.05) except in control and *Salvia officinalis* added recipe (Table 4.11). These results indicate that color of sucuks did not change significantly (P>0.05) during the ripening period. Decrease in the L-values showed that the dark color formation due to the browning (Bozkurt, 2006). It can be concluded that color of sucuk did not change (P>0.05) to dark color because of the browning reaction. However, Bozkurt (2006), Kayardı and Gök (2003) found that Hunter L-values of sucuk generally decrease during the 15 days of ripening. The average of L -values of sucuks was 35.65 at the end of the ripening period. Control sucuk had the lowest (P<0.05) and *Urtica dioica* added sucuk had the highest (P<0.05) L-values at the end of the ripening period.

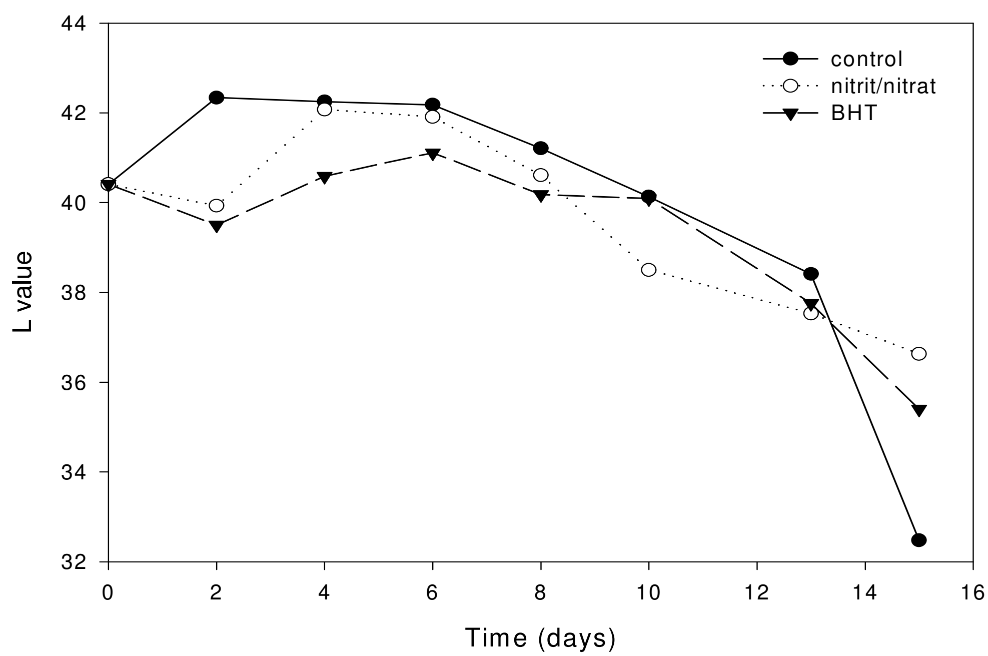

Figure 4.16. Hunter L-values of sucuks prepared with non, nitrite/nitrate, and BHT

Table 4.11. Statistical results for Hunter L-values of sucuk recipes during the ripening period

Time (days)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	40.41aA	40.41aA	40.41aA	40.41aA	40.41aA	40.41aA	40.41aA	40.41aA	40.41aA	40.41abA	40.41aA
2	42.34a A	39.93aA	39.50aA	41.61aA	40.43aA	41.82aA	40.54aA	39.62aA	41.14aA	42.36aA	39.83aA
4	42.25aA	42.07aA	40.59aA	40.01aA	40.73aA	40.64aA	39.73aA	40.51aA	40.42aA	39.28abA	39.39aA
6	42.18aA	41.91aA	41.11aA	38.56aA	41.00aA	41.82aA	39.25aA	39.93aA	39.27aA	38.25abA	40.02aA
8	41.21aA	40.61aA	40.18aA	39.92aA	41.68aA	41.33aA	39.30aA	38.91aA	38.21aA	38.16abA	38.88aA
10	40.13aA	38.50aA	40.09aA	38.34aA	39.49aA	39.37aA	38.48aA	38.77aA	37.13aA	37.98abA	39.23aA
13	38.41abA	37.53aA	37.75aA	37.56aA	38.85aA	38.80aA	38.16aA	37.29aA	37.13aA	37.73abA	37.18aA
15	32.48bA	36.63aA	35.40aA	35.82aA	36.72aA	36.17aA	33.93aA	35.82aA	36.04aA	35.06bA	35.49aA

S1: Control, no antioxidant added recipe; S2: Nitrite/Nitrate (150/300 ppm) added recipe; S3: 300 ppm BHTadded recipe; S4: 300 mg soluble solid/kg of *Urtica dioica* extract added recipe; S5: 600 mg soluble solid/kg of *Urtica dioica* extract added recipe; S6: 300 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S7: 600 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S8: 300 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S9: 600 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe and S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe.

Different small letters indicate statistical difference at α=0.05 level in each column.

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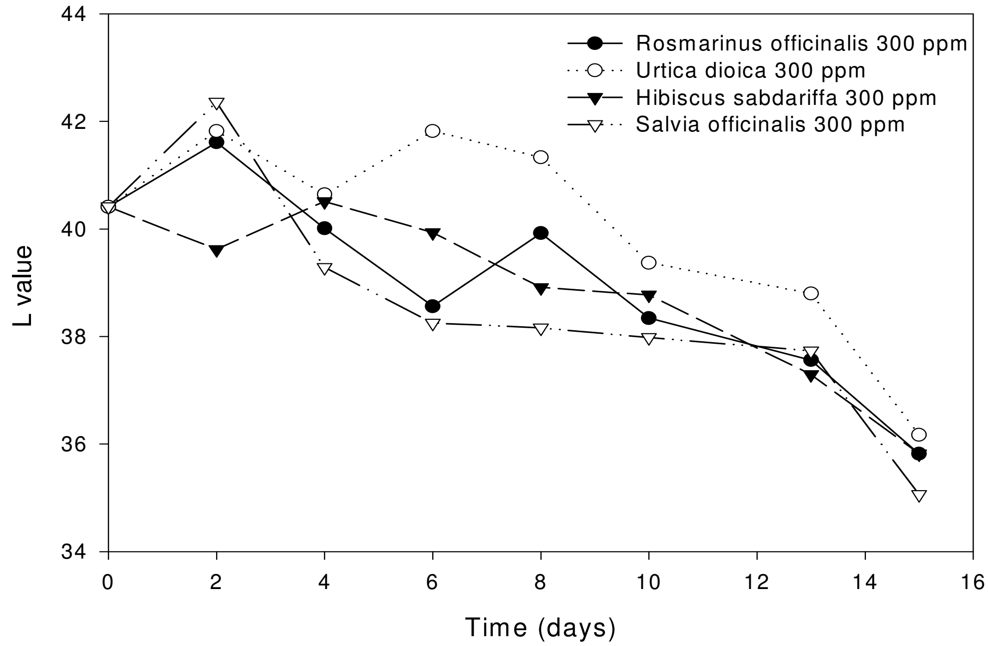

Figure 4.17. Hunter L-value of sucuks prepared with 300 ppm natural antioxidants extracts

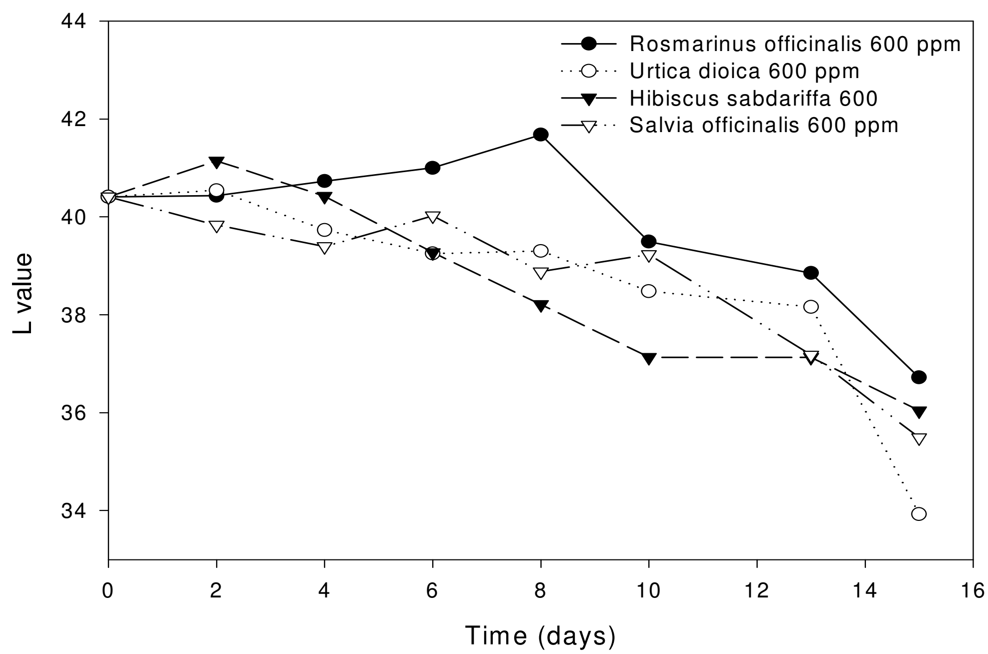

Figure 4.18. Hunter L-value of sucuks prepared with 600 ppm natural antioxidants extracts

Hunter L–value of retailed sucuks is shown in Table 4.12. The Hunter L-values of sucuks were in the range of 30.27 – 42.69. Statistical analysis indicate that Hunter L-values of retailed sucuks were different (P<0.05) from each other. Generally, the Hunter L-values of retailed sucuks were higher than that of prepared sucuk in this study. These differences could be due to the differences in manufacturing process and recipe.

Table 4.12. Hunter L-values of retailed sucuk

Sample	Hunter L value	Sample	Hunter L value
R1	32.53	R11	39.31
R2	32.65	R12	36.66
R3	36.59	R13	36.39
R4	38.74	R14	41.06
R5	30.46	R15	39.16
R6	31.22	R16	36.54
R7	42.69	R17	36.58
R8	41.05	R18	37.36
R9	31.03	R19	38.02
R10	30.27		

Redness (a-) values are shown in Figure 4.19 – 4.21. Statistical results are shown in Table 4.13. The Hunter a-value increased (P<0.05) during the ripening periods. During this period nitrogenous compounds present in meat combined with myoglobin to produce the desired red pigment (nitrosomyoglobin). Therefore Hunter a-values would be high (Bozkurt, 2006). The a-value of control recipe was lower (P<0.05) than the other recipes at the end of ripening period. The results of statistical analysis given in Table 4.13 indicate that nitrite/nitrate and *Urtica dioica* (600 ppm) added sucuks had the most effective (P<0.05) enhancing Hunter a-values. The color is one of the most important quality attributes of sucuk that attracts the preferense of the consumers (brick-red color). The attraction of sucuk is attained when desired brick-red color is formed during the ripening period, so its values increase. Therefore, to get more attracted sucuk nitrite, nitrate and natural antioxidants could be used. The Hunter a-values of all sucuks increased significantly (P<0.05) in 2 days of ripening and after that time except *Rosmarinus officinalis* (600 ppm) there was no difference (P>0.05) in Hunter a-values between antioxidant added recipes.

Table 4.13. Statistical results for Hunter a-values of sucuk recipes during the ripening period

Time (days)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	7.40aA	7.40aA	7.40aA	7.40aA	7.40aA	7.40aA	7.40aA	7.40aA	7.40aA	7.40aA	7.40aA
2	10.51bA	9.70bA	10.25bA	10.68bA	10.30bA	9.87bA	9.40bA	10.05bcA	10.22bA	9.76bA	9.71bA
4	11.21bAB	11.08bAB	10.44bAB	10.72bAB	11.26bAB	11.23bAB	11.90cA	10.67bcAB	11.28bAB	9.88bB	10.59bAB
6	10.64bA	10.31bA	11.37bA	11.02bA	11.01bA	10.51bA	11.10bcdA	10.80bcA	10.60bA	10.90bA	9.69bA
8	10.49bA	11.38bA	11.02bA	11.02bA	11.07bA	11.18bA	11.28cdA	10.85bcA	10.05bA	11.17bA	10.86bA
10	10.18bA	10.63bA	11.57bA	10.22bA	10.96bA	11.27bA	11.16bcdA	11.18cA	10.52bA	11.04bA	10.93bA
13	10.65bA	10.39bA	10.05bA	10.09bA	10.71bA	10.38bA	11.07bcdA	9.30bA	10.32bA	9.82bA	10.41bA
15	8.01aA	10.36bB	9.77bB	10.32bB	10.71bB	9.89bB	9.56bdAB	10.14bcB	9.88bB	9.70bB	9.60bAB

S1: Control, no antioxidant added recipe; S2: Nitrite/Nitrate (150/300 ppm) added recipe; S3: 300 ppm BHTadded recipe; S4: 300 mg soluble solid/kg of *Urtica dioica* extract added recipe; S5: 600 mg soluble solid/kg of *Urtica dioica* extract added recipe; S6: 300 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S7: 600 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S8: 300 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S9: 600 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe and S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe.

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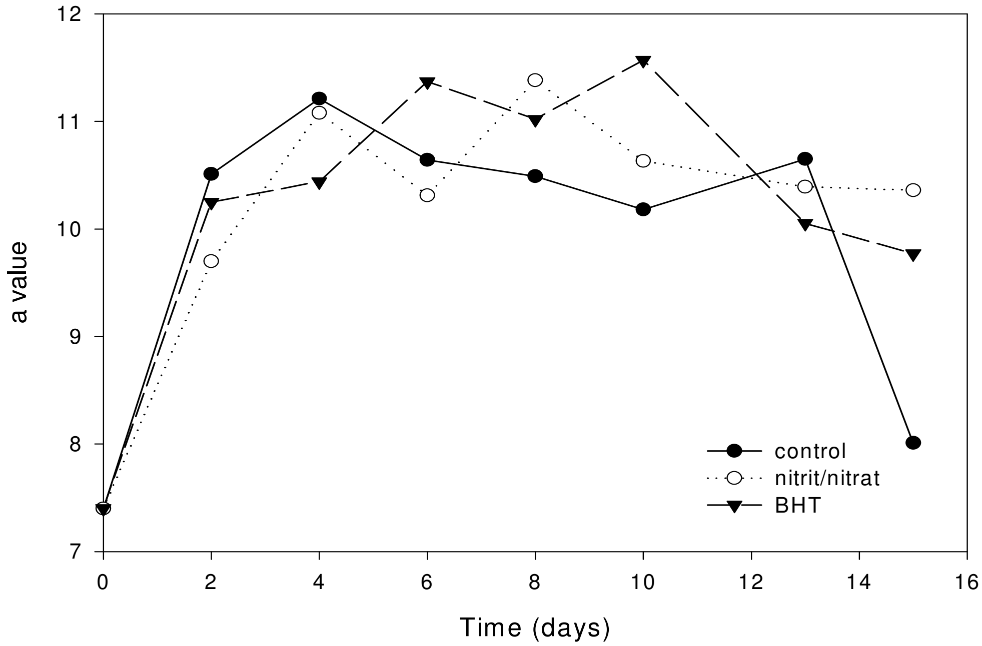

Figure 4.19. Hunter a-value of sucuks prepared with non, nitrite/nitrate, and BHT

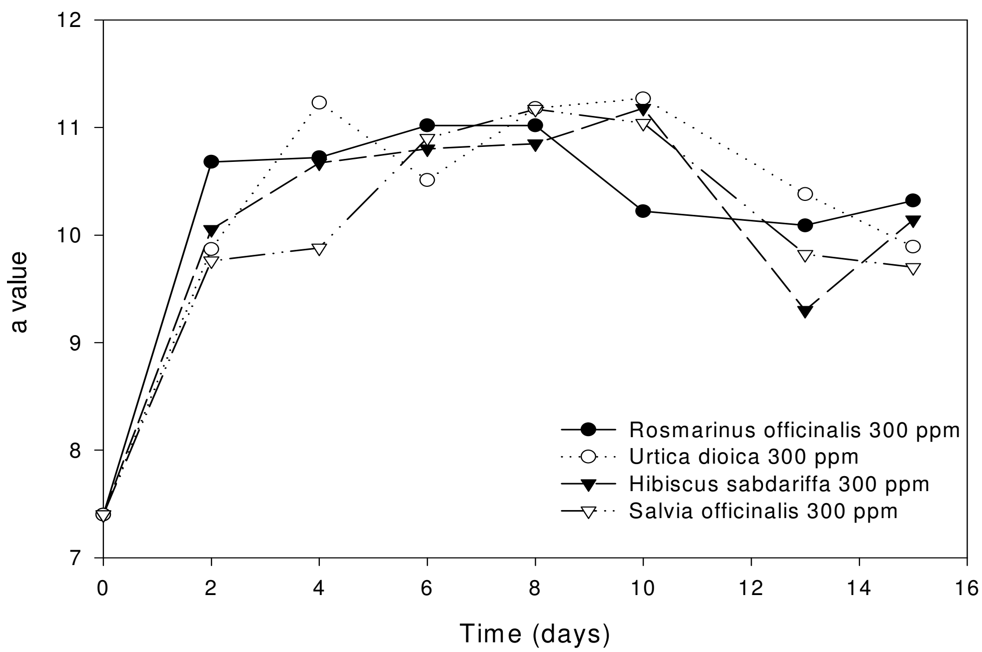

Figure 4.20. Hunter a- value of sucuks prepared with 300 ppm natural antioxidants extracts

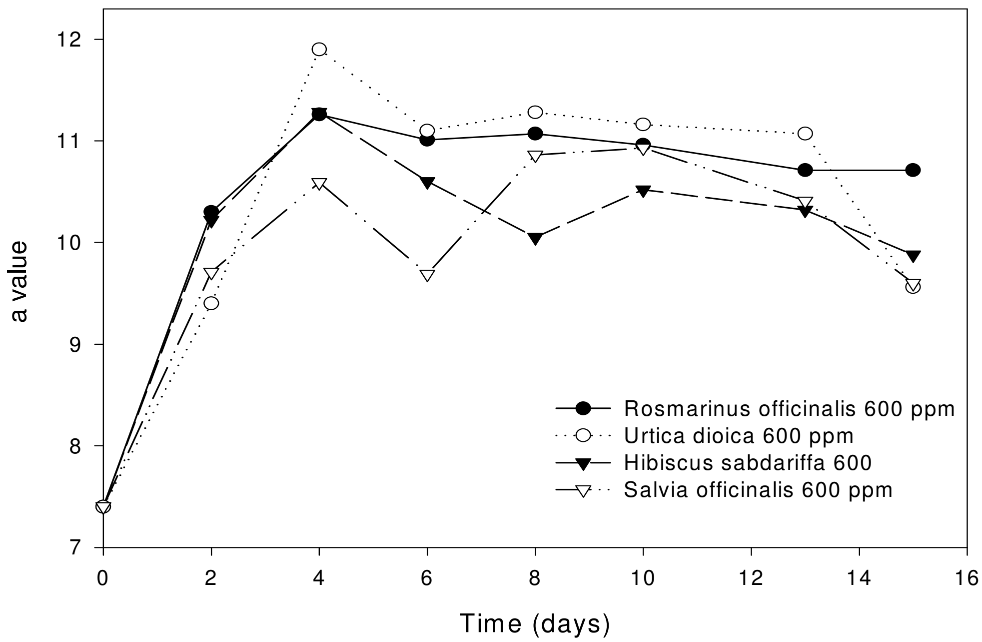

Figure 4.21. Hunter a- value of sucuks prepared with 600 ppm natural antioxidants extracts

Hunter a-values of retailed sucuks are given in Table 4.14. Statistical analysis was carried out to determine the differences in the Hunter a-values of sucuk. It was observed that Hunter a-values were significantly different ($P<0.05$). This could be due to the recipe and process differences. Retailed sucuks had higher Hunter a-values than the prepared sucuks in this study at the end of the ripening period. So, it can be concluded that retailed sucuks had more red color than prepared sucuk.

Table 4.14. Hunter a-values of retailed sucuk

Sample	Hunter a-value	Sample	Hunter a- value
R1	15.83	R11	17.20
R2	16.28	R12	18.03
R3	17.70	R13	18.64
R4	16.96	R14	15.44
R5	14.58	R15	16.68
R6	16.11	R16	16.75
R7	13.09	R17	17.44
R8	13.89	R18	12.51
R9	16.89	R19	12.01
R10	18.01		

Yellowness (b-) values of sucuk during the ripening period are given in Figures 4.22-4.24. Their statistical results are shown in Table 4.15. Statistical analysis (one way anova) indicate that Hunter b-values of sucuks were affected ($P<0.05$) from ripening time. However, they were not affected significantly ($P>0.05$) from the addition of antioxidants. It was found that their values decreased ($P<0.05$) from 12.58 to about 10.53 at the end of the ripening period. At that time the lowest Hunter b-value was 9.13 in control sucuk. Similar results were observed by Perez-Alvarez et al. (1999) that Hunter b-values of Spanish sausages decreased during the fermentation and ripening periods. The decrease in the Hunter b-value indicated that the color of sucuks turned to blue rather than yellow. This could be due to the browning reactions, because browning reactions yield melanoidins which have brown color (Bozkurt, 2006).

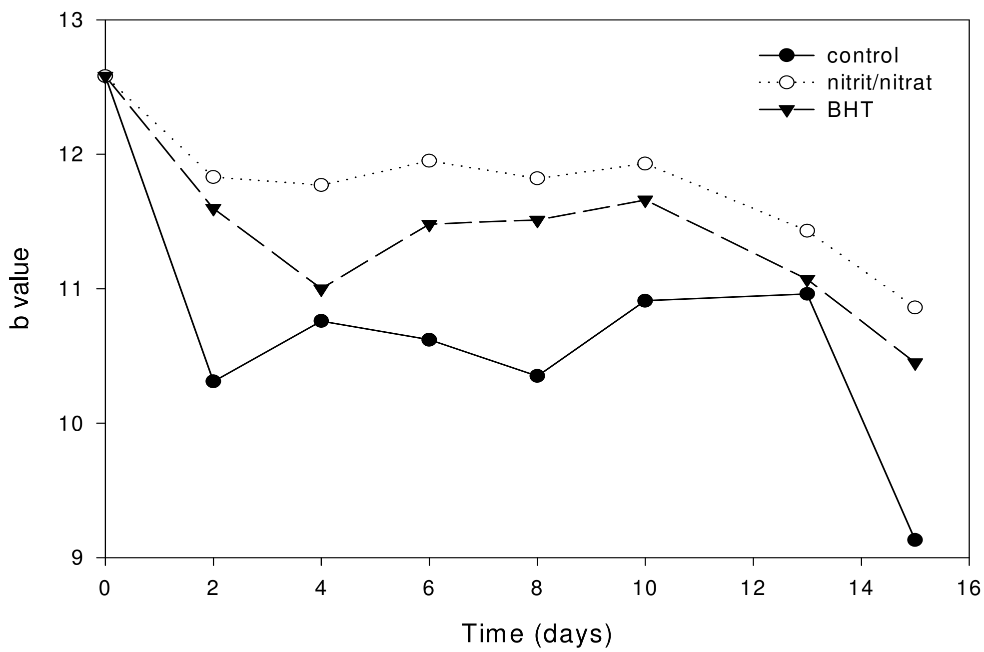

Figure 4.22. Hunter b-value of sucuks prepared with non, nitrite/nitrate, and BHT

Table 4.15. Statistical results for Hunter b-values of sucuk recipes during the ripening period

Time (days)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	12.58aA	12.58aA	12.58aA	12.58aA	12.58aA	12.58aA	12.58aA	12.58aA	12.58aA	12.58aA	12.58aA
2	10.31bA	11.83aA	11.60abA	11.94aA	11.98aA	11.40aA	12.23aA	11.83aA	11.90aA	11.25abA	11.40abA
4	10.76abA	11.77aA	11.00abA	11.30aA	12.19aA	11.94aA	12.65aA	11.76aA	11.82aA	11.65abA	11.39abA
6	10.62bA	11.95aA	11.48abA	11.39aA	11.66aA	11.42aA	12.07abA	11.36abA	11.66aA	11.79abA	11.61abA
8	10.35bA	11.82aA	11.51abA	11.18aA	11.79aA	11.48aA	11.30abA	10.98abA	11.70aA	11.17abA	11.25abA
10	10.91abA	11.93aA	11.66abA	10.85aA	11.72aA	11.38aA	11.46abA	10.90abA	11.29aA	10.73abA	10.98abA
13	10.96abA	11.43aA	11.07abA	10.71aA	11.82aA	11.34aA	11.27abA	10.94abA	11.34aA	10.62abA	10.27abA
15	9.13bA	10.86aAB	10.45bAB	10.87aAB	11.46aB	10.87aAB	10.09bAB	9.55bA	10.80aAB	10.25bAB	10.08bAB

S1: Control, no antioxidant added recipe; S2: Nitrite/Nitrate (150/300 ppm) added recipe; S3: 300 ppm BHTadded recipe; S4: 300 mg soluble solid/kg of *Urtica dioica* extract added recipe; S5: 600 mg soluble solid/kg of *Urtica dioica* extract added recipe; S6: 300 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S7: 600 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S8: 300 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S9: 600 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe and S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe.

Different small letters indicate statistical difference at α=0.05 level in each column.

Different capital letters indicate statistical difference at α=0.05 level among products at each time.

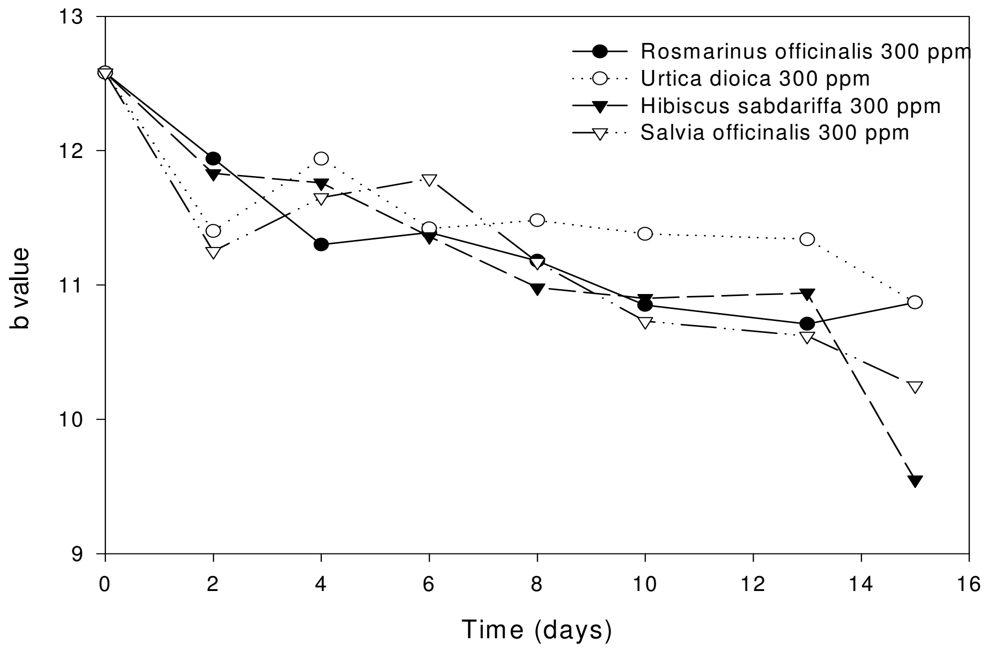

Figure 4.23. Hunter b- value of sucuks prepared with 300 ppm natural antioxidants extracts

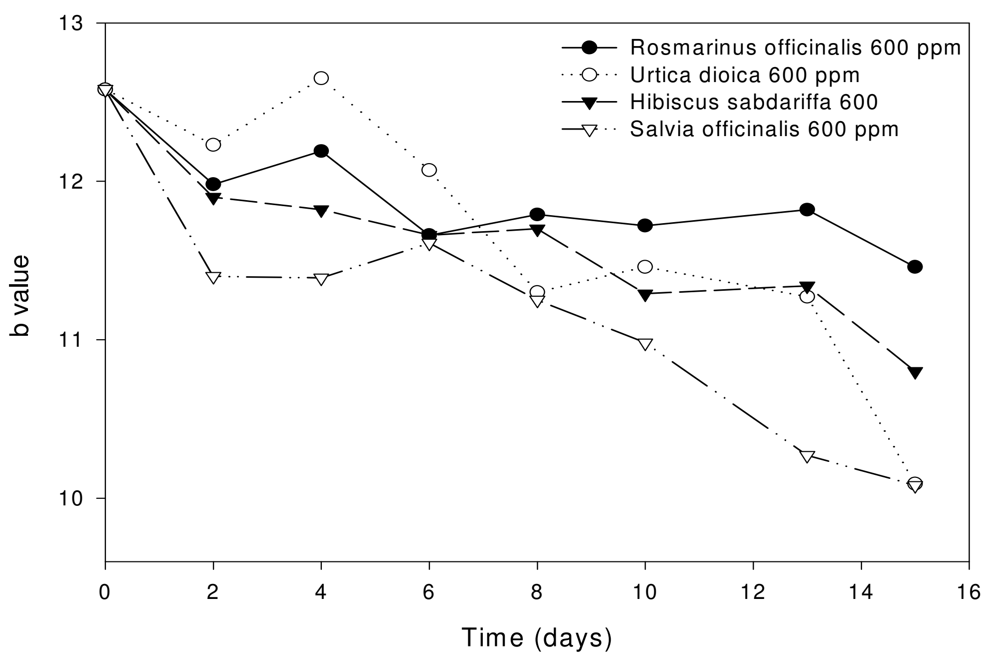

Figure 4.24. Hunter b- value of sucuks prepared with 600 ppm natural antioxidants extracts

Hunter b-values of retailed sucuks are given in Table 4.16. Statistical analysis was carried out to determine the differences between Hunter b-values of sucuks. It was observed that Hunter b-values were significantly different ($P<0.05$). This could be due to the recipe and process differences. Generally retailed sucuks had higher Hunter a-values than the prepared sucuks in this study at the end of the ripening period. The decrease in the Hunter b-value indicated that color of sucuk turned to blue rather than yellow. So, it can be concluded that retailed sucuks had more yellow than prepared sucuks.

Table 4.16 retailed sucuk Hunter b-values

Sample	Hunter b-value	Sample	Hunter b-value
R1	10.37	R11	11.77
R2	9.99	R12	12.53
R3	11.22	R13	12.32
R4	11.24	R14	9.91
R5	9.06	R15	9.70
R6	9.83	R16	12.11
R7	10.89	R17	12.35
R8	10.64	R18	12.52
R9	9.80	R19	11.84
R10	10.00		

4.5. Overall Sensory Quality

Overall sensory quality of sucuks depends on formation of desired aroma, color and texture (resistance to cut). Sucuks are fermented during the first days of ripening periods. Lactic acid bacteria produce acids, aldehydes and ketones which give aroma to sucuk during the fermentation (Lücke, 1994; Gökalp *et al.*, 1999; Ordonez *et al.*, 1999). Coloring compounds (nitrosomyoglobin) are formed and gelatinization of protein occurs (increasing cutting scores). These changes increase overall sensory quality of sucuks.

Overall sensory quality scores were followed by flavor, color and cutting scores. It was calculated from flavor (%50), color (%25) and cutting scores (%25) of the sausages (Bozkurt, 2002).

Overall sensory quality scores and their statistical analysis results are shown in Figures 4.25 – 4.27 and Table 4.17, respectively. It was found that overall sensory quality scores of sucuks were affected significantly ($P<0.05$) from ripening time. Overall sensory quality scores increased ($P<0.05$) from 3.25 to about 9 during the ripening period. These results were in agreement with Bozkurt (2006) that overall sensory quality scores of sucuk increased ($P<0.05$) during the ripening period. This difference could be due to the ripening condition and recipe. Acids, aldehydes and ketones give aroma to sucuks which producing by lactic acid bacteria during the fermentation (Lüke, 1994). Although TBARS value of control sucuk was found to be the highest, sensory flavor changes were partially masked by the spices. So, final overall sensory quality scores of control were not different ($P>0.05$) from the others.

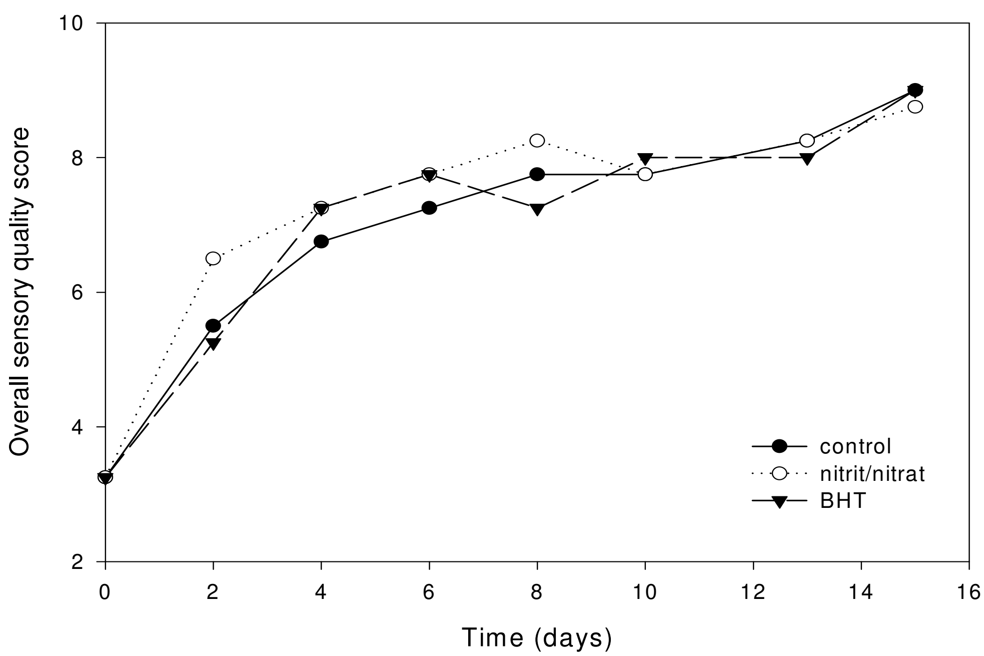

Figure 4.25. Overall sensory quality score of sucuks prepared with non, nitrite/nitrate, and BHT

Overall sensory quality scores of sucuks were not affected significantly (P<0.05) from the addition of antioxidants. In all sucuk recipes same trends were observed during ripening period. Also, at the end of the ripening, no significant (P>0.05) differences were observed in overall sensory scores between usages of natural antioxidants and nitrite/nitrate, and BHT (Table 4.17). At two level *Hibiscus sabdariffa* added sucuks overall sensory quality scores were lower than the other sucuks.

Overall sensory quality scores of retailed sucuks are shown in Table 4.18. According to statistical analysis overall sensory qualities were significiantly (P<0.05) differrent. The lowest score (P<0.05) was observed in 18th and 19th sucuk samples, where the highest (P<0.05) were in 14th and 15th samples. Overall sensory quality scores of prepared sucuks in this study at the end of the ripening period were about 9 and were higher than those of retailed sucuks. This result indicates that prepared sucuks were more acceptable than the retailed ones. So, it can be concluded that natural antioxidants could be used to increase overall acceptability with various health beneficials.

Table 4.18. Overall sensory qualities scores of retailed sucuk

Sample	Overall sensory score	Sample	Overall sensory score
R1	8.00	R11	8.25
R2	8.00	R12	8.50
R3	8.50	R13	8.50
R4	8.50	R14	9.00
R5	8.00	R15	9.00
R6	8.75	R16	8.25
R7	8.75	R17	8.25
R8	8.75	R18	6.75
R9	8.00	R19	6.75
R10	8.00		

Table 4.17. Statistical results for overall sensory quality scores of sucuk recipes during the ripening period

Time (days)	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	3.3aA	3.3aA	3.3aA	3.3aA	3.3aA	3.3aA	3.3aA	3.3aA	3.3aA	3.3aA	3.3aA
2	5.5bAB	6.5bB	5.3bC	6.0bAB	6.0bAB	5.8bAB	6.0bAB	6.0bAB	5.5bAB	6.0bAB	5.5bAB
4	6.8cA	7.3bcA	7.3cA	7.3cA	7.0bcA	7.0cA	7.0bcA	6.8bcA	6.8cA	6.8bcA	7.0cA
6	7.3cdA	7.8bcdA	7.8cA	7.8cdA	7.8cdA	7.8cdA	7.0bcA	7.0bcdA	8.3dA	7.0bcdA	7.3cA
8	7.8cdA	8.3cdA	7.3cA	8.0cdA	7.8cdA	8.0cdA	7.8cdA	7.3cdA	8.3dA	8.0deA	8.0cdA
10	7.8cdAB	7.8bcdAB	8.0cdAB	8.0cdAB	8.3cdAB	8.0cdAB	7.8cdAB	7.5cdAB	8.8dA	7.3cdB	7.5cAB
13	8.3deA	8.3cdA	8.0cdA	8.8dA	7.8cdA	8.0cdA	8.8dA	8.0deA	8.0dA	8.0deA	8.0cdA
15	9.0eA	8.8dA	9.0dA	9.0dA	8.8dA	9.0dA	9.0dA	8.8eA	8.8dA	8.8eA	9.0dA

S1: Control, no antioxidant added recipe; S2: Nitrite/Nitrate (150/300 ppm) added recipe; S3: 300 ppm BHTadded recipe; S4: 300 mg soluble solid/kg of *Urtica dioica* extract added recipe; S5: 600 mg soluble solid/kg of *Urtica dioica* extract added recipe; S6: 300 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S7: 600 mg soluble solid/kg of *Rosmarinus officinalis* extract added recipe; S8: 300 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S9: 600 mg soluble solid/kg of *Hibiscus sabdariffa* flowers extract added recipe; S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe and S10: 300 mg soluble solid/kg of *Salvia officinalis* extract added recipe.

Different small letters indicate statistical difference at α=0.05 level in each column.

Different capital letters indicate statistical difference at α=0.05 level among products at each time.

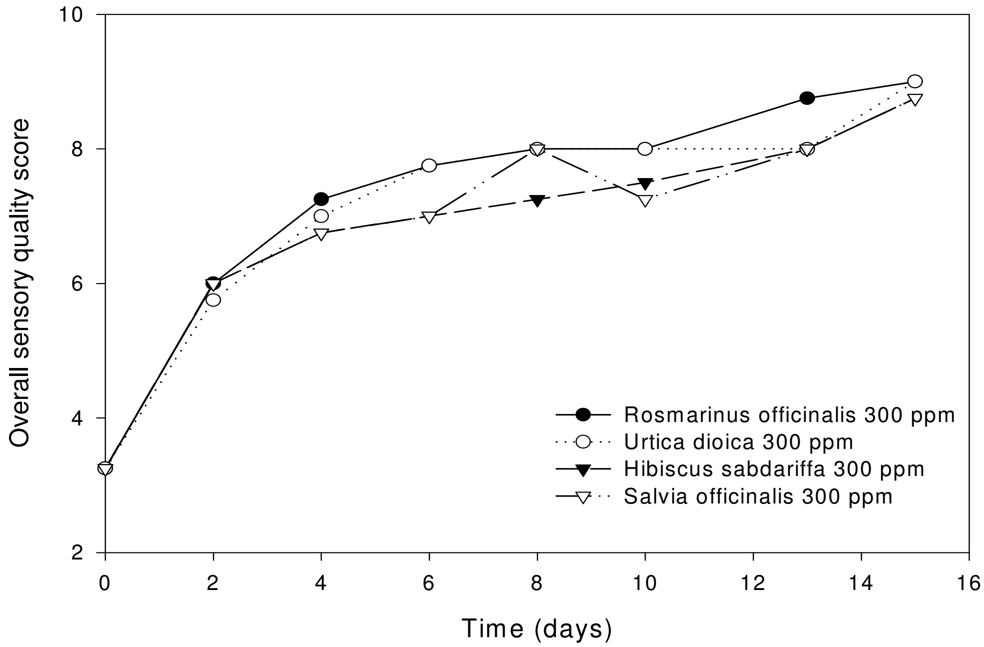

Figure 4.26. Overall sensory quality score of sucuks prepared with 300 ppm natural antioxidants extracts

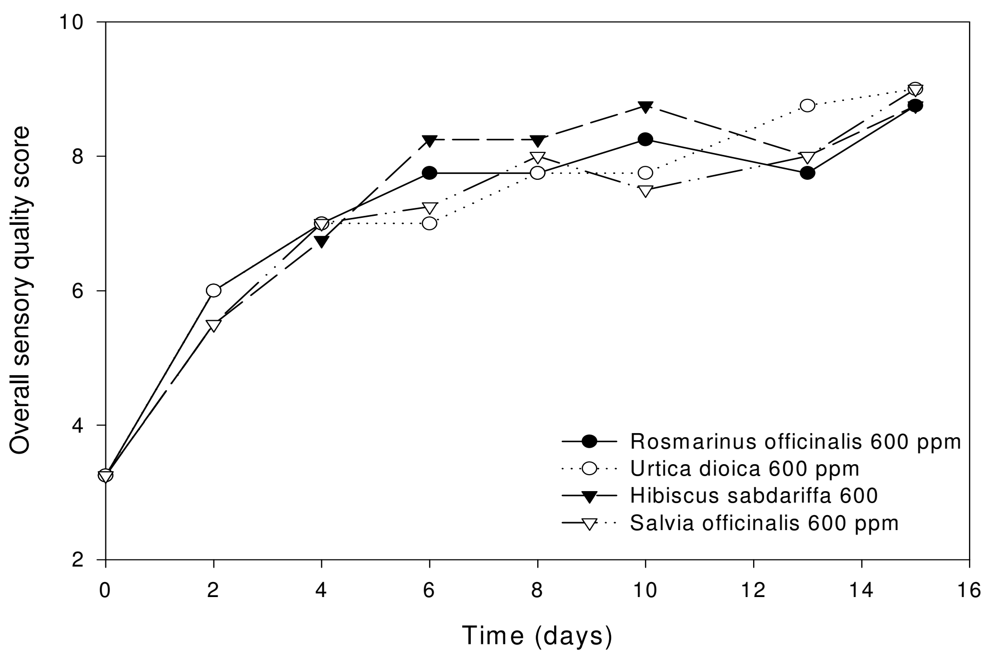

Figure 4.27. Overall sensory quality score of sucuks prepared with 600 ppm natural antioxidants extracts

CHAPTER V

CONCLUSION

In this study, effect of *Salvia officinalis L., Urtica dioica, Rosmarinus officinalis L.* and *Hibiscus sabdariffa* and buthylatedhydroxytoluene, and nitrite/nitrate on the pH, TBARS value, biogenic amine, Hunter L, a and b- value and sensory attributes of sucuk were investigated during the ripening periods.

The following results were obtained in sucuk during the ripening.

- pH values of sucuks were affected (P<0.05) from ripening time, however addition of antioxidants did not change (P>0.05) the trend of pH during the ripening period.
- The highest (P<0.05) pH value was observed in *Salvia officinalis* (600 ppm) extract added recipe while the lowest in control recipe.
- About 42 % of retailed sucuks had higher pH value than 5.4 which is the maximum level declared in Turkish Food Codex, while 26 % of retailed sucuks were lower than 5.2 that is the minimum level for Turkish Food Codex.
- TBARS values increased significantly (P<0.05) during the first 4 days. At the end of the ripening period, TBARS values were ranged between 0.35 (*Salvia officinalis*) and 0.81 mg/kg (in control).
- Sucuks containing natural antioxidant extract had significantly lower (P<0.05) TBARS value when compared to the control recipe. Also, the natural antioxidants extracts appeared to be more effective (P<0.05) than BHT and nitrite/nitrate.
- *Salvia officinalis* extracts reduced (P<0.05) TBARS values more than the others.
- During the first 2 days of ripening, histamine concentration increased significantly (P<0.05).

- The highest (P<0.05) histamine concentration was determined in the control recipe.
- *Urtica dioica* and *Salvia officinalis* were more effective (P<0.05) on decreasing histamine concentration than the other antioxidants.
- Histamine concentrations of retailed sucuk were found to be ranged from 0.682 to 210.993.
- Putrescine concentration in recipes increased (P<0.05) from 1.13 to about 1.53 mg/kg during the first 2 days.
- The lowest (P<0.05) putrescine concentration was observed in nitrite/nitrate added recipe.
- In 3 of 19 retailed sucuks, putrescine was not detected.
- Tyramine concentration increased significantly (P<0.05) during the ripening period.
- *Rosmarinus officinalis* (300 ppm) and nitrite/nitrate added recipe had the lower (P<0.05) tyramine concentration than the other recipes while the control recipe had highest P<0.05) tyramine.
- Hunter L-values were not affected (P>0.05) from ripening time and addition of antioxidants into recipe except control and *Salvia officinalis* added recipe.
- Control sucuk had the lowest (P<0.05) and *Urtica dioica* added sucuk had highest (P<0.05) L-values at the end of the ripening period.
- The Hunter a-value increased (P<0.05) during the ripening periods.
- The a-value of control recipe was lower (P<0.05) than the other recipes at the end of ripening period.
- Hunter b-values decreased (P<0.05) from 12.58 to about 10.53 at the end of the ripening period.
- Overall sensory quality scores increased (P<0.05) from 3.25 to about 9 during the ripening period.
- In all sucuk recipes same trends in overall sensory quality scores were observed during ripening period.

The results of this study showed that natural antioxidants are more effective than nitrite/nitrate and BHT. So, these natural antioxidants could be used in sucuk to enhance quality and provide safer products.

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