EFFECTS OF SOME STORAGE CONDITIONS AND ADDITIVES ON QUALITY AND STABILITY OF SUCUK (TURKISH DRY-FERMENTED SAUSAGE)

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ABSTRACT

EFFECTS OF SOME STORAGE CONDITIONS AND ADDITIVES ON QUALITY AND STABILITY OF SUCUK (TURKISH DRY-FERMENTED SAUSAGE)

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In this work, aerobic plate counts (APC), mold and yeast counts and lactic acid bacteria (LAB) counts; pH values, 2-thiobarbituric acid (TBA) values, nitrosomyoglobin conversion rate, residual nitrite level and biogenic amine formations; and overall sensory quality were monitored for microbial, chemical and organoleptic changes in sucuk (Turkish dry-fermented sausage) during the ripening and storage periods (20, 30 and 40°C with 50, 65 and 80 % RH). These analyses were also determined in sucuks collected from local markets (firm-made) and butchers (traditional).

APC and LAB increased ($P<0.05$) during the first 10 days of ripening and decreased ($P<0.05$) during the further ripening and storage periods. Starter culture had no effect ($P>0.05$) on the reduction of APC. Additives (nitrite, nitrate, α-tocopherol, ascorbic acid, potassium sorbate, potassium pyrophosphate, di-potassium hydrogen phosphate) had significant effect on the reduction of APC, LAB, and mold and yeasts counts. Increasing the storage temperature and % RH increased APC and LAB count significantly ($P<0.05$).

pH values of sucuks were sharply decreased ($P<0.05$) during the ripening. Additives and starter culture decreased TBA values but increased pH values and nitrosomyoglobin conversion rate. Increasing % RH of storage room increased the TBA and nitrosomyoglobin conversions values but not pH values. The residual nitrite level sharply decreased ($P<0.05$) in sucuks made with nitrate/nitrite during the

first five days of ripening periods. The amount of residual nitrite was low ($P<0.05$) in sucuks stored at 30°C.

Histamine ranged from 0.00 to 292.66 mg/kg during the ripening and storage periods. Starter culture and additives limited ($P<0.05$) the histamine, tyramine and putrescine formation. Histamine formation was highest ($P<0.05$) at high RH (80 %) storage. The lowest ($P<0.05$) tyramine, histamine and putrescine concentrations were observed in sucuk type B9. Putrescine concentration changed inversely related with the pH values of sucuks. Its formation decreased ($P<0.05$) with addition of additives and starter culture into sucuks. It was found that additives had no effect ($P>0.05$) on the tryptamine and polyamines (spermidine and spermine) formation. It was observed that 1,7-diamino heptane, serotonin, spermidine and spermine concentrations were changed between 0.00-43.54, 0.00-57.26, 0.00-30.60 and 0.00-31.29 mg/kg, respectively, in sucuks during the ripening and storage periods.

Overall sensory quality increased during the first days of ripening. The lowest overall sensory quality was observed in sucuk type A1. Starter culture and additives increased ($P<0.05$) overall sensory quality. Nitrite/nitrate played important role in the formation of desired color (nitrosomyoglobin) and flavor; ascorbic acid, and α-tocopherol reduced the lipid oxidation; and phosphates improved textural properties of sucuks.

Empirical equations were used to predict the chemical, microbial and overall sensory qualities of sucuks during the storage periods. It was found that empirical equations were well fitted to the experimental data.

Traditional sucuks had higher APC, and mold and yeasts counts than that of firm-made sucuks. pH values and residual nitrite level of firm-made sucuks were higher ($P<0.05$) than that of traditional sucuks. Traditional sucuks were spicier and the dominant aroma was from spices. Generally, firm-made sucuks had no desired aroma.

Key Words: Sucuks, stability, quality, safety, biogenic amine, TBA value, residual nitrite level

ÖZ

BAZI SAKLAMA KOŞULLARININ VE KATKI MADDELERİNİN SUCUĞUN KALİTESİ VE DAYANIRLILIĞI ÜZERİNE ETKİLERİ

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Bu çalışmada, sucuklarda olgunlaşma ve saklama (20, 30, ve 40°C'de % 50, 65 ve 80 nisbi nem'de) sürecinde meydana gelen mikrobiyal, kimyasal ve duyusal değişimleri takip etmek için aerobik bakteri (APC), laktik asit bakteri (LAB) ve küf ve maya sayıları; pH, 2- tiobarbitürik asit (TBA) sayısı, nitrosomyoglobin dönüşüm oranı, kalıntı nitrit miktarı, ve biyojenik aminlerin oluşumları belirlenmiş ve toplam duyusal kalite analizleri yapılmıştır. Bu analizler piyasadan toplanan, fabrikada ve geleneksel olarak üretilen (kasaplardan toplanan) sucuklarda da tespit edilmiştir.

APC ve LAB sayılarının olgunlaşma süresinin ilk on gününde arttıkları ($P<0.05$) ve daha sonraki süreçlerde ise azaldıkları belirlenmiştir. Starter kültür kullanımının sucuklarda APC sayısı üzerine herhangi bir etkisi olmadığı görülmüştür. Katkı maddeleri (nitrit, nitrat, α-tokoferol, askorbik asit, potasyum sorbat, potasyum pyrofosfat, di-potasyum hidrojen fosfat) sucuklarda APC, LAB ve küf ve maya sayılarını azaltmıştır. Saklama sıcaklığı ve nisbi nemin artmasıyla APC ve LAB sayılarının arttığı belirlenmiştir.

Sucukların pH değerleri olgunlaşma süresinde hızla azalmıştır. Katkı maddeleri ve starter kültür kullanımı TBA sayısını azalmış ($P<0.05$) fakat pH ve nitrosomyoglobin dönüşümü oranını arttırmıştır ($P<0.05$). Nisbi nemin artmasıyla TBA sayısı ve nitrosomyoglobin dönüşüm değerleri artmış ($P<0.05$) fakat bu pH değerinde herhangi bir değişime neden olmamıştır. Kalıntı nitrit miktarı nitrit/nitrat eklenen sucuklarda olgunlaşma süresinin ilk beş gününde hızla azalmıştır ($P<0.05$). Kalıntı nitrit miktarı 30°C' de saklanan sucuklarda en az olduğu bulunmuştur.

Histamin miktarı sucukların olgunlaşma ve saklama süreleri boyunca 0.00 ile 292.66 mg/kg aralığında değiştiği görülmüştür. Starter kültür ve katkı maddeleri sucuklarda histamin, tyramin ve putresin oluşumunu sınırlamıştır ($P<0.05$). En fazla ($P<0.05$) histamin oluşumu 80 % RH' de saklanan sucuklarda olmuştur. Tyramin, histamin ve putresin miktarlarının B9 numaralı sucuk tipinde en az ($P<0.05$) oldukları gözlenmiştir. Putresin miktarının pH miktarıyla ters orantılı olduğu tespit edilmiştir. Putresin oluşumu katkı maddesi ve starter kültür kullanımıyla azalmıştır ($P<0.05$). Katkı maddelerinin tryptamin ve poliaminler (spermidin ve spermin) oluşumu üzerine herhangi bir etkisinin olamadığı ($P>0.05$) tespit edilmiştir. 1,7-diamino heptan, serotonin, spermidin ve spermin miktarlarının sucukların olgunlaşma ve saklama süreleri boyunca sırasıyla 0.00-43.54, 0.00-57.26, 0.00-30.60 ve 0.00-31.29 mg/kg değerleri arasında değiştiği bulunmuştur.

Sucukların toplam duyusal kalitesi olgunlaşma süresinin ilk günlerinde artmıştır. En az ($P<0.05$) toplam duyusal kalite sucuk tipi A1'de olduğu gözlenmiştir. Starter kültür ve katkı maddeleri sucukların toplam duyusal kalitesini arttırmışlardır ($P<0.05$). Nitrit/nitrat sucuklarda arzu edilen rengin (nitrosomyoglobin) ve aromanın oluşumunu arttırmış; askorbik asit ve α-tokoferol lipid oksidasyonunu azaltmış; ve fosfatlar yapısal özelliği arttırmıştır.

Sucukların saklama süresinde mikrobiyal, kimyasal ve toplam duyusal kalitesini tahmin etmek için basit denklemler kullanılmıştır. Basit modellerin deneysel verilere tam anlamıyla uydukları tespit edilmiştir.

Geleneksel olarak üretilen sucukların APC ve küf ve maya sayıları fabrikada üretilen sucuklara göre daha yüksek ($P<0.05$) bulunmuştur. pH değeri ve kalıntı nitrit miktarlarının fabrikada üretilen sucuklarda geleneksel olarak üretilen sucuklara göre daha yüksek olduğu ($P<0.05$) bulunmuştur. Geleneksel olarak üretilen sucukların daha baharatlı olduğu ve baskın aromanın baharatlardan kaynaklandığı gözlenmiştir. Genellikle, fabrikada üretilen sucuklarda ise arzu edilen aromanın oluşmadığı tespit edilmiştir.

Anahtar Kelimeler: Sucuk, dayanırlılık, kalite, güvenirlik, biyojenik aminler, TBA sayısı, kalıntı nitrit miktarı

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To My Family

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

RH	: Relative Humidity
APC	: Aerobic Plate Count
LAB	: Lactic Acid Bacteria
TBA	: 2-Thiobarbituric Acid
Trad	: Traditional
Firm	: Firm-made
TA	: Tryptamine
PA	: β-phenyethyl amine
PUT	: Putrescine
Cd	: Cadaverine
HA	: Histamine
Dia	: 1,7-Diamino Heptane
Ser	: Serotonin
TYR	: Tyramine
Spd	: Spermidine
Sm	: Spermine
HPLC	: High Performance Liquid Chromatography
NED	: N-(1-Naphtyl) ethylene-diamine dihydrochloride
TEP	: 1,1,3,3- tetraethoxypropane
hr	: hour

CHAPTER I

INTRODUCTION

Sucuk, the Turkish dry-fermented sausage, is a fermented dry sausage produced considerably in various parts of the Turkey as traditional methods. Production sucuk types and its constituents vary form region to region depending upon the taste of the people.

Sucuk is very popular meat preserved product in Turkey and similar products are known in most Middle East Countries and in Europe (Erkmen, 1997). Traditionally produced sucuks ripened at ambient climate. Sucuk is made from a mixture of lamb or beef. Meat (about 18 % fat) is mixed with tail fat, salt, sugar, cleaned dry garlic, spices, $NaNO_2$, $NaNO_3$, and vegetable oil (Erkmen, 1997). Sucuk dough is filled into natural or artificial casings and dried under the environmental conditions, and therefore under these circumstances some undesired bacterial contamination can occur which effect their stability and safety (Gökalp *et al.*, 1999).

A standardized and technological method has not been developed and adapted for sucuk production throughout the country (Gökalp, 1995). Thus, serious economical and health problems are may arise the lack of good processing technologies, and standardized methods. Many quality characteristics of the sucuk, i.e. color, flavor, odor, texture may not be developed satisfactorily; also some toxic materials such as biogenic amines are formed during fermentation (Gökalp, 1995; Erginkaya and Var, 1989; Bozkurt and Erkmen, 2002a, b). Traditional sucuks are produced by chance inoculation of lactic acid bacteria. Sometimes, amine-positive bacteria may be contaminated from the environment during the ripening and storage periods of the sucuks (Gökalp, 1995). This may result in the losses of economical values as well as safety of the consumption.

Biogenic amines are toxic substances; they can cause nausea, respiratory distress, hot flushes, sweating, heart palpitation, bright red rash, oral burning and hyper or hypotension (Gonzales-de-Liano *et al.*, 1998). Histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenyl ethylamine, spermine, and spermidine are the most important biogenic amines (Shalaby, 1996). Histamine intake of 8-40 mg, 40-100 mg and higher than 100 mg, may cause slight, intermediate and intensive poisoning, respectively (Parente *et al.*, 2001). Nout (1994) pointed out that maximum histamine contents may be 50-100 mg/kg in sucuks. The allowable maximum level of tyramine in foods is 100-800 mg/kg and 1080 mg/kg of tyramine is toxic (Shalaby, 1996). Putrescine, spermine, spermidine, and cadaverine have no adverse health effect, but they may react with nitrite to form carcinogenic nitrosamines and also can be proposed as indicators of spoilage (Hernandez-Jover *et al.*, 1997a; Eerola *et al.*, 1997). Tyramine, histamine, and tryptamine were find in sucuk that were retailed in local markets (Ayhan *et al.*, 1999; Şenöz *et al.*, 2000).

Nitrate and nitrite can give rise to very toxic and carcinogenic effect (Shalaby, 1996). There are three potential problems currently associated with cured meat due to the presence of residual nitrite (consumption of which increases the total body burden of nitrite), nitrosamines and biogenic amine. Since nitrate can bacterially reduced to nitrite that is precursor of the various N-nitrosamines, which are very carcinogenic compounds

Lipids become rancid as a result of oxidation and this oxidative rancidity is a major cause of food deterioration and decrease the shelf life of foods. The lipid oxidation gives products that change the meat quality, e.g. the color, flavor, odor, texture and even the nutritional value. Therefore, the lipid oxidation has been accepted as one of the major mode of quality deterioration in meat products (Fernandez *et al.*, 1997). As a result of oxidative bitterness, malonaldehyde is formed in meat and meat products. It can cause cancer and mutation (Brewer and Wu, 1993).

Chemical and microbiological changes occur during the processing and storage of the foods. These changes affect the quality, safety and stability of foods. Predictive modeling including mathematical equation is used to define these changes in the food material. Models are valuable tools for predicting the chemical and microbial products during the production and storage periods of the foods (Whiting and Buchanan, 1994). Microbial models can estimate potential risk from pathogens and spoilage microorganisms in a food after normal storage period. Chemical models can also predict the products of chemical reaction occurring during the process and storage periods. These chemical and microbial models can aid in the prediction of microbial or chemical toxins production during the process and storage in the foods by means of these models can highlight the safety of the foods (Labuza *et al.*, 1992).

1.1. Aim of the Research

In this work, aerobic plate count (APC), mold and yeast count, and lactic acid bacteria (LAB) count were monitored as microbial content in sucuk during the ripening and storage periods. pH values, 2-thiobarbituric acid (TBA) values, nitrosomyoglobin conversion, residual nitrite level and biogenic amine formations were monitored for chemical changes in sucuks during the ripening and storage periods. Organoleptic properties (color, flavor and cutting scores) of sucuks were followed by overall sensory quality of sucuk types during the ripening and storage periods. These microbial, chemical and organoleptic attributes were also determined in sucuks retailed in local markets (firm-made) and butchers (Traditional).

Therefore, aim of the research was to;

1. monitor the **microbial** (aerobic plate, lactic acid bacteria, and mold and yeast counts) changes during the ripening and storage periods
2. monitor the **chemical** (pH, TBA values, nitrosomyoglobin conversion, residual nitrite and biogenic amine formation) changes during the ripening and storage periods
3. monitor **sensory attributes** (color, flavor and cutting scores) of sucuk which occur during the ripening and storage.
4. detect the effect of various **additives (nitrite, nitrate, α-tocopherol, ascorbic acid, potassium pyrophosphate, di-potassium hydrogen phosphate)** on microbial, chemical and sensory attributes of sucuk during the ripening and storage periods
5. detect the effect of **pH changes** on microbial, chemical and sensory attributes of sucuk during the ripening and storage periods
6. detect the effect of **storage temperature** on microbial, chemical and sensory attributes of sucuk during the ripening and storage periods
7. detect the effect of **% RH** on microbial, chemical and sensory attributes of sucuk during the ripening and storage periods
8. develop **predictive empirical equations** that describe the microbial, chemical and organoleptic changes in sucuk during the storage periods.

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. Typical values for the different components in skeletal muscle (lean) are given in Table 2.1; the actual composition for a given muscle will vary with the breed, age, sex, and diet of the animal as well as its anatomical location.

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 is must 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. Sucuk

Sucuk (Turkish dry-fermented sausage) is a fermented dry meat product. Minced meat and fat are mixed with salt, sugar, spices, and little amount of other additional substances. These are filled into natural or artificial casings. Then, at specific temperature and moisture content they are ripened and dried so these products are called as "fermented dry meat products".

Sucuk is a very popular meat product in Turkey and similar products are known in most Middle East countries and in central Europe. Sucuk is made from a mixture of fresh meat that may include beef or lamb. Meat (90 kg meat with 18 % fat) is mixed with tail fat (10 kg), salt (2 kg), sugar (sucrose 0.4 kg), clean dry garlic (1 kg), spices (cumin, cinnamon, allscipe, clove, red pepper, and black pepper) $NaNO_3$ (0.033 kg), $NaNO_2$ (0.005 kg) and vegetable oil; and this mixture is known as sucuk dough. Sucuk dough is filled into casings. Sucuk is then hung on sticks to ripening and dried at a controlled temperature and a relative humidity, stored at refrigeration temperatures later (Erkmen, 1997). Composition of sucuk collected

Table 2.1 Approximate composition of lean meat (percent wet basis)

Component	Composition (%)
Water	75.0 (range 65-80 %)
Protein	18.5 (range 16-22 %)
Lipids	3.0 (range 1.5-13 %)
Non-protein nitrogenous substances	1.5
Free amino acids	0.3
Carbohydrates	1.0
Inorganic constituents	1.0

from retail market is given in Table 2.2 (Kolsarıcı *et al.*, 1986). Some products can contain flavor enhancers, polyphosphates and other emulsifiers, vitamin C, coloring matter and various protein supplements (derived from soy milk and possibly cereals and peanut) (Kolsarıcı *et al.*, 1986).

The production process is usually considered to be finished when 50 % of the original weight has been lost. Initial salt contents are low and phosphate is usually not needed. The percentage of salt increases proportionally as sucuk dries. The rate of drying must be carefully controlled to prevent the outside of sucuk drying out too rapidly creating a case-hardened surface which would inhibit the drying of the center and hence cause spoilage. Humidity control is therefore important throughout the drying stages (Lücke, 1994).

In Europe, sausage is most important in total meat production. Bratwurst, mettwurst, wienerwurst, the many hard salamis of Italian, German and Swiss origin are very popular in their market. Over a hundred million kg of sausages are produced in the United States annually. In USA sausages named as "fermented sausage" are categorized into two groups which are dry and semi-dry sausages. Dry sausages (Italian salami or Italian-style salami "Milano", "Sicilian", "Genoa", German salami, pepperoni, and hard salami), their ripening periods change between 10 and 100 days, and these type products are not smoked. Dry sausages ready to sell contain about 35 % of water (Oliphant, 1998).

Table 2.2 Composition of sucuk

Components	Composition		
	Minimum	Maximum	Mean
Water (%)	36.84	49.22	42.7
Fat (%)	25.13	40.15	33.23
Protein (%) Nx6.25	10.85	21.13	17.31
Ash (%)	2.73	4.66	3.79
Salt (%)	2.18	3.30	2.65

2.2.1. Selection and Preparation of Raw Material for Sucuk Production

In sucuk production, meats should complete rigor mortis phase. Use of very old animal meats is not preferred because of excessive connective tissue. If very young animal meats 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).

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. As well as, 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, characteristics of 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 meats has high water holding capacity so causes some problems during the ripening. Excessive amount of microorganisms must not present in meats. Meat storage temperature must not exceed 2°C. Meats must be high quality and fresh (Hamm, 1986).

2.2.2. Casings

Generally, casings are divided into two major groups. These are natural casings and artificial casings (Honikel, 1989; Oğan, 1996).

Natural casings are processed from various parts of the alimentary tract of cattle, hogs or sheep. About 1 % of sucuk weight consists of casing. Casings are normally packed in salt. Before use they should be thoroughly soaked and washed in lukewarm water at about 29 to 32°C. After washing the casings should be kept wet at all times until they are filled. Some smaller manufacturers still prefer to use them (Oliphant, 1998; Piette and Postec, 1993).

Three natural casings are used; the hog-born for liver sausage, the small intestines of the hog, and for a variety of European type sausages; the sheep intestines are generally used for breakfast link sausage. For example, skinless wieners are made with an artificial casing which is peeled off after smoking. Various kinds of sausages are on the market such as cold cuts and lunch meats. Some of them are precooked, other require cooking.

The advantages of **artificial casings** over natural casings lie in their uniformity of size, the absence of risk of contamination from improper preparation and the ability in most cases to be used without preliminary washing, soaking, etc. They are of several types such as cellulose, polyvinyl chloride (PVDC), PVDC/fibrous and collagen casings (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 amounts of peptides and amino acids and small amount of sugar (glucose and glucose-6-phosphate). The content of these fermentable sugars 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 only 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 amounts 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. Factors Affecting Fermentation, Ripening and Storage of Sucuk

Some factors affect the product quality during the fermentation steps of sucuk. Parameters affecting the quality of sucuks are given in Table 2.3. They are divided into two major groups; external factors and internal factors (Lücke, 1994; Oliphant, 1998; Gökalp *et al.*, 1999).

2.2.4.1. External Factors

External factors important for the microbial growth and chemical reaction in a food include the environmental conditions in which it is produced and stored. These are relative humidity, temperature and air condition.

2.2.4.1.1. Temperature

Temperature is the most important factor with respect to microbial growth and chemical reaction. Chemical reaction rate (lipid oxidation and browning) is decreased with the reduced temperature. When the foods are exposed to temperatures beyond the maximum and minimum growth temperatures of microorganisms, cells die rapidly as a result of the irreversible denaturation of proteins. Temperature has an effect on the pH, texture, a_w and water portion of sucuks. Normally, sucuks are ripened at 12-24°C and stored at 10-15°C (Lambert *et al.*, 1991; Lücke, 1994; Gökalp, 1995).

2.2.4.1.2. Relative Humidity

It is the degree of saturation between air and water vapor. When foods having a high water activity are stored in atmosphere of low relative humidity, water will transfer from the food to the atmosphere. So, drying of the food material is attained. Drying rate is important because sucuk should be sliceable without breakage and has a proper texture. After filling of sucuks, they are stored at 60 % relative humidity (RH) between 2 and 6 hr at 20-25°C. During the ripening; in the first three days, 20-25°C and 90 % RH are applied and then RH is decreased to 85 %. Through the end of maturity RH must be 80 %. Relative humidity of the environment changes between 70 and 75 % (Lambert *et al.*, 1991).

2.2.4.1.3. Air-Circulation

Velocity of air is measured by anemometer (meter/second). Air circulation affects the drying of sucuk. If air circulation is very high, edges of sucuk dry quickly and a dry cover occurs at the outside of sucuk. Air circulation must be turbulent and must not be one way to get proper drying and so texture. At first days of ripening, air circulation velocity must be 0.5-1 m/s, and decreased to 0.1-0.2 m/s in other days. In the storage of sucuks, air circulation must be 0.05-0.1 m/s (Gökalp, 1995).

2.2.4.2. Internal Factors

2.2.4.2.1. Raw Material

Raw materials for sucuk are meat and fat. Meat composition (amount of carbohydrate, lipid, water and protein) determines the progress of the fermentation and ripening (Heperkan and Sözen, 1988; Lambert *et al.*, 1991). Microorganisms, like all living systems, have specific carbon, nitrogen, mineral and vitamin requirements. Meat and meat products are generally considered a rich source of nutrients. Accordingly, they can support the growth of a wide variety of microbial species. However, addition of specific nutrients to meat formulations has been shown to influence the physiological characteristics of contaminating microorganisms. For example, the addition of certain spices to sucuk stimulates bacterial lactic acid production. This stimulatory effect is due to the spices acting as a supplemental source of manganese and trace mineral that is a cofactor for lactic acid production in *Lactobacillus* and *Pediococcus* and that is generally limiting in meat products.

2.2.4.2.2. pH

pH decreases during fermentation of sucuk with the lactic acid accumulation. Normally, the initial pH of sucuk is about 6.10-6.20. The final pH of sucuk is largely dependent on the amount of fermentable carbohydrate in the formulation. For sucuk pH should be in the range from 4.80 to 5.40 with optimum 5.10-5.20. If pH is higher than 5.80, that sucuk easily spoils (Gökalp *et al.,* 1999; Turkish Food Codex, Anonymous, 2000).

The initial pH (6.1-6.2) of sucuk is well within the range that permits growth of most bacteria, yeasts and molds (Jay, 1978), and it would be expected that a wide range microbial species could effectively compete in such an environment (Buchanan, 1986).

2.2.4.2.3. Water Activity

Microorganisms need water to survive and multiply. Water activity is the measure of water useful for microorganisms. Meat and meat products that have high water activity spoils at less tie than the low pH products such as sucuk. The water

Table 2.3 Parameters affecting the quality of sucuks (Lücke, 1994)

Parameter	Variables (examples)	Guidelines
Raw material	Animal species (beef/poultry)	pH ≤5.8; good microbial quality; no antibiotics
	Age at slaughter	
	Fats/oils	
	Types of fatty tissue (back/belly)	
	Formulation (fat content)	
Additives	Sodium chloride	Initially a_w 0.955-0.965
	Nitrite/Nitrate	Addition of 300/500 ppm
	Sugar content	0.2-0.7 %
	Sugar type (glucose, sucrose, lactose)	0.2-0.5 % of rapidly fermentable sugar
	Lactic acid bacteria	pH reduction to ≤ 5.3 during fermentation
	Micrococcaceae	
	Ascorbate	
	Spices	
Comminution	Method (grinder/cutter)	Low temperature (to avoid melting of fat)
	Degree (coarse/fine)	
Filling	Filling equipment	No air inclusions
	Casing material (natural/collagen based/cellulose based)	Permeability high for vapor and smoke, low for oxygen; shrinkable, peelable
	Casing diameter	
Ripening	*Fermentation climate*	
	- temperature	≤ 25°C
	- time	Until pH ≤ 5.3
	- humidity (ERH %)	No vapor condensation; ERH in chamber 5-10 units below ERH of product
	Ageing/drying climate	
	- temperature	≤ 15°C until a_w < 0.90
	- humidity (ERH %)	ERH in chamber 10-15 units below ERH of product
	- air movement	Uniform drying
	- time	
Surface treatment	Smoke	No growth of undesired molds
	Mold starter	

activity level of sucuk decreases with water loss during ripening of sucuk. Adjustment of sucuk water activity is possible by changing the salt and fat amount added.

In general, decreasing the water activity of a meat system by addition of NaCl and other humectants or by partial dehydration shifts the microflora to more salt tolerant genera such as *Lactobacillus, Streptococcus, Staphylococcus, Vibrio* and yeasts and molds (Buchanan, 1986).

During ripening of sucuk, the initial relative humidity (90%) should be decreased after third day (Gökalp, 1999).

2.2.4.2.4. Oxygen and Other Gases

Processed meat products are highly reduced, and accordingly the oxygen content can vary considerably within the product. For example in sucuk-type products, the surface of the meat will be highly aerobic, favoring the growth of aerobes and facultative anaerobes (Lücke, 1986). However, a short distance beneath the surface, the amount of available oxygen is greatly decreased and microaerophiles, anaerobes and facultative anaerobes would be expected to predominate. It could be anticipated that more aerobically oriented genera such as "*Moroxella*" or "*Brochothrix*" would predominate on the surface, while microaerophiles such as *Lactobacillus* would thrive in the interior (Buchanan, 1986).

The use of a casing that reduces the oxygen content of the atmosphere surrounding the product retards the growth of aerobic or noncompetitive facultative anaerobic species (e.g; *Pseudomonas, Brochothrix* and *Moroxella*), while faster the growth of microaerophiles such as *Lactobacillus* and yeast.

2.2.4.2.5. Salt and Sugar

The acid formation during the fermentation and ripening depends on the amount and kind of the sugar added. Growth of acid reduces surface micro-flora. The enough pH reduction can be provided with the addition of fermentable sugar as little as 0.2 %. If excess sugar is added undesired flavor and acid formation (lactic acid) occur, so a sour taste is obtained. Salt is used for taste and is effect the chemical and microbiological evidence (Alperdem, 1986). According to TSE salt content of sucuk must not exceed 5 %.

2.2.4.2.6. Initial Number of Starter Culture

The length of the apparent lag phase before measurable acid formation depends on the initial number of starter culture and adapted to conditions. Addition of lactic acid bacteria reduces the fermentation time, provide color stability and aroma during drying period, reduces nitrate and inhibits pathogenic microorganisms (Turantaş and Ünlütürk, 1993).

2.2.4.2.7. Antimicrobial Preservatives

The inhibition of the growth and activity of microorganisms is the main purpose of the use of antimicrobial preservatives. These antimicrobials deteriorate the cell wall and membrane of microorganisms, and inhibit the enzymes which play important roles in metabolism of microorganisms.

The most common preservatives used in sucuk production and other meat products are sodium nitrate and sodium nitrite. These compounds are well known for their activity against clostridial species such as *Clostridium botulinium* and *Clostridium perfringens*. Nitrate has also been shown to inhibit *Staphylococcus aureus,* although this activity is effectively limited to anaerobically cultured cells (Smith and Palumbo, 1981; Turantaş and Ünlütürk, 1993).

Nitrates are decomposed to nitric acid, which forms nitrosomyoglobin when it reacts with the heme pigments in meats and thereby forms stable red color (nitrosomyoglobin; Figure 2.2). Nitrates probably act as reservoir for nitrite and their use is being restricted. Moreover, nitrites can react with secondary and tertiary amines to form nitrosamines, which are known to be carcinogenic (Frazier and Westhoff, 1988).

The other antimicrobial preservatives used in sucuk production are sorbic acid and its Na^-, Ca^- and K^- salts, benzoic acid sodium benzoate, and acetic acid. Salt and sugar have indirect antimicrobial effects (Gökalp, 1999). Sugar increases the acidity of sucuk and changes the osmotic pressure by decreasing water activity.

2.3. Microbial Activity in Sucuks during the Ripening and Storage Periods

Two basic microbial activities proceed during the fermentation period. Namely, reduction of pH by lactic acid bacteria, and formation nitric oxide by nitrate and nitrite reductase activity of microorganisms (Ordonez *et al.*, 1999). The most important microbial activity occur during the ripening and storage periods of dry sucuk belongs to lactic acid bacteria (genera *Lactobacillus, Staphylococcus* and *Micrococcus)*,and molds and yeasts. Microbial load in sucuks ranges from 10^5 to 10^6 CFU/g before fermentation and increased up to 10^8 to 10^9 CFU/g during the fermentation.

2.3.1. Lactic Acid Bacteria

Lactic acid bacteria (LAB) as presently consist of the following four genera: *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* (Erkmen and Fadıloğlu, 2001). The most commonly LAB isolated from dry-fermented sucuk used in starter cultures are *Lactobacillus sake, L. curvatus, L. plantarum, Pediococcus pentosaceus* and *P. acidilactici* (Gürakan *et al.*, 1995; Ordonez *et al.*, 1999). The nitrate and nitrite reducing bacteria present in the fermented sucuk belong to *Staphylococcus* and *Micrococcus* genera (*S. carnosus, S. xylosus, M. varians*, etc). Sharp increase in aerobic and lactobacilli counts and small increase in *Micrococcaceae* count are observed during the fermentation period (Ordonez *et al.*, 1999). LAB has a number of beneficial effects on the manufacturing process, and quality and shelf-life of sucuks. These are (Ordonez *et al.*, 1999);

1. It facilitates the conservation process that inhibits undesirable microorganisms.
2. It helps to develop the texture due to reduction of water-retention capacity of proteins, also enhances the drying process.
3. It accelerates the gelatinization.
4. It controls enzymic activities that contribute to aroma.
5. It favors to color formation

2.3.1.1. *Lactobacillus*

Lactobacilli are rod-shaped bacteria which react positively in gram-staining, are unable to form spores and are usually not motile. Characteristic of them is their behavior towards air oxygen and their fermentative metabolism. Lactobacilli cannot make use of air oxygen as a source of energy, that is, they cannot "breathe" and therefore cannot breakdown organic matter (e.g. sugars) completely into carbon dioxide and water. They "ferment" carbohydrates into lactic acid. A sub group of lactobacilli, known as heterofermentative strains, produce not only lactic acid but also large quantities of acetic acid, ethanol and carbon dioxide. In contrast to "obligate" anaerobic bacteria lactobacilli can usually tolerate air oxygen and thus also grow on aerobically incubated culture media. They are relatively easy to recognize on it because, unlike almost all other gram-positive, aerobically growing rods, they produce no-catalase. Catalase breaks down hydrogen peroxide, a product of the reaction of air oxygen with certain cell constituents. Hydrogen peroxide is also produced in large quantities from air oxygen by many *Lactobacillus* strains. The fact that they nevertheless grow aerobically is due to their great tolerance to hydrogen peroxide (Erkmen and Fadıloğlu, 2001).

The acids formed by sugars from lactic acid bacteria are a great help in suppressing undesirable bacteria in the dry sucuk. Lactobacilli are greatly responsible for carbohydrate break down and thus for the acid aroma component of dry sucuk (Lücke, 1994), and also affect texture and hygienic stability (Sanz *et al.*, 1997).

2.3.1.2. *Micrococcus* and *Staphylococcus*

Bacteria of the genera *Staphylococcus* and *Micrococcus* are gram-positive spherical bacteria. They have catalase and grow anaerobically and the species *Staphylococcus xylosus, S. saprohyticus, S. carnosus* and *Micrococcus* varians have been mainly found in dry sucuk (Lücke, 1994; Erkmen and Fadıloğlu, 2001). The numbers of these bacteria increase during the first days of ripening period (fermentation) due to the high water activity and then decrease during the further ripening and storage periods (Ordonez *et al.*, 1999). They are sensitive to the decrease in pH. Therefore, fermentation is a critical process in the manufacturing of dry-fermented sucuks and pH reduction should be slowly to allow the growth of *Micrococcaceae*. If the *Micrococcaceae* do not grow well, reduction of nitrates and nitrites are hindered (Lücke, 1994).

Bacteria of *Micrococacceae* family, naturally developed or added as starter culture, accomplish an essential role to the reduction of nitrate to nitrite by means of their nitrate reductase activity in dry fermented sucuks. This activity allows the production of nitrite which contributes to preservation and both color and flavor (Sanz *et al.*, 1997). The catalase of *Micrococacceae* which breaks down peroxides, including hydrogen peroxide produced by Lactobacilli and rancidity is inhibited. *Micrococacceae* play an important role in the fermentation of sucuks such as their ability to break down fat (lipolysis) and protein (proteolysis) which have great importance in the development of flavor, also stabilize the color in dry sucuk (Lücke, 1994; Bover-Cid *et al.*, 1999; Erkmen and Fadıloğlu, 2001). Proteolysis occurs during the fermentation of sucuks and favored by the denaturation of proteins as a result of the acidity, drying and the effect of salt (sodium chloride).

2.3.2. Molds and Yeasts

A surface flora consisting of molds and yeasts is formed during the drying of dry sucuks provided that the relative humidity of the ripening chamber is not too low. Because of the relatively low temperatures prevailing during drying the mold flora consists principally of *Penicillum* species, *Penicillum verrucosum* species being those isolated most often. *Aspergillus* species (mainly representatives of the *Aspergillus glaucus* group) are more likely to be found on products that have been stored too warm. The yeasts present are chiefly strains of the genus *Debaryomyces* adopted to high common salt concentrations (Erkmen and Fadıloğlu, 2001).

The surface flora of many air-dried dry sucuks, which consists mainly of molds of the genus *Penicillum*, protects the products from harmful effects of air oxygen and light, makes drying sucuks easier and gives them their typical aroma by breaking down fats, proteins and lactic acid (Lücke, 1994).

2.3.3. Starter Cultures and Their Effects

The primary genera of bacteria, which have been successfully utilised as fermented sucuk starter cultures are *Lactobacillus, Micrococcus* and *Pediococcus, Staphylococcus* and of yeasts *Debaryomyces,* and *Penicillium* (Erkmen and Fadıloğlu, 2001) Among the micrococcus, the widely used one is *Micrococcus aurantiacus,* and 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. Researchers have also determined that the addition of certain strains of molds and yeasts, especially yeast *Debaryomyces hansenii*, to the European fermented sausage mixture, had been advantageous and utilized for unique and more aromatic flavor and better and homogeneous color development and inhibiting surface "flowering" (Lücke, 1994).

Table 2.4 lists microbial species available as a starter culture in the fermentation of sucuk. Many of these, in particular the psychotrophic, salt tolerant *Lactobacillus* species (such as *L. sake* and *L. curvatus*), *Micrococcus* species, nonpathogenic *Staphylococcus* species, *Debaryomyces hansenii* and *Penicillium nalgiovense* (Anderson, 1997; Lücke, 1994).

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, preventing the formation of some biological amines such as histamine, tyramine, it inhibits the formation of nitrosamine from nitrates which is added to dough as curing component, it increases the nutritive values of product and as a result their assist in sucuk to be higher in quality standard and long shelf life of sucuk formation (Smith and Palumbo, 1981, Vural and Öztan, 1991).

The microorganisms should have following properties for using as sucuk starter culture:

1. Microorganism should be halophilic and nitrite tolerant.
2. Microorganisms should be active between temperatures of 15-43°C.
3. They shouldn't produce biogenic amines and metabolites that show pathogenic, toxic and mutagenic properties.
4. Starter cultures shouldn't be pathogenic.
5. Certainly, they should produce lactic acid. If lactic acid bacteria are used, they should be homofermentative.

Table 2.4 The list of sausage starters and the role of microorganisms in sausage fermentation

Microbial group	Species used as starters	Useful metabolic activity	Benefits to sausage fermentation
Lactic acid Bacteria	*L. plantarum, L. pentosus, L.sake, L. curvatus, P.pentosaceus, P. acidilactici*	Formation of lactic acid and bacteriocins	Inhibition of pathogenic and spoilage bacteria Acceleration of color formation and drying
Catalase-positive Cocci	*S. carnosus, S. xylosus M. varians*	Nitrate reduction and oxygen consumption	Color formation and stabilization Removal excess nitrate
		Peroxide destruction	Delay of rancidity
		Lipolysis	Aroma formation
Yeasts	*Debaryomyces hansenii*	Oxygen consumption	Delay rancidity
		Lipolysis	Aroma formation
Molds	*Penicillium nalgiovense* biotype 2, 3, 6	Oxygen consumption	Color stability
		Peroxide destruction	Delay of rancidity
		Lactate oxidation	Aroma formation
		Peoteolysis	Aroma formation
		Lipolysis	Aroma formation

6. The selected lactic acid bacteria shouldn't be proteolytic or lipolytic.
7. Microorganisms should tolerate other starters and be synergist.
8. Microorganisms should reduce nitrates, be catalase positive and not produce hydrogen peroxide.
9. Microorganisms should affect positive to the flavor of product.

Sucuk has been made with chance inoculation of lactic acid bacteria (Gökalp *et al.*, 1999). The pure cultures of *Lactobacillus casei* var *alactosus* or *Pediocuccus cerevisiae* (Özer and Özalp, 1968) and *L. plantarum* and *P. cerevisiae* could be used as sucuk starter cultures.

2.4. Chemical Changes in Sucuk during the Ripening and Storage

2.4.1. Acid Formation (pH Reduction)

In fermented products lactic acid bacteria, especially lactobacilli have a great importance. Acids formed (mainly lactic acid) from the sugars by lactic acid bacteria following glycolitic pathway (Figure 2.1). 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).

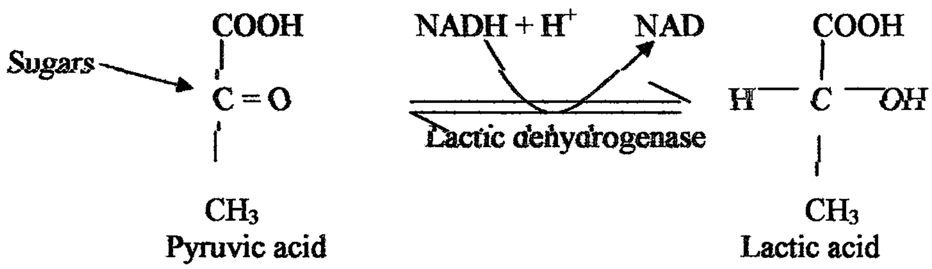


Figure 2.1 Glycolitic pathway for lactic acid formation

Microbial acidity has a great importance on the quality of final product from the microbiological point. Acid formation rate depends on the activity of lactic acid bacteria, ripening temperature and ability of lactic acid bacteria to ferment sugars.

The rate of acid production is determined by the type and amount of added carbohydrate, the species of lactic acid bacteria, the rate of drying and the temperature. The initial water activity also affects rate of acid formation. Acid formation may be too slow to inhibit *Staphylococcus aureus* at a_W values below 0.955 (Lücke, 1994). Generally, glucose, sucrose, and maltose are fermented rapidly, and lactose slowly, but the different sugar fermentation pattern of various lactic acid bacteria must be taken into account (Lücke, 1994).

2.4.2. 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 (Gray, 1978; 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).

In the meat products, besides microbiological, chemical and physical spoilage oxidative rancidity are also important. Determination of some intermediates, which occur as a 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 which occurs as a result of oxidative bitterness in meat and meat product is terms of human health because of the effect of this component, that causes cancer and mutation. It is desired to be occurring at low level during the storage of product and their marketing (Stu and Draper, 1978). Although sensory analysis is one of the most sensitive methods available, it is not practical for routine analyses and generally lacks reproducibility. Consequently, many chemical and physical methods have been developed to quantify oxidative deterioration with the object of correlating the data with off flavor development.

A number of methods are available for determination of lipid oxidation in foods (Pikul *et al.*, 1989). The 2-thiobarbituric acid (TBA) test has been widely used for measuring of oxidative rancidity in fat containing foods especially muscle of foods. In the result of the oxidation of unsaturated fatty acid malonaldehyde occurs as a color formation component. Color development during the TBA test is usually assessed by measuring the absorbance of the red pigment at 532 nm. Furthermore, compounds other than malonaldehyde may have demonstrated absorbance at the wavelengths used in the analysis (Melton, 1983; Rhee, 1978) and red color may be developed in the presence of certain carbohydrates.

The TBA test is a colorimetric analytical technique in which the absorbance of red chromogen formed between TBA and malonaldehyde is measured spectrophotometrically. The TBA test can be performed on:

1. Direct method; is the reaction between TBA reagent with whole food and extraction of the red pigment formed (Hoyland and Taylor, 1991).
2. Distillation method; reaction of steam distillate with TBA reagent (Tarladgis *et al.*, 1960).
3. Aqueous acid extract of the food (Salih *et al.*, 1987)
4. High performance liquid chromatographic (HPLC) methods (Christensen and Holmer, 1996).
5. Reaction of previously extracted lipid with TBA (Fernandez *et al.*, 1997).

6. Spectrofluorometric method; reaction of fluorescence compound with TBA reactive compounds (Williams *et al.*, 1983).

The distillation method is the one of the most used methods and while in efficient extraction of malonaldehyde may result from the method, its considered to be more sensitive than the whole sample procedure (Salih *et al.*, 1987). Results have not been entirely satisfactory when the more direct method of whole sample analysis (Ahn *et al.*, 1995) is used, due to the two phase system which is developed in the process. Considerable controversy exists as to the results when traditional TBA methods of analysis to the presence of acid and heat during analysis (Tarladgis *et al.*, 1964; Ahn *et al.*, 1995). Modification of the test procedures might well include addition of antioxidants in order to prevent further oxidation of lipids during analysis (Salih *et al.*, 1987).

2.4.3. Color Formation (Nitrosomyoglobin Conversion)

The characteristic color of dry-fermented sucuk is produced by interaction between the meat pigments (myoglobin) and nitrite and nitrate which are added to sucuk dough (Figure 2.2). Nitrite is the only toxic substance used in the foodstuff and/or allowed for consumption. It has been forbidden to add 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. When nitrite is first added to meat, the color is changed from the purple red color of myoglobin to the brown of metmyoglobin. With time and reducing conditions, the color is converted to the rather dark red of nitrosomyoglobin. Adding more nitrite may cause the formation of effective carcinogenic nitrosamines while in the case of lower quantity there appear a color problem in the product or nitrite could not demonstrate the excepted anti-microbial effect.

The red cured color is formed when the muscle pigment myoglobin reacts with the nitric oxide (NO) produced from nitrite in the acidic medium (Figure 2.2).

Micrococcaceae are responsible for reduction of nitrates and nitrites to nitric oxide and this nitric oxide is used for the production of nitrosomyoglobin during the fermentation period (Vural and Öztan, 1992a; Talon *et al.*, 1999). The same reaction takes place with hemoglobin, the blood pigment which is also to be found in small concentration in the raw materials used for sucuks. Hemoglobin is present in meat and sometimes also in the blood plasma. The nitric oxide/myoglobin or hemoglobin compound, "cured red", is relatively stable to light and oxygen, but is particularly heat-stable. Thus a cured meat product that has been heat treated, such as frankfurter-type sausages, looks red, whilst a product made with no curing substances, i.e. only with common salt, has a grayish-brown color.

The production of nitric oxide from nitrite and the reaction nitric oxide from nitrite and the reaction with muscle or blood pigment is influenced by factors such as temperature, pH, oxygen, and reducing substances, and this means that the development of a good cured color is very dependent on technology (Figure 2.2).

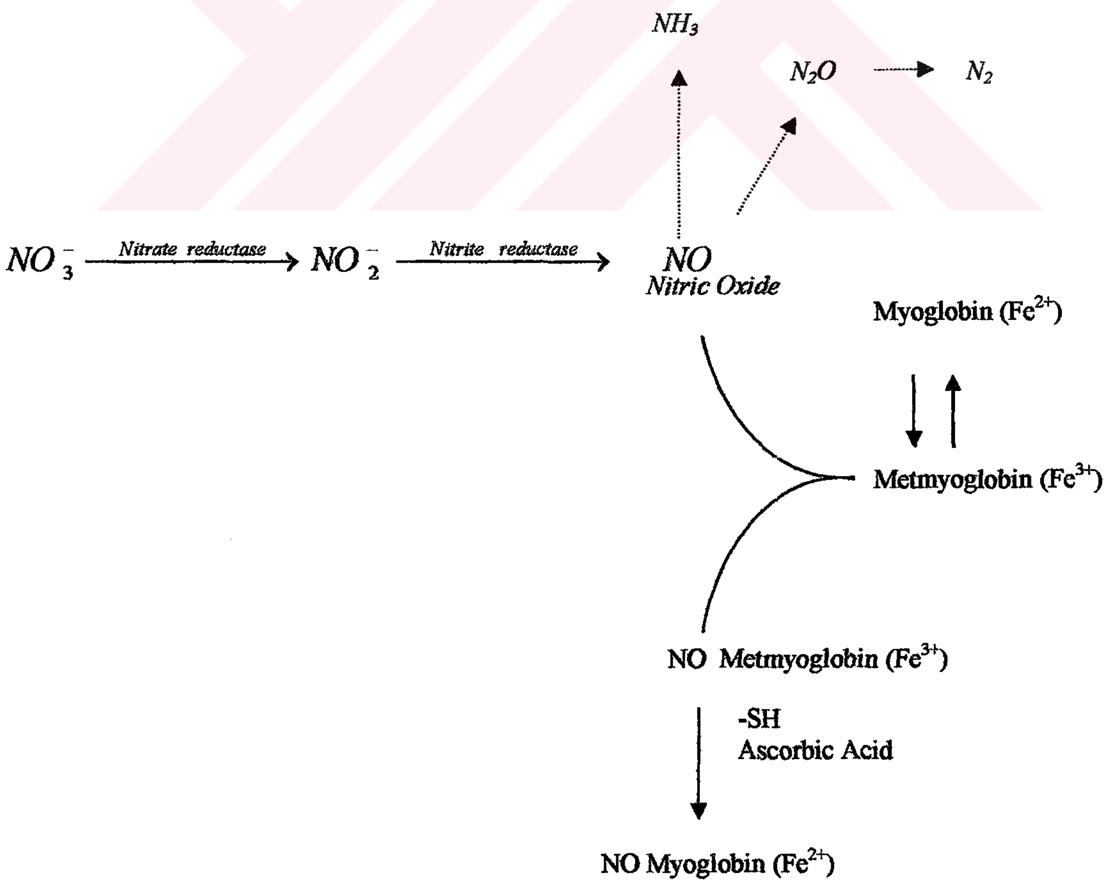


Figure 2.2 Formation of nitrosomyoglobin (Ordonez *et al.*, 1999)

The color formation in meat product in meat product has a close relationship with the pH (Öztan, 1999). The development of color is optimum between pH 5.4-5.7 (Kamarei and Karel 1982). In the sausages, since the level of pH is higher than this limit as technological obligation, the extra average of other components is needed for the development of color (Wirth, 1986; Agullo *et al.*, 1990; Arihara *et al.*, 1993).

Nitrite has an important preserving effect on meat products whereas even at high concentration does not seem to affect the microorganisms growth, at relatively low doses of nitrite inhibits the growth of some bacteria species (Ordonez *et al.*, 1999).

2.4.4. Formation of Biogenic Amines

Biogenic amines may be classified as aliphatic (mono-, di- and poly amines), aromatic, or heterocylic in their structure (Shalaby, 1996). Biogenic amines could be found in meat, sausages, sucuk, milk, chocolate, cheese, fish and some beverages (Eerola, *et al.*, 1993; Shalaby, 1993; Hernandez-Jover *et al.*, 1997a, b; Ordonez *et al.*, 1997; Durlu-Özkaya *et al.*, 2001; Bozkurt and Erkmen 2002a, b).

Biogenic amines are toxic substances; they can cause nausea, respiratory distress, hot flushes, sweating, heart palpitation, bright red rash, oral burning, gastric, intestinal problems, and hyper– or hypotension (Gonzales-de-Liano *et. al.*, 1998; Durlu-Özkaya *et al.*, 2001). Histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenyl ethylamine, spermine, and spermidine are the most important and common biogenic amines found in foodstuffs (Shalaby, 1996). Histamine intake of 8-40 mg, 40-100 mg and higher than 100 mg, may cause slight, intermediate and intensive poisoning, respectively (Parente *et al.*, 2001). Nout (1994) pointed out that maximum histamine contents is 50-100 mg/kg for sausages. The allowable maximum level of tyramine in foods is 100-800 mg/kg and 1080 mg/kg of tyramine is toxic for humans (Shalaby, 1996). Putrescine, spermine, spermidine and cadaverine have not adverse health effect, but they may react with nitrite to form carcinogenic nitrosamines and also can be proposed as indicators of spoilage (Hernandez-Jover *et al.*, 1997b; Eerola, *et al.*, 1997).

Biogenic amines can be found in meat products due to the decarboxylase activity of microorganisms during the manufacturing and storage. The formation of biogenic amines depends on the ripening and storage conditions of product, presence of amine positive microorganisms, free amino acids required for amine formation, quality of raw materials and hygienic conditions of processing environment. They have been proposed as possible indicators of poor hygienic quality of raw materials and poor process conditions of some foods (Vidal-Carou *et al.*, 1990; Maijala, *et al.*, 1995; Shalaby 1996; Gonzalez-de-Liano *et al.*, 1998; Ordonez *et al.*, 1999; Durlu-Özkaya *et al.*, 2001).

Some symptoms for biogenic amines are reported in the literature (Shalaby, 1996). Tryptamine has toxic effect on the human beings as increasing the blood pressure therefore causes hypertension, but there is no regulated the maximum amount in some countries for tryptamine consumption in sausages. β-phenyl ethylamine releases noradrenaline from sympathetic nervous system, increases the blood pressure and causes migraine. Putrescine leads to hypotension, bradycardia, lockjaw, paresis of extremities and potentiate the toxicity of the other amines. Also, putrescine is important for their capability of pyrrolidine, which is a one of the carcinogenic nitrosamine, is formed when sucuks are heated. Polyamines (spermidine and spermine) have physiological functions related to cell stabilization and DNA, RNA and protein synthesis; however, they have some pathological cases (tumors) (Shalaby, 1996).

2.4.4.1. Meat and Meat Products

Tyramine, cadaverine, putrescine, spermine, and spermidine have been reported in meat and meat products (Eerola *et al.*, 1993; Shalaby, 1996; Hernandez-Jover *et al.*, 1997a; Ayhan *et al.*, 1999; Şenöz *et al.*, 2000; Gardini *et al.*, 2002; Bozkurt and Erkmen, 2002a). Most important biogenic amines in fermented sucuk are putrescine, cadaverine, histamine and tyramine. A HPLC based method used for the determination of eight biogenic amines (Cadaverine, histamine, β- phenyl ethylamine, putrescine, spermine, tryptamine and tyramine) in fermented sucuk.

Fermentation may be important in the formation of histamine in certain types of sucuks. Semi-dry sucuks are fermented for short periods often with lactic acid cultures added, while dry sucuks are allowed to ferment from the action of natural microflora for a longer period (Öztan, 1999; Martuscelli *et al.*, 2000). During sucuk ripening process the histamine concentration increases at least ten-fold during the first three days of ripening (Dierick *et al.*, 1974). In general, quite variable quantities of biogenic amines were reported for sucuks. 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 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 ripening (Shalaby, 1996).

Table 2.5 Biogenic amines in foods and their pharmacological effects (Shalaby, 1996)

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

2.5. Functions of Nitrite in Sucuks

Nitrite has several function in meat products such as;

1. Nitrite provides the formation of at bright red-pink color in cured meat products, with the appropriate pH.
2. It helps the curing of the meat product to gain flavor and aroma.
3. The antioxidant feature of nitrite, prevent the rancidity at a large scale, which formed with oxidation of lipids in meat products.
4. It prevents the augmentation of *Clostridium botulinum* in meat products and the formation of toxins, with salts together (Wirth, 1986; Christiansen and Holmer, 1996).

2.5.1. Color Development

Function of nitrite in the color development is given in section "2.4.3 Color Formation (Nitrosomyoglobin Conversion)".

2.5.2. Aroma Development

A typical odor and flavor are produced when nitrite is allowed to act upon meat or meat products and this is clearly distinguishable from those of products treated only with common salt. It is called the "cured aroma". The aroma of heat treated, cured meat products are again different from those of raw cured meat products. Different or additional flavor-providing or number of meat constituent with nitrite or nitric oxides, the reaction partners so far identified are alcohols, aldehydes, inosine, hypoxanthine and, particularly, compounds containing sulfur.

2.5.3. Preservation

Nitrite is inhibitory to a various range of bacteria. A level of 200 mg/ kg at pH 6.0 was sufficient to inhibit strains of *Escherichia, Flavobacterium, Micrococcus, Pseudomanas* and others, although *Salmonella* and *Lactobacillus* species were more resistant. Of most practical importance though is the ability of nitrite to inhibit spore-forming bacteria such as *Clostridium botulinium* which will survive at the heat process applied to many cured meats. To achieve this commercially, initial levels of nitrite greater than 100 mg/kg are used.

Recently, even in relatively low concentrations, inhibits the growth of many spices of micro-organisms. Even those species that cause food poisoning *Clostridium botulinium, Salmonella, Staphylococci* have their growth restricted at concentrations of 80 to 150 ppm. This fact had an important influence on the use of nitrite right up to our own time. But the preservative action of nitrite must always be look at together with other factors such as water activity, pH and temperature.

2.5.4. Antioxidative Effect

Nitrite is an effective antioxidant and has been found to retard oxidation in cooked beef when used at 50 ppm. It was found that nitrite has higher antioxidant power than other antioxidants commonly used as a preservative (Ordonez *et al.*, 1999).

Only lately, it was realized that nitrite also has an inhibiting effect on oxidative fat breakdown in meat products. The effect is to be seen in the completing of pro-oxidative substances in the product, e.g., of iron only products made with common salt is usually subject to a quicker flavor breakdown, and this is particularly true of finely minced product such as frankfurter-type sausage, into which a great deal of air, and thus of oxygen, is mixed during the manufacturing process. Products made with nitrite will keep for a much longer time before definite signs of flavor breakdown become evident than products made only with common salts.

Many cured meats contain substantial amounts lipid, but with the exception of bacon, relatively little consideration has been given to reaction of lipid with nitrite. Bacon has been given an exceptional case because of the relatively frequent occurrence of small quantities of nitrosopyrrolidine in it after heating. The probable pathway of formation is nitrosation of proline, followed by decarboxylation.

2.5.5. Curing Aids

Dietary intake of nitrite is low, generally less than two mg $NaNO_2$ per day and comes mainly from cured meat, although it is also present in fish, cheese, cereals and vegetable products. Nitrate is also of concern since it can be reduced to nitrite by the bodies own microflora. Cured meats are not a significant source; vegetables contribute more than 75 % of the dietary intake of nitrate, although water can be an important source in some areas.

The most effective and successful curing aids for color stabilization is sucuks are ascorbic acid. Ascorbic acid acts more quickly than ascorbate, but this characteristic is not particularly important. Experience has shown that there are no advantages in adding large amounts, in fact if a large overdose is added undesirable reactions are observed (greening and, with ascorbate, a flavor resembling lye.).

Ascorbic acid is a powerful reduction agent, releasing NO from NO_2:

$$\underset{\text{Ascorbic acid}}{C_6H_8O_6} + 2\,HNO_2 \longrightarrow \underset{\text{Dehydroascorbic acid}}{C_6H_6O_6} + NO + 2H_2O$$

With the larger supply of NO a larger quantity of myoglobin is converted into nitric oxide myoglobin ("cured red").

2.5.6. Formation of Nitrosamines

The use of nitrite and nitrate in food has attracted scrutiny since it was discovered in the 1950s that N-nitrosamines, formed by the reaction of nitrite with secondary amines, especially at low pH, can be carcinogenic. Concern developed that they may be present in food or formed in the body as a result of ingestion of nitrate or nitrite with food. Surveys have identified that cured meats, particularly fried bacon, beer and in same countries, fish, make the most significant contribution to dietary intakes of nitrosamines, although a US surveys made the point that a smoker inhealth about 100 times the amount of volatile nitrosamines per day as were provided by cooked bacon (Cassens *et al.*, 1979).

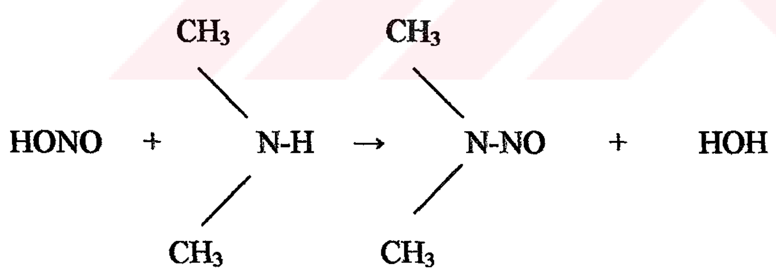
Awareness of the problem has led to changes in the production practices for cured meats such as the use of low levels of nitrite in preference to nitrate and the increased the use of ascorbic acid which inhibits the nitrosation reaction. These measured have produced significant reductions in nitrosamine levels.

The two basic reactions of nitrite to nitrosation are diazotization, and deamination. Nitrosation is of greatest interest to meat curing. Even though N-nitrosation has been considered most often because of formation of N-nitrosamines (usually carcinogenic compounds), C- and S-nitrosation also occur (Özdemir *et al.*, 1984).

In essence, the question is one of the risk/benefit, involving risk from preformed nitrosamines and benefit from protection against botulism. It is complicated, with the usual factors of improved analytical sensitivity on the one hand and freedom of choice on the other. Factors unique to the problem include an extremely long history of apparently safe usage, some legal interpretation involving color fixative or additive status.

With primary aliphatic amines, the reaction results in wide variety of deamination products. N-nitroso compounds are produced from the reaction of nitrite with tertiary amines (Cassens *et al.*, 1979). USDA allows 0 – 3 ppb of nitrosamines in sucuk. Example for nitrosamine formation;

HONO + Dimethylamine → Dimethyl nitrosamine + HOH



Pseudonitrosites formed across the two double bonds of the triglyceride. When this compound was heated with morpholine in lipid solvent, nitrosation of the secondary amine occurred; they related their finding to the formation of N-nitrosopyrrolidine in bacon (Cassens *et al.*, 1979).

2.5.7. Residual Nitrite

The salts of nitrite are soluble in water and are considered reactive. Nitrite may react in numerous ways with functional group and has many industrial applications. It oxidizes hemoglobin to methmyoglobin and is lethal in humans at a dose of about 1 g.

In meat, the usually mild acid conditions lead to formations of only a small quantity of nitrous acid. Nitrite is a week reducing agent and is oxidized to nitrite only by strong chemical oxidants or by nitrifying bacteria. Nitrite oxidizes many reduced substances.

The sodium nitrite added to sucuk dough is reduced in only a few hours to nitric oxide by enzymes present in the muscle (Ertaş *et al.*, 1989). Moreover, in sausage quantity of residual nitrite must not exceed to limit of 15 ppm in European countries and 30 ppm in codex Alimentarius (Wirth, 1986; Öztan and Vural, 1991). Much usage of nitrite causes the quantity of residual nitrite to increase and the formation of carcinogen effective nitrosamine (Özdemir *et al.*, 1984).

2.6. Organoleptic Properties of Sucuks

Organoleptic characteristics of sucuks could be divided into three groups; appearance, cooking and eating properties.

2.6.1. Appearance Properties

Casings shouldn't have torn, it should have characteristic taste, odor and good appearance. Desired taste in sucuk is lactic acid taste because sucuk is a fermented product.

When sucuk is cut cross-section surface should have pink color. When we apply a force with a finger it should give a resistance and not collapse. It shouldn't be too hard and too soft. When it is cut with a sharp knife it shouldn't be brittle or sticked to knife.

When it is cut there shouldn't be high color difference between central side and outer surface, color of meat and fat shouldn't mix each other.

Sucuk should have smooth cross-section surface and have not air bubbles. Fat ratio, water ratio, and salt ratio shouldn't exceed 30 %, 40 %, and 5%, respectively. It should contain protein at least 22 %. Sucuks with high quality should have pH 5.2-5.4 (Gökalp *et al.*, 1999).

2.6.2. Cooking Properties

Under normal conditions of frying or grilling sucuks should not loose more than 10 % of their weight as water or fat and a loss exceeding 20 % is not acceptable. As well as suffering only a limited weight loss, sucuks should not shrink or distort excessively after cooked.

Deformation of sucuks on cooking is also an important criterion of quality. This may take the form of excessive shrinkage of the filling for reasons are already given. When natural casings are used the skin may contract over the length of sucuk, giving a dump-bell appearance it is not always easy to determine whether this is caused by expansion of the filling or contraction of the casing, but the latter is probably the more likely especially as this rarely occurs with synthetic casings.

2.6.3. Eating Properties

Finally, mention should be made of the eating properties expected of a good sucuk. The casing should be render-a condition not always met by natural skins but invariably met by synthetic casings and the filling firm and succulent but not rubbery. The latter criteria can only be met by using a reasonable quality of meat mix. Flavor is highly subjective and difficult to quantify. In general large manufacturers with a wide distribution tend to produce sucuk with a rather bland inoffensive flavor.

2.7. Quality of Sucuk

There are four basic quality criteria for sucuks (TS 1070; Anonymous, 1983). These are; 1- physical criteria, 2- chemical criteria, 3- microbiologic criteria and 4- organoleptic criteria. Chemical, microbial and organoleptic criteria for sucuk are given in Table 2.6.

2.7.1. Physical criteria

Color and appearance of sucuks can be detected according to physical structure, texture, appearance and outer surface. Sucuks must be at the standard diameter and dimensions and packaged under vacuum. Packing material must be transparent and has a pleasant appearance. Sucuk has its special taste and flavor. Fat layer must not present on surface. Outer color of sucuk must be red-brown, inner color must be pink-red. When it is pressed with a finger on sucuk, it must be resistant and not be exceedingly hard or soft. When it is cut with a knife, it must not stick on the knife (Turgut, 1986; TS 1070).

Deep red is an appropriate color of sucuk which might be occurred when nitrites are reduced to nitric oxide by starter culture. After that, nitric oxide combines with myoglobin to produce red pigment (nitrosyl myoglobin or nitrosomyoglobin). In sucuk, the stability of the color is assisted by the residual nitrite in the product. The movement in recent years to keep nitrite levels as low as possible because of their formation of the biogenic amine or nitrosamine from nitrite (Özçelik, 1982).

In Turkey, there were no maximum or minimum standard values for amounts of nitrosomyoglobin, biogenic amine and nitrosamine. The maximum allowable residual nitrite in sucuk at the retail point must not be higher than 50 ppm according to the Turkish Food Codex (Anonymous, 1997). However, in European countries residual nitrite limit in sausage should be maximum 15 ppm and in codex of Alimentarius stated that it is not higher than 30 ppm in sausages. Turkish Food Codex stated that pH value for fermented sucuks should be maximum 5.4 (Food Codex 2000/4, Anonymous, 2000). For biogenic amine contents only histamine and tyramine limit are declared in the literature is as follows; histamine contents should be in the range of 50-100 mg/kg and the allowable maximum level of tyramine is 100-800 mg/kg in sausages (Nout, 1994; Shalaby, 1996). The FDA's limit for histamine in fish is 50 ppm.

2.8. Defects

General **Defects** detected in sucuks are: appearance, color, aroma and flavor (Turgut, 1986; Oğan, 1996).

2.8.1. Appearance Defects

They are divided into; shape deformation, microbial colonies, saltiness, oil diffusion, unstabilized color, deformation of casings, and points of brown, yellow and black color on the surface of sucuks. These defects are occurring during the manufacturing and storage of sucuk. Shape and color deformation occurs in the manufacturing of sucuk because of the undesired drying as one-way air drying, rapidly drying, very hot air usage, and very dry air usage. It can be observed during the storage as a result of very humid storage and after unproper filling cause's cavity in sucuk. Microbial colonies appear at different color on sucuk. Most of these colonies are produced by mold and yeast. These are occurred because of the insanitary manufacturing and storage conditions. They may oxidize to lipids (*Candida lipolytica*), and cause to greening of the surface (*Penicilium* and *Aspergillus* spp.). Saltiness of surface appears due to the diffusion of small salt crystals during the hanging period. Oil diffusion may take place because of the diffusion of high melting point fats through the surface of sucuk during the manufacturing and storage. Unstabilized color appearances occur due to low nitrite reduction with the microorganisms, too much drying and undesired mold growth on the surface of sucuk (Vural and Öztan, 1996; Göğüş, 1986).

Table 2.6 Standards for sucuk (TS 1070)

Chemical Criteria	Limits
Moisture (mass)	max. 40 %
Salt (mass)	max. 5 %
Coloring matter	None
pH	min. 5.4 - max. 5.8
Fat (mass) for first quality	max. 30 %
for second quality	max. 40 %
for third quality	max. 50 %
Protein (mass) Nx6.25	
for first quality	min. 22 %
for second quality	min. 20 %
for third quality	min. 20 %
Microbial Criteria	
Total Aerobic Mesophilic bacteria (CFU/g)	10^5-10^6 in 2 sample out of 5 sample
Escherichia coli (CFU/g)	None
Staphlococcus aureus (CFU/g)	0-100
Salmonella (CFU/g)	None in 25 g sample
Mold and yeast (CFU/g)	0-100
Clostridium perfingens (CFU/g)	10-100 in 2 sample out of 5
Coliform bacteria (CFU/g)	Maximum 10
Organoleptic criteria	
Flavor	Must be in its original flavor
Taste	No rancid, no sour, no bitter
Appearance	Must be in appropriate color and texture

2.8.2. Color Defects

Color defects occur as; unsufficient and unstabilized color formation, core color defects, dark and rancid color formation. Unsufficient and unstabilized color formation take place due to the addition low amount of nitrite, low amount of myoglobin present in the meat (because of young animal meat), high fat content, high pH reduction during the fermentation and low inoculum level of starter culture used. Core color defects may be due to microbial origin as the presence of homofermentative lactic acid bacteria and proteolytic bacteria. They produce H_2O_2 and H_2S, respectively. These compounds are bind to meat pigment to produces green color in the core of sucuk. Dark color formation is occurred because of the high amount of myoglobin in meat. Rancid color formation is take place due to the high ripening and storage temperature. Lipids are oxidized at high temperatures and desired colors of sucuk are deformed (Turgut, 1986; Oğan, 1996).

2.8.3. Flavor and Taste Defects

Flavor and taste defects are; souring, microbial, taste defects, bitter and rancidity. Heterofermentative bacteria produce acetic acid, tartaric acid and lactic acid. These acids affect the flavor and taste of sucuk. Using too much sugar, starter culture and high fermentation temperature (above 25°C) may cause to sour defects. Production of NH_3 and H_2S from the microorganisms affect the flavor and taste of sucuk, as a microbial flavor and taste defects. These defects may result from unhygienic manufacturing and storage conditions. Bitter taste defects are due to excess sodium nitrate usage and presence of Mg in the salt. Rancidity is formed due to the oxidation of unsaturated fatty acid. Unsaturated fatty acids naturally are low in meat but they are produced by means of microbial action. So, sucuks are susceptible for the rancidity.

2.9. Prediction of Quality and Stability

Various foods exhibit ecological parameters that significantly affect the behavior of microflora and chemical reaction. A number of interrelated factors influence the shelf-life and keeping quality of foods, specifically; pH, moisture content or a_w, holding temperature and most importantly microorganisms (Lambert *et al.*, 1991). All of these factors, either alone or in combination with one another, can result in changes in the color, odor, texture, and flavor of the foods. These parameters can increase or decrease the rate of chemical and microbial decomposition. It is difficult to estimate either the magnitude or direction of their combined influence on the quality of food (Labuza *et al.*, 1992).

Models are applied in a variety of ways to ensure the microbial and chemical safety and quality of foods: to determine shelf-life, product formulation, food storage, and hazard analysis critical control point (HACCP) studies (Zwietering *et al.*, 1990; Labuza *et al.*, 1992; Bozkurt and Erkmen, 1999; Walker and Jones, 1994).

Predicting the shelf-life and safety of a food system with several different components becomes a problem when there are several modes of deterioration on each mode has its own temperature and other environmental factor sensitivities (Labuza, 1982). The shelf-life of whole product at any given temperature is determined by the mechanism in any component which proceeds fastest and that causes the shortest life. Growth of microorganisms and production chemical substances can be predicted in a food after normal or expected abuse storage period. These models can aid estimation of time for attaining the specified population of spoilage or pathogenic microorganisms and specified formation of chemical substances (Whiting and Buchanan, 1994). A major question for organoleptic spoilage of sucuk is whether as a result of chemical or microbial actions. Since different microbial activity and chemical reactions are affected differently by temperature changes (each has its own T, pH, a_w, and gas dependence). In order to predict, one needs a shelf-life plot based on major modes of deterioration including microbial or chemical deterioration as a function of environmental factor.

$$f(A) = \int k_{[f(T)]} dt \qquad (1)$$

where f(A) = quality function and $k_{[f(T)]}$ = time/temperature dependence of quality loss rate or growth rate of microbes (Labuza *et al.*, 1992).

There are several reasons that explain why prediction of development of an unsafe food is difficult. If spoilage organisms grow faster than pathogens or chemical spoilage occurs faster than toxin production below a certain critical temperature, the food probably would spoil before pathogens growth or toxin production occurs. If competitions exist between the mixed floras, the situation is more complex, making prediction is complicated (Labuza *et al.*, 1992).

Models can aid in the development of Quality control programs by showing what conditions permit growth of microorganisms, production of chemical compound and thereby identifying critical control points. Models may aid the identification of critical control points by providing information on the likely growing of spoilage or pathogenic microorganisms and chemical decomposition at particular stages of a process. In addition, once a stage has been identified as critical it is important to identify the target value for control (for example pH or temperature) (Walker and Jones, 1994; Bozkurt and Erkmen, 1999; Bozkurt and Erkmen, 2001).

CHAPTER III
MATERIALS AND METHODS

3.1. Materials

Sucuk (the Turkish dry-fermented sausage) additives nitrite, nitrate, α-tocopherol, ascorbic acid, potassium sorbate, potassium pyrophosphate and di-potassium hydrogen phosphate were obtained from Merck (Darmstadt, Germany). Sodium nitrate, sodium nitrite, sulfanilamide, N-(1-Naphtyl) ethylene-diamine dihydrochloride (NED), HCl and glacial acetic acid were obtained from Merck (Darmstadt, Germany); 1,1,3,3- tetraethoxypropane (TEP), 2-thiobarbituric acid were obtained from Sigma (St. Louis, MO). β-phenylethylamine hydrochloride, histamine dihydrochloride, serotonin hydrochloride, cadaverine dihydrochloride, spermine diphosphate, spermidine diphosphate, 1,7-diamino heptane, putrescine dihydrochloride, tryptamine hydrochloride, and tyramine hydrochloride used as biogenic amine standards were obtained from Sigma (St. Louis, MO); and sodium hydroxide, L-histidine, L-tyrosine and 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) were used in HPLC analysis. All chemicals except acetonitrile which was HPLC grade were of analytical grade (extra pure).

Aerobic plate count agar (for aerobic plate count), potato dextrose agar (for mold and yeast count), De-Man Rogosa sharp, MRS, broth (Merck, Darmstadt, Germany) and MRS broth with 1.5 % agar (Merck, Darmstadt, Germany; for lactic acid bacteria) were used as microbial culture medium.

A sucuk culture mixture of *Pediococcus acidilactici*, *Lactobacillus plantarum* and *Staphylococcus carnosus* was obtained from Biocarna (Wiesby, Germany).

3.2. Experimental Design

An experimental design for full plant production was chosen to investigate the effect of temperature and relative humidity on the quality and safety of sucuk.

The storage conditions of sucuks are given in Table 3.1. The storage temperatures and relative humidities (RH) were 20, 30 and 40°C and 50, 65 and 80 %, respectively. These preselected relative humidity and temperature values are the prevailing ranges in Gaziantep region (Gaziantep Meteorology Department). Thus the effect of these climatic conditions on the quality and safety of sucuk were taken into a consideration.

Different recipes were followed for sucuk preparation to observe the selective effect of internal factors on the quality and stability of sucuk during the manufacturing and storage period. Sucuk recipes were prepared according to the followings:

- ***Using with and without starter culture.*** A mixture of starter culture was used to observe the effects of starter culture on microbial, chemical and organoleptic changes.
- ***Using different ingredients.*** Different ingredients were used to reduce nitrate and nitrite usage, to improve quality and safety by means of stabilizing the color and texture, and to reduce lipid oxidation and microbial growth.

3.3. Sucuk Preparation

Sucuk dough was prepared from followings; lamb (from 1 year old animal) containing about 18 % fat was mixed with tail fat, salt, sugar, cleaned dry garlic, spices, $NaNO_2$, $NaNO_3$, vegetable oil (generally, olive oil), antioxidants and antimicrobials (Bozkurt and Erkmen, 2002a, b). Meat, fat, and spices were added according to the following recipe; 900 g lamb (standard meat was used), 200 g tail fat, 5.5 g cumin, 1.1 g cinnamon, 11.42 g allscipe, 0.48 g clove, 5.5 g red pepper, 11 g black pepper, 20.76 g garlic, 4.4 g sugar, 18 g salt and 2.1 g olive oil were used to prepare sucuk dough. Chemical additives, which are given in Table 3.2, were added into sucuk dough for the preparation of different types of sucuk. Starter culture inoculation was adjusted to $1x10^9$ CFU/g. Following chart was used for preparation of sucuk (Figure 3.1).

Table 3.1 Full factorial experimental design for storage conditions of sucuks

Run Number	Temperature (°C)	Relative Humidity (%)	Duration (days)
1	20	50	60
2	20	65	61
3	20	80	29
4	30	50	51
5	30	65	53
6	30	80	29
7	40	50	36
8	40	65	48
9	40	80	29

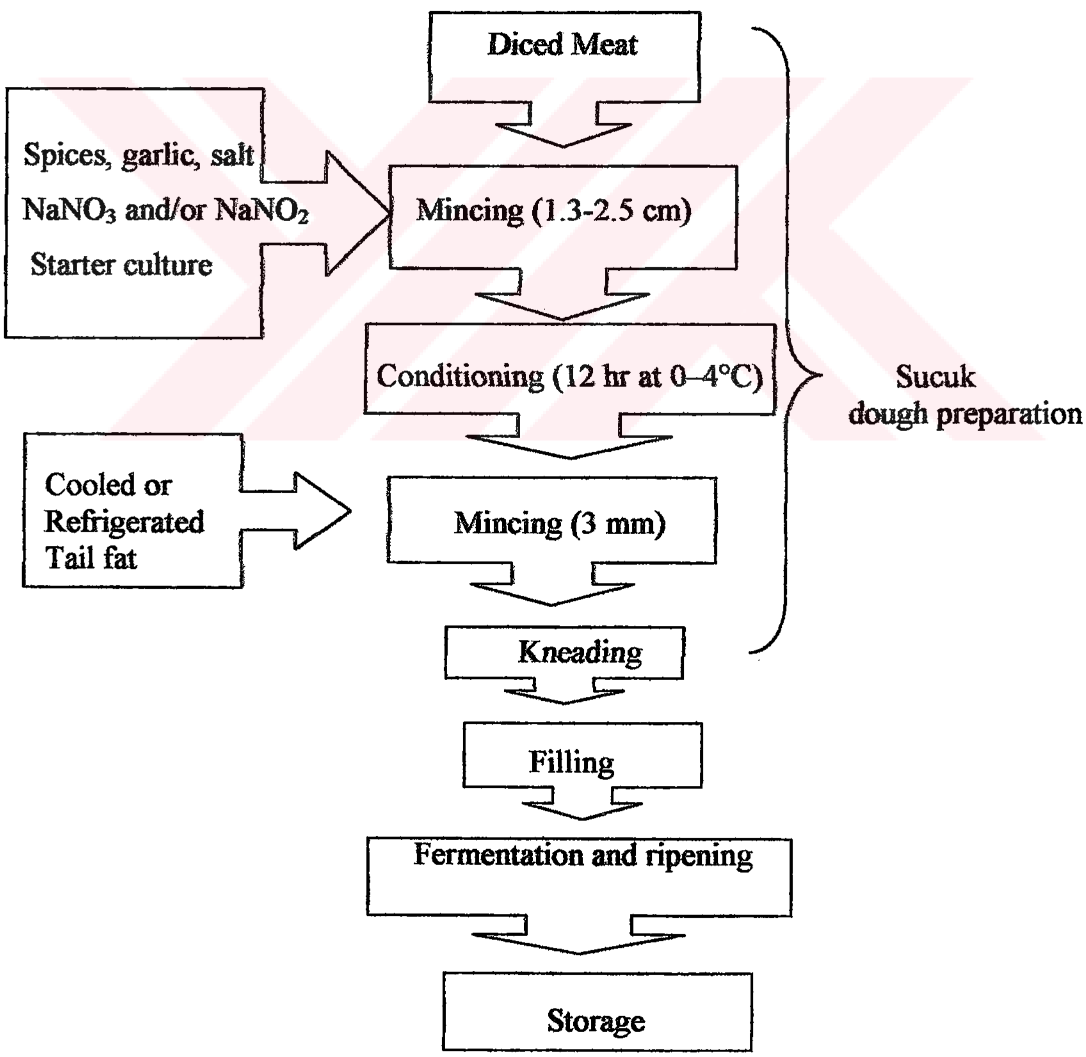


Figure 3.1 Production flow-chart of sucuk (Bozkurt and Erkmen, 2002a, b)

Table 3.2 Composition of the prepared sucuk

Additives	Sucuk types											
	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
Nitrate (ppm)	0	150	300	0	0	0	150	150	150	300	300	300
Nitrite (ppm)	0	75	150	0	0	0	75	75	75	150	150	150
Potassium pyrophosphate (g/kg)	0	1.25	2.5	0	1.25	2.5	0	1.25	2.5	0	1.25	2.5
Di-potassium hydrogenphosphate (g/kg)	0	1.25	2.5	0	1.25	2.5	0	1.25	2.5	0	1.25	2.5
Ascorbic acid (ppm)	0	250	500	0	250	500	0	250	500	0	250	500
α-Tocopherol (ppm)	0	100	200	0	100	200	0	100	200	0	100	200
Potassium sorbate (ppm)	0	100	200	0	100	200	0	100	200	0	100	200

A1-A3 indicate that sucuks were prepared without starter culture

B1-B9 indicate that sucuks were prepared with starter culture (*Pediococcus acidilactici /Lactobacillus plantarum/ Staphylococcus carnosus,* 20 g for 100 kg meat)

After the mixing of ingredients and starter culture, sucuk dough was stuffed into the artificial collagen casings (Naturin RL2, Germany) of a 38 mm diameter at 2°C using filling apparatus of meat grinder (Tefal Prep'Line 1600, France). The prepared sucuks were about 100 g of unit. About 912 unit of sucuks were prepared for data point with two replicate. Moreover, some of additional sucuks were also prepared to check the results. Thus, more than 1824 sucuks were prepared.

Ripening conditions were selected as indicated for sucuk by Gökalp (1995). The selected ripening conditions are given in Table 3.3.

After the ripening of sucuks they were stored at different temperatures and relative humidities as given in Table 3.1.

During the ripening and storage conditions chemical, microbial, and organoleptic characteristics were determined. The experiments were repeated two times.

3.4. Sampling and Sample Preparation

Each 100 g of unit sucuk types was removed to analyze at sampling time. Sucuks were cut into small pieces and stored at –20°C for chemical analysis.

3.5. Microbiological Analysis of Sucuk

3.5.1. Sample Preparation for Microbiological Analysis

Surface of casing of sucuk were sterilized using 70 % ethyl alcohol. They removed under aseptic condition. Twenty-five g of ground sucuk was aseptically weighed in a sterile polyethylene bag. Then it was homogenized in 225 ml of a sterile peptone-water (0.1 %) in a sterile mechanical blender cup (Erkmen, 2000). Serial decimal dilutions were prepared using a sterile peptone-water (0.1 %) solution.

3.5.2. Aerobic Plate Count (APC)

Aerobic plate count was made with the aerobic spread plate count method as described by Erkmen (2000). In this count, aerobic plate count agar (PCA; Merck, Darmstadt, Germany) was used.

Table 3.3 Predetermined conditions for sucuk ripening

Days	Temperature (°C)	Relative humidity (%)
1-2	25	90
3-4	20	80
5-7	18	75
8-10	18	67
11-15	18	60

A 0.5 ml of the dilution was transferred into corresponding labeled plate and spread plated over the aerobic plate agar surface. Inoculated PCA plates were incubated at 35°C for 24 hr to 72 hr. Colonies on the agar plates in the range from 30 to 300 colonies were counted. Average value was taken from the duplicate count and then APC were recorded as APC per g of sucuk.

3.5.3. Lactic Acid Bacteria Count (LAB)

Lactic acid bacteria were counted with the method using de-Man Rogosa Sharp (MRS; Merck, Darmstadt, Germany) agar as described by Erkmen (2000).

A 0.5 ml of the dilution was transferred into corresponding labeled plate and spread plated over the agar surface. Inoculated MRS agar plates were incubated at 30°C for 1 to 4 days. Colonies on the agar plates in the range from 30 to 300 colonies were counted. Average value was taken from the duplicate count and then the count recorded as LAB per g of sucuk.

3.5.4. Mold and Yeast Counts

Mold and yeast counts were counted with the aerobic spread plate count as described by Erkmen (2000). In this count potato dextrose agar (PDA; Merck, Darmstadt, Germany) was used.

A 0.5 ml of the dilution was transferred into corresponding labeled plate and spread plated over the agar surface. Inoculated PDA plates were incubated at 25°C for 2 to 5 days. Colonies on the agar plates in the range from 30 to 300 colonies were counted. Average value was taken from the duplicate count and then the count were recorded as mold and yeast counts per g of sucuk.

3.6. Chemical Analysis of Sucuk

3.6.1. 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).

3.6.2. Determination of TBA (2-Thiobarbituric Acid) Value

Formation of malonaldeyhde was followed by TBA value. TBA value was determined by Spectrophotometric method at 538 nm by using TBA reagent as described by Byun *et al.* (2001).

Two g of ground sucuk was taken and was extracted with 10 ml of 0.4 M perchloric acid for two times and made up to 25 ml with 0.4 M perchloric acid. Extracted solution was centrifuged for 5 min at 1790Xg. After centrifugation, 1 ml of extract was poured into another glass stoppered test-tube. A 5 ml of TBA reagent was added into tube. Meanwhile, 1 ml of distilled water with 5 ml of TBA reagent was added into glass stopperred test-tube as a blank solution. Tubes were heated in a boiling water bath for 35 min. After cooling under tap water, the absorbance of sample was obtained at 538 nm against blank using a standard spectrophotometer (Novaspec II, Pharmacia Biotech., Cambridge, England). A standard curve was prepared using 1,1,3,3- tetraethoxypropane (TEP).

3.6.3. Determination of Nitrosomyoglobin Conversion

Nitosomyoglobin conversion was found as;

$$\% \text{ Nitrosomyoglobin conversion} = \frac{\text{ppm nitrosoyl - heme - pigments x 290}}{\text{ppm total - heme - pigments x 680}} \, x \, 100$$

3.6.3.1. Determination of Nitrosomyoglobin

Nitrosomyoglobin is the pigment that gives the bright red color to sucuk. It was determined by spectrophotometeric method at 540 nm by using extraction against blank solutions (Zaika *et al.*, 1976).

A 10 g of ground sucuk was taken into 50 ml flask. A 40 ml acetone and 3 ml water were added, mixed and set aside for 5 min. After the filtration and centrifugation for 5 min at 1790Xg, absorbance value was obtained with respect to the blank solution of 40 ml acetone and 3 ml water at 540 nm. Result was calculated as ppm nitrosoyl-heme-pigments.

3.6.3.2. Determination of Total Pigment

Total pigment was determined by using extraction against blank solutions measuring the absorbance values at 640 nm with spectrophotometer (Zaika *et al.*, 1976).

A 10 g of ground sucuk was taken into 50 ml flask. A 40 ml acetone plus 2 ml water and 1 ml concentrated HCl were added, mixed and set aside for 1 hr in a dark place. After filtration and centrifugation for 5 min at 1790Xg, absorbance values with respect to the blank solution of 40 ml acetone, 2 ml water and 1 ml of concentrated HCl were obtained at 640 nm using spectrophotometer. Result was calculated as ppm total-heme-pigments.

3.6.4. Determination of Residual Nitrite

Residual nitrite was determined by the spectrophotometric method (AOAC, 1990). In this method NED reagent, sulfanilamide reagent and nitrite standard solutions were used.

A 5 g of ground sucuk was taken into 500 ml flask and 40 ml heated (80°C) water was added, and completed to 300 ml with hot water then mixed thoroughly. The mixture was heated in the boiled water for 2 hr. Then the mixture was cooled to room temperature and volume was completed to 500 ml with distilled water. Following filtration 45 ml of filtrate was transferred into 50 ml volumetric flask and 2.5 ml sulfanilamide was added and then set aside for 5 min. A 2.5 ml of NED reagent was added and waited for 15 min for the color development. The absorbance values were obtained at 540 nm against blank of 45 ml water, containing 2.5 ml sulfanilamide reagent, and 2.5 ml NED reagent.

3.6.5. Determination of Biogenic Amines

High Performance Liquid Chromatographic method was used for the determination of the biogenic amines (Eerola *et al.* 1993). Peaks were detected at 254 nm by using the Hewlett Packard UV detector.

Apparatus

The HPLC system used in this research had a quadratic gradient pump a Shimadzu Solvent Delivery Module (LC-10ADvp, KYOTO, JAPAN), Hewlett Packard UV detector, RP-18 guard column, and a computer including Borwin package program. The HPLC column was Spherisorb ODS2, 10 μm, 200x4.60mm (Phenomnex)

LC mobile phases

Ammonium acetate (0.1 M) was prepared by dissolving 7.7 g ammonium acetate in 100 ml of triple distilled water, filtered through a 0.45 μm Millipore filter (Bedford, USA) and acetonitrile (HLPC grade) filtered through a 0.45 μm Millipore filter (Bedford, USA).

Sample Preparation and Extraction

Two g frozen ground sucuk (about –20°C) was thawed and then homogenized in 10 ml of 0.4 M perchloric acid with blender. Homogenized sample was centrifuged for 10 min at 1790Xg and rinsed supernatant into a flask through filter paper. Extraction was repeated with 10 ml of 0.4 M perchloric acid solution. Supernatants were combined and adjusted to 25 ml with 0.4 M perchloric acid.

Derivatization of sample Extracts and Mixed Standards

To 1 ml sample extract, 200 μl of 2 N NaOH solution was added and the addition of 300 μl saturated sodium bicarbonate was followed. After two ml dansyl chloride solution (10 mg/ml) addition into each sample, the samples were incubated for 45 min at 40°C. Residual dansyl chloride was removed by adding 100 μl ammonia (25 %). After allowing to stand for 30 min at room temperature, the volume of the samples were adjusted to 5 ml by adding acetonitrile, centrifuged for 5 min at 1790Xg, supernatants were filtered through 0.45 μm filter, and then 20 μl sample was injected into HPLC.

Chromatographic Separation

Gradient elution program was used with mobile phases as acetonitrile solvent A and 0.1 M ammonium acetate as solvent B. Gradient program was begin at 50 % solvent A and 50 % solvent B, and ended with 90 % solvent A and 10 % solvent B in 30 min. Flow rate was 0.4 ml/min. A 20 µL of filtrated sample was injected.

Standard Curve Preparation

Different amounts of standard solution was diluted to 1 ml with 0.4 M perchloric acid to obtain concentrations from 0.5 to 10 µg/ml. Dansyl derivatives of calibration standard mixtures were prepared as previously described.

3.7. Determination of Organoleptic Properties

Panelist evaluated sensory attributes (cutting, color of cut surface, and flavor) of sucuk samples during the ripening and storage periods. Sensory analysis (flavor, color, and resistance to cut) was made by 10 panel number. Panelist gave score for each of sample with respect to their perceptions of each sensory attribute for flavor and color using a hedonic scale as 1 (worst) to 10 (best). Resistance to cut (cutting) scores was evaluated during the slicing by the panelists. The overall sensory qualities of sucuks were evaluated according to expression described by Bruna *et al.* (2000) as:

Overall sensory quality= (colorx0.25) + (cuttingx0.25) + (flavorx0.50)

3.8. Identification of Starter Culture Properties

Lyophilized starter cultures were activated in 10 ml of MRS broth and incubated for 24 hr at 30°C anaerobically in an incubator (Nüve, Turkey).

3.8.1. Catalase Test

Catalase activity of starter cultures was tested by using H_2O_2 (10 %) as described by Erkmen (2000).

Aseptically, 1 ml of culture was added into small clean test tube. One ml of H_2O_2 (10 %) was added into culture. The liberation of free oxygen as gas bubbles indicated the production of catalase with culture.

3.8.2. Nitrate and Nitrite Reductase Activity

Nitrate reductase activity of starter cultures was assessed by conversion of nitrate to nitrite. Nitrite reductase activity of starter cultures was determined by conversion of nitrite to nitric oxide by means of culture.

Aseptically, 2 ml of activated starter cultures were taken and added into clean 150 ml flask. Nitrate (2 %) was added into starter culture and incubated at 30°C for 24 hr. During this incubation periods, nitrite formation from nitrate was determined. Production of nitrite from nitrate represented nitrate reductase activity of starter culture.

Aseptically, 2 ml of activated starter culture was taken and added into clean 150 ml flask. Nitrite (2%) was added into starter cultures and incubated at 30°C for 24 hr. During this incubation periods, nitrite concentration was determined. Decreasing the concentration of nitrite indicated nitrite reductase activity of starter culture.

3.8.3. Test of Starter Culture for Production of Biogenic Amines

Starter cultures (0.1 ml) was transfer to a tube containing MRS broth supplemented with 0.1 % L-histidine and 0.1 % L-tyrosine, and incubated for 24 hr at 30°C anaerobically. Formation of biogenic amines by starter culture was checked by inoculating a loopful of mixture of culture to tubes containing 10 ml supplemented MRS broth (Roig-Sagues and Eerola, 1997). These inoculated tubes were incubated at 20°C for 72 hr and biogenic amines were determined from duplicate samples as described in Section 3.6.5.

3.9. Determination of Quality Attributes of Retailed Sucuks

Retailed sucuks were divided into two main parts; traditional and firm-made sucuks. Traditional sucuk samples were obtained from generally local butchers. Firm-made sucuk samples were manufactured and marketed by companies. Hundred types (25 types traditional and 75 types firm-made) of sucuks were collected from local markets and butchers between December 2000 and February 2002. Microbiological, chemical and organoleptic analyses were applied to all of sucuks.

3.9.1. Microbiological Count

APC in retailed sucuks were determined as it described in Section 3.5.2, LAB counts were found as described in Section 3.5.3, and mold and yeast counts determined as described in Section 3.5.4.

3.9.2. Chemical Analyses

Chemical analyses of retailed sucuks were made; pH values as described in Section 3.6.1, TBA values as described in Section 3.6.2, nitrosomyoglobin conversions as described in Section 3.6.3, residual nitrite levels as described in Section 3.6.4, and biogenic amine concentrations as described in Section 3.6.5.

3.9.3. Determination of Organoleptic Properties

Organoleptic properties of retailed sucuks were determined by following overall sensory quality as described in Section 3.7.

3.10. Statistical Analysis

Statistical analysis was performed using the SPSS 8.0 (SPSS Inc, USA) software for Windows. The analysis of variance (ANOVA test) was performed for chemical, microbial and organoleptic changes with factors (time, additives, temperature and % RH) to determine any significant differences within the 95% confidence interval. If there were significant difference among these factors (additives, temperature and % RH) Duncans multiple range tests were carried out for determination the differences between these factors, also for finding the highest and lowest of these factors during the ripening and storage periods. To determine a relationship between determined attributes (microbial, chemical and overall sensory quality) of sucuk, Pearson's correlation coefficients were used.

Statistical analyses were done between sucuks made in this research and retailed samples in local markets and butchers.

3.11. Predictive Model

Different empirical formulas were applied to describe the microbial (APC, LAB and mold and yeast counts) and chemical (pH, TBA, nitrosomyoglobin conversion, residual nitrite level and histamine, tyramine and putrescine concentrations) changes and overall sensory quality of sucuks (B1-B9) during the storage periods. Multi-parameter non-linear regression analysis was performed to find the predicted data for these changes using the experimental data. Non-linear regression was carried out by using Marquardt-Levenberg algorithm in SigmaPlot for Windows version 6.00 a package program (SPSS Inc., USA). Validations of empirical equations were decided by comparing statistically the predicted and experimental data. Statistical analyses were carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is P<0.0001. This lowest level of significance indicates that regression is significant at α=<0.0001 levels, in other words, empirical formulas were well fitted to the experimental data. Example for the non-linear empirical formula;

$$\text{APC (log CFU/g)} = a + b\,t + c\,A + d\,T + e\,R + f\,T^2$$

where a, b, c, d, e and f are the constants derived from a non-linear regression, t is the storage time in day, A is the additives, T is temperature in °C and R is the relative humidity of the storage in %. Significance of the constants indicate that presence of this constant necessary or not, if significance level is lower than P<0.0001 it is necessary to put this constant into equation. Additives given in Table 3.2 were coded for regression analyses as; for B1 1.0, for B2 1.1, for B3 1.2, for B4 2.0, for B5 2.1, for B6 2.2, for B7 3.0, for B8 3.1 and for B9 3.2.

CHAPTER IV
RESULTS AND DISCUSSION

4.1. Microbial Changes in Sucuks

In this work, aerobic plate, mold and yeast, and lactic acid bacteria (LAB) counts were monitored in sucuk during the ripening and storage periods. These microbial changes were also determined for sucuks collected from local markets (firm-made) and butchers (traditional).

4.1.1. Aerobic Plate Count

Aerobic plate counts (APC) of sucuks (A1-A3 and B1-B9) are given in Figures 4.1-4.9 during the ripening and storage periods at 50, 65 and 80 % RH with 20, 30 and 40°C, respectively. APC increased ($P<0.05$) during the first days of ripening periods. This would be due to available ripening conditions especially, temperature and % RH of the medium for microorganisms. Further ripening and storage periods, the number of organisms decreased ($P<0.05$).

4.1.1.1. Aerobic Plate Count during the Ripening Periods

During the first five days of the ripening, APC generally increased (about 1.0 log cycle; Figures 4.1-4.9). After that they decreased ($P<0.05$) or became nearly constant during the further ripening periods. During the first days of the ripening period, % RH was adjusted to 80-90 %, and temperature was set to 22-25°C (Table 3.3). This increased ($P<0.05$) the number of aerobic bacteria. During the further ripening period, relative humidity and temperature were decreased gradually to 60 % and 18°C, respectively (Table 3.3). Thus, environmental conditions limited the aerobic bacterial growth.

Number of APC was constant during the 15 days of ripening in sucuk types A3, B7, B8, and B9 (Figure 4.2). These sucuks were made with high level of nitrite and nitrate. It was observed from Figures 4.1-4.9 that sucuks were made with high level of additives had low number of bacteria ($P<0.05$) than the other sucuks. The effect of starter culture on the APC was lower ($P<0.05$) than the effect of additives during the ripening period.

These results indicated that reduction in the number APC of sucuks could be restricted by strictly adjusting and controlling ripening conditions.

4.1.1.2. Aerobic Plate Count during the Storage Periods

Figures 4.1, 4.2 and 4.3 show the changes of APC in sucuks (A1-B9) during the storage periods at 50, 65 and 80 % RH, respectively, at 20°C. APC gradually decreased ($P<0.05$) during the storage periods. The highest ($P<0.05$) level of APC was observed in sucuk types B1 and B2 stored at 50 % RH; in sucuk types A1, B1, B2 and B3 stored at 65 % RH; and A1, B1, and B2 stored at 80 % RH. This could be due to the absence of nitrite/nitrate in these sucuks (Table 3.2). Low number of APC were observed in sucuk types A3, B7, B8, and B9 made with high level of nitrite/nitrite after 60 days at 20°C and at 65 % RH. Reduction of APC in sucuks made with nitrite/nitrate were significant ($P<0.05$) during the storage periods.

Figures 4.4, 4.5 and 4.6 show the changes of aerobic plate count of sucuks during the storage periods at relative humidity of 50, 65 and 80 %, respectively, at 30°C. APC decreased ($P<0.05$) during the storage periods (except sucuks stored at 80 % RH; Figure 4.6). Increasing % RH of storage increased ($P<0.05$) APC. In general, APC of sucuks stored at 30°C with 50% RH was lower ($P<0.05$) than sucuks stored at 30°C with 65 % RH and 80 % RH. Lower number of APC was observed in sucuk type B2 after 42 days, stored at 30°C and 50 % RH.

The changes of APC of sucuks during the storage periods at 40°C with 50, 65 and 80 % RH are given in Figures 4.7, 4.8 and 4.9, respectively. APC of sucuks decreased ($P<0.05$) gradually during the storage periods. The lowest ($P<0.05$) level of APC was observed in sucuk types A2, A3, B7, B8 and B9 stored at 40°C with 65 % RH. These sucuks contained high level of nitrite/nitrate and other additives (Table 3.2), but APC were higher in sucuks stored at 40°C than sucuks stored at 30 and 20°C.

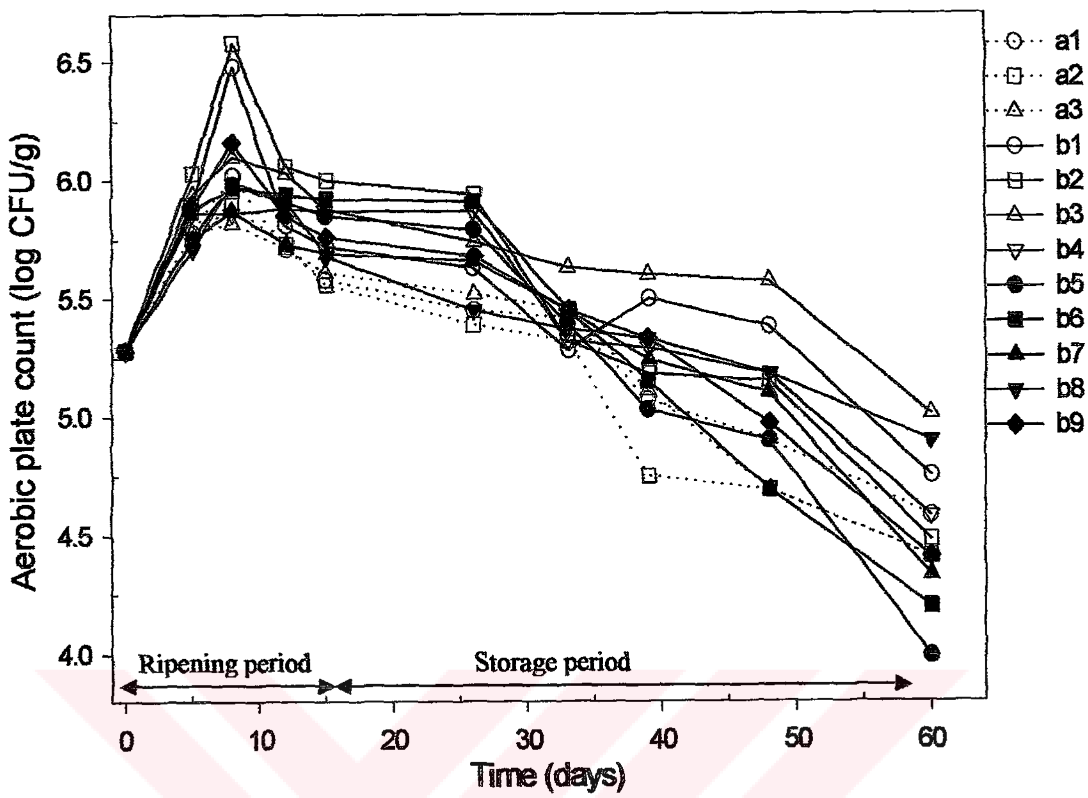


Figure 4.1 Changes of APC of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

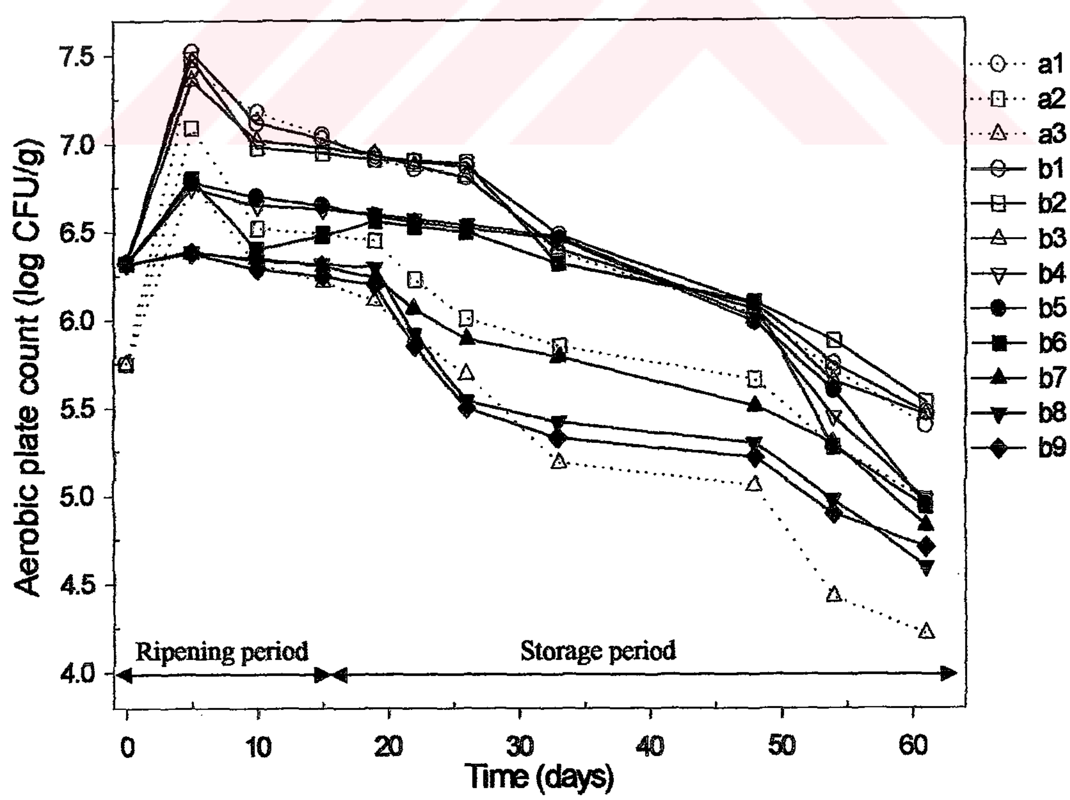


Figure 4.2 Changes of APC of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

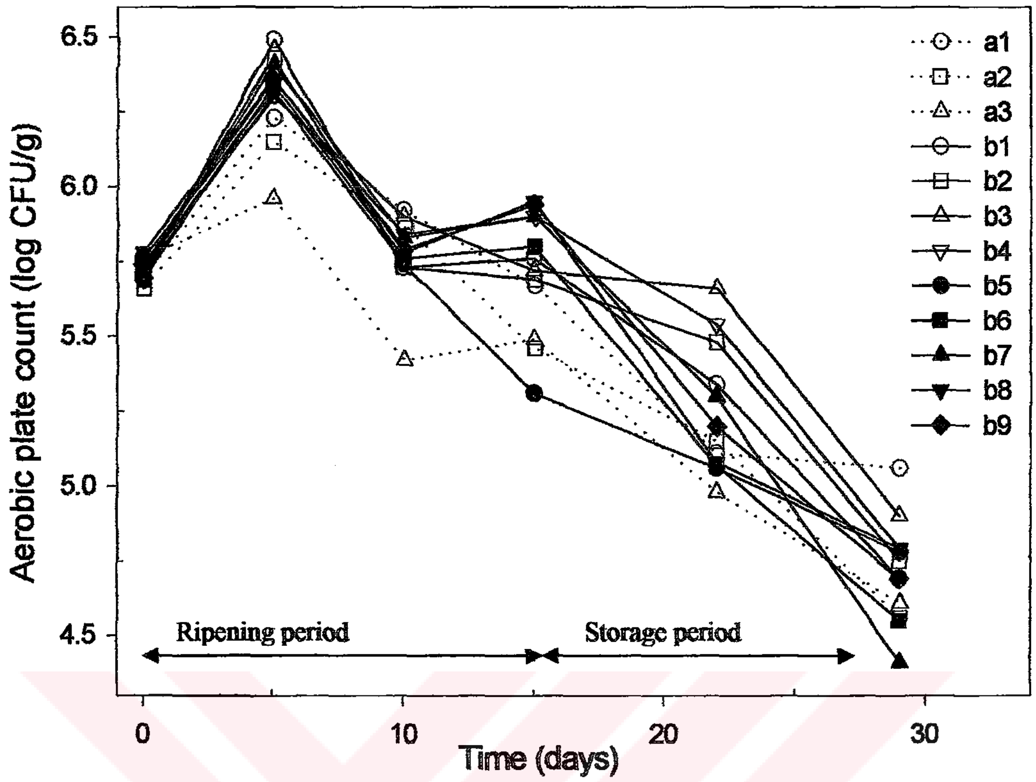


Figure 4.3 Changes of APC of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

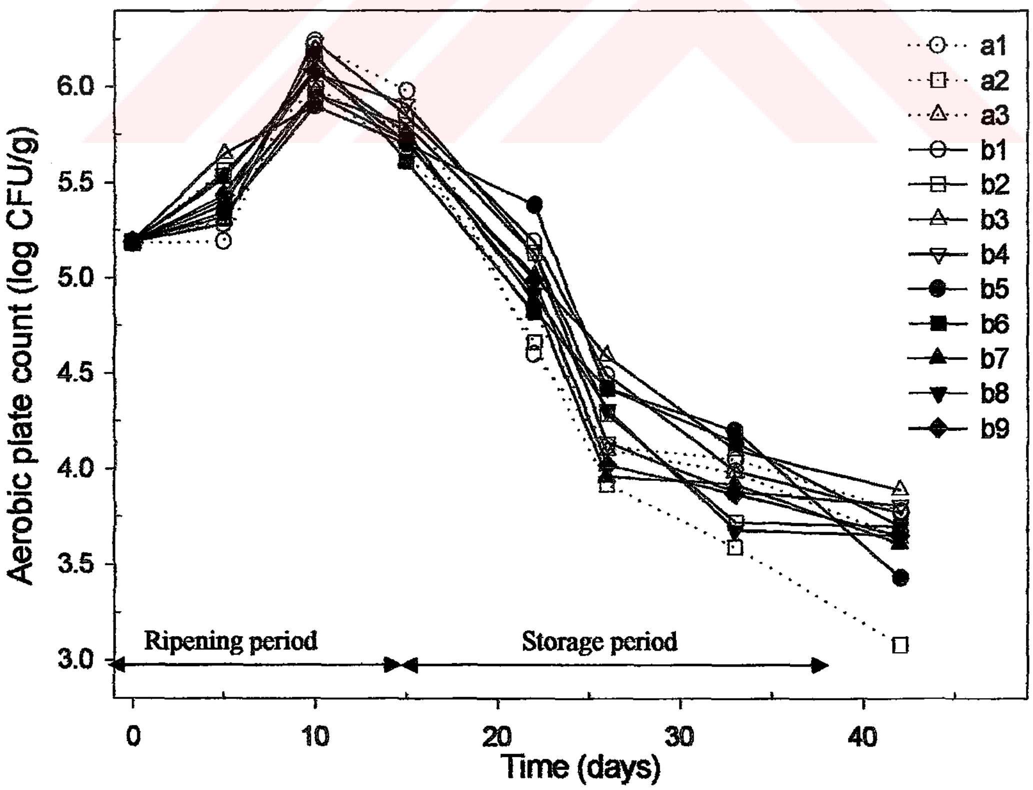


Figure 4.4 Changes of APC of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

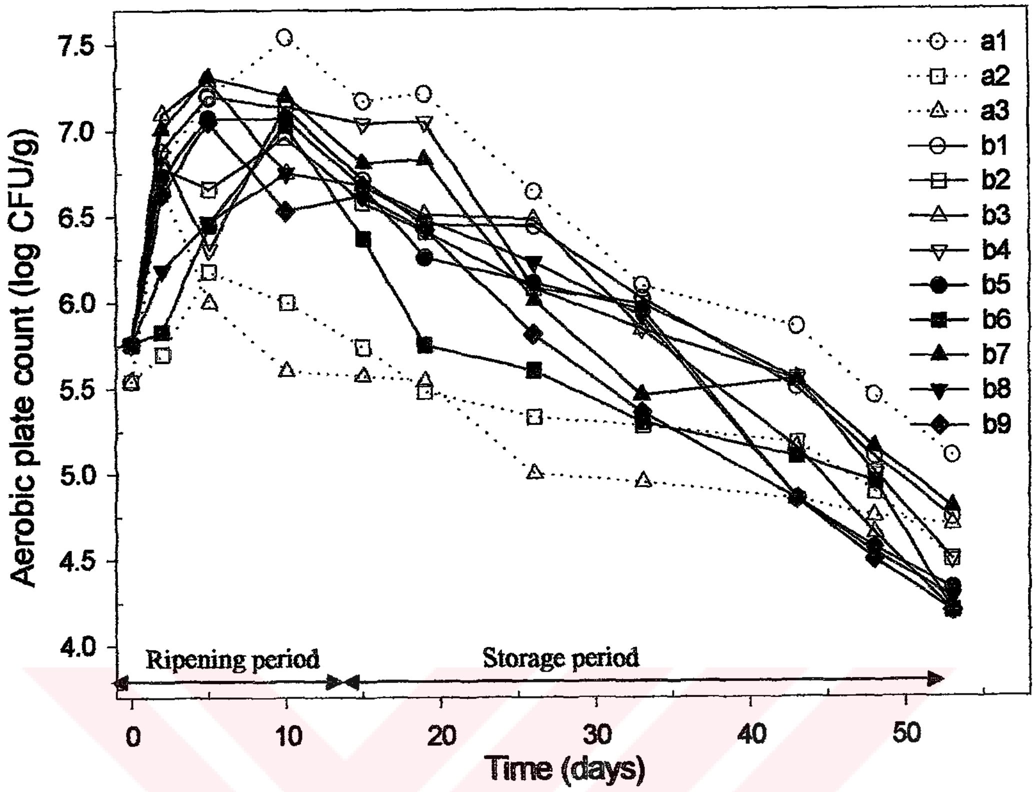


Figure 4.5 Changes of APC of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

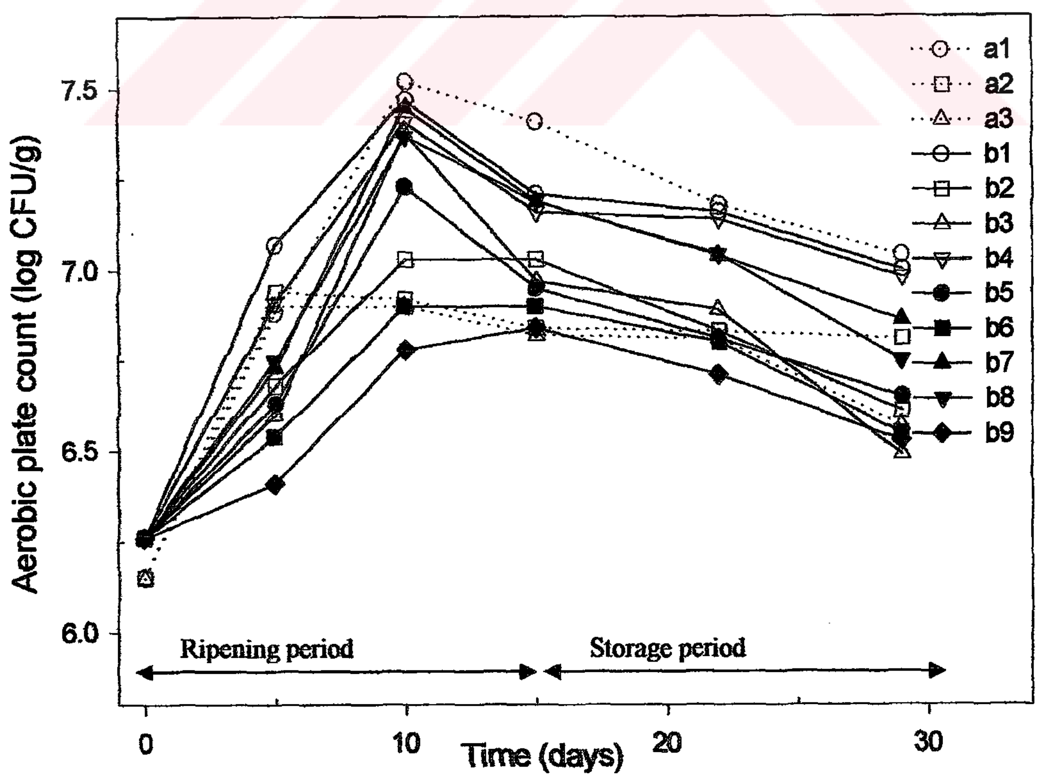


Figure 4.6 Changes of APC of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

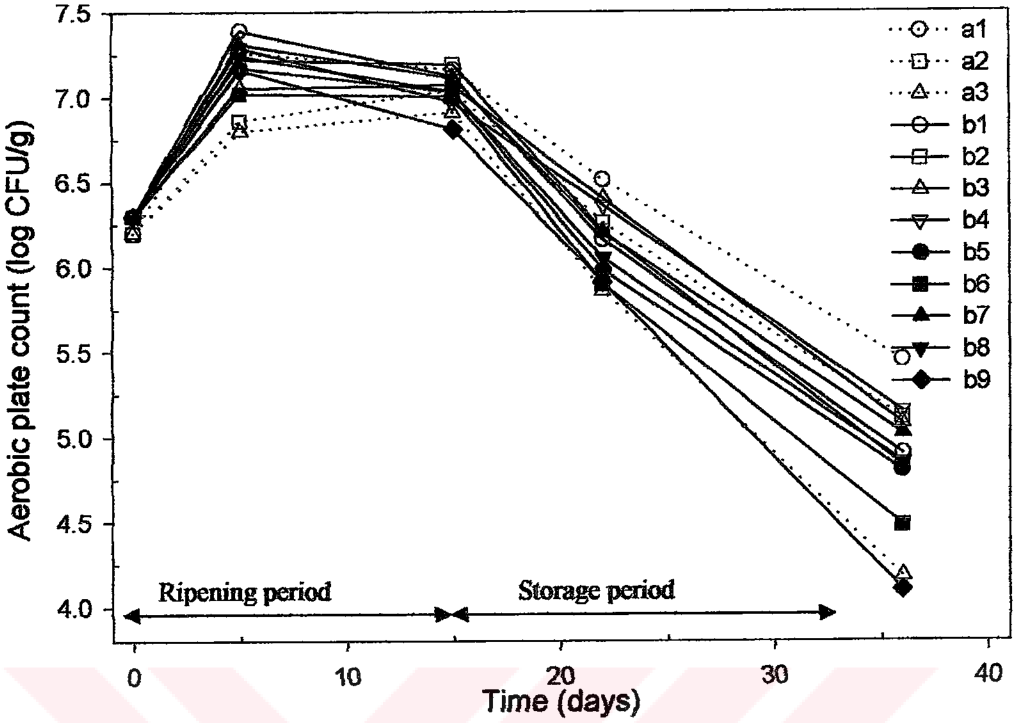


Figure 4.7 Changes of APC of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

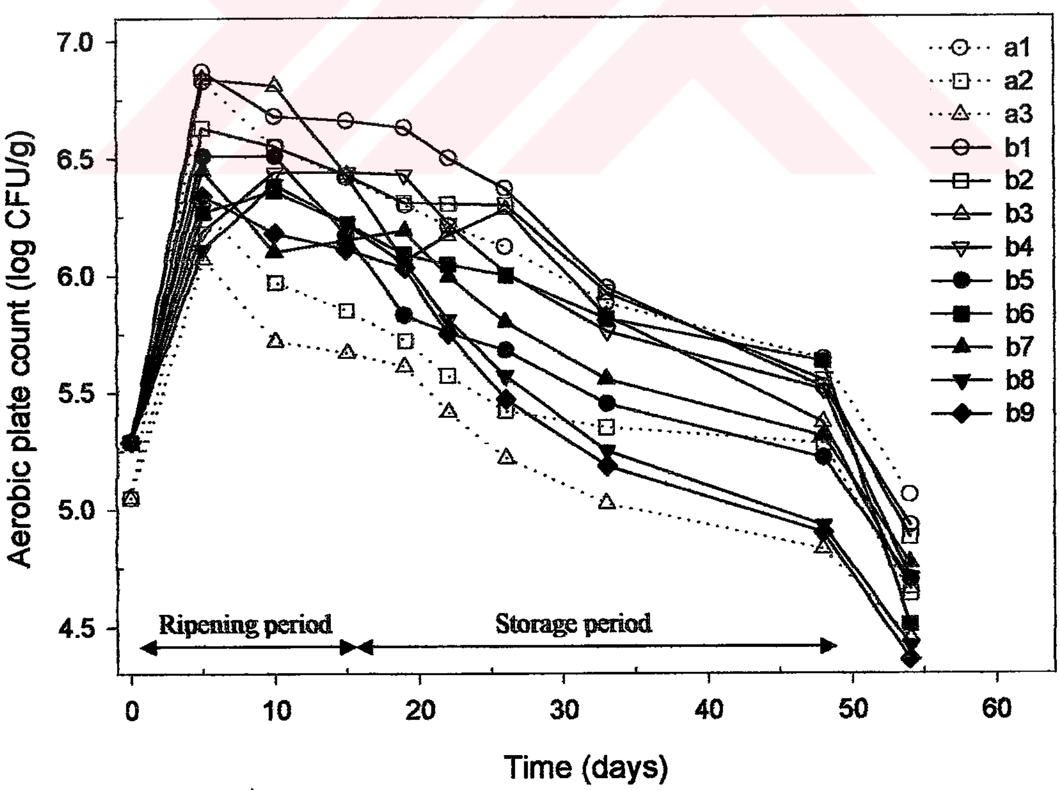


Figure 4.8 Changes of APC of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

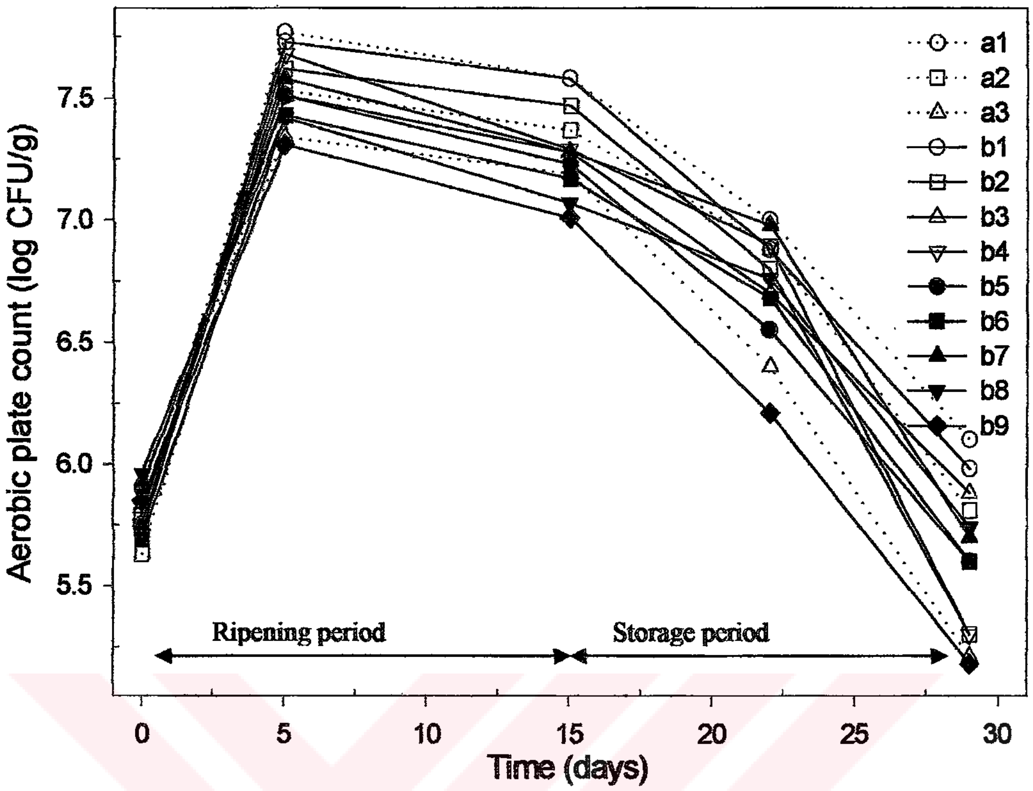


Figure 4.9 Changes of APC of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

Statistical analyses were applied to determine the effects of starter culture inoculation on the APC level of sucuks. Statistical analyses showed that starter culture inoculation had no significant effect ($P>0.05$) on the APC level of sucuks.

Additives had significant effect ($P<0.05$) on the reduction of APC in sucuks. In general, sucuk types A2, A3, B5, B6, B7, B8, and B9 had low ($P<0.05$) APC than the others. These sucuks had nitrite/nitrate and high level of other additives (potassium pyrophosphate, di-potassium hydrogen phosphate, ascorbic acid, potassium sorbate and α-tocopherol). Reduction effects of nitrite/nitrate on the APC are more ($P<0.05$) than the other additives. Nitrite, nitrate, and potassium sorbate have an antibacterial activity in foods (Frazier and Westhoff, 1988). Brewer *et al.* (1992) reported that potassium sorbate inhibited the psychrotrophs, anaerobic and facultative anaerobic mesophiles, and lactobacilli during the refrigerated storage. Statistical analysis showed that increasing the storage temperature increased APC significantly ($P<0.05$).

Microorganisms need water to grow in foods, so they can easily grow at high RH environment. It was found from statistical analysis that there was a linear relationship ($P<0.05$) between % RH of the storage and APC of sucuks. Increasing % RH of the storage increased the number of APC (Figure 4.6).

Two-way (additives - temperature, additives - % RH, and temperature - % RH) interactions and three-way (additives - temperature - % RH) interaction were examined by means of analysis of variance (ANOVA). Statistical analysis showed that additive – temperature, and additives - temperature - % RH interactions were not significant ($P>0.05$) on the reduction of APC, but additive - % RH and temperature - % RH interactions were significant ($P<0.05$) on the reduction of APC. This means that increasing both % RH and temperature increased ($P<0.05$) APC much more than the increasing one factor (temperature or % RH) alone. Therefore, temperature and % RH of storage were more important in the control of APC than using one factor alone. These parameters could be used as hurdle factors. It can be concluded from additives - % RH interaction that effect of nitrite/nitrate and other additives increased ($P<0.05$) the inactivation of APC with increasing the % RH of the storage (Figure 4.1-4.9).

Positive significant correlation ($P<0.05$) were found from Pearson test. The Pearson correlation coefficients were 0.830, 0.294, 0.198, 0.204 and 0.236 between APC and LAB count, mold and yeast counts, overall sensory quality, putrescine and histamine concentration, respectively. In other words, there was a linear relationship ($P<0.05$) between these pairs. This means that the lower the APC, the lower the LAB count, mold and yeast counts, overall sensory quality, putrescine and histamine concentration. Negative correlations were observed between APC and pH, residual nitrite level, TBA values, and TYR concentration; the correlation coefficients were -0.294, -0.163 and -0.208 between APC and pH, residual nitrite level and TYR concentration, respectively. Negative correlation means the higher the pH, residual nitrite level and TYR concentration the lower the APC. A non-significant ($P>0.05$) correlation was found between APC and nitrosomyoglobin conversion and TBA values in sucuks. These results indicated that there were no relationship between APC and nitrosomyoglobin conversion and TBA values of sucuks.

4.1.1.2.1. Prediction of Aerobic Plate Count during the Storage

The following empirical formula was applied to describe the APC in sucuks (B1-B9) which were analyzed during the storage period. Multi-parameter non-linear regression analysis was performed to find the predicted APC. Validation of empirical equation was decided by compared statistically the predicted and experimental APC results. Statistical analyses were carried out between experimental and predicted data by means of one-way ANOVA with a significance level (P value) of $P<0.0001$. This lowest level of significance indicates that empirical formula was well fitted to the experimental data.

$$\text{APC (log CFU/g)} = a + b\,t + c\,A + d\,T + e\,R + f\,T^2 \qquad [1]$$

where a, b, c, d, e and f are the constant derived from non-linear regression, t is the storage time in day, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significance levels are given in Table 4.1. Significance of the constants indicates that presence of this constant necessary or not, if significance level is lower than $P<0.0001$, it is necessary to put this constant into equation. Residual APC versus time (days) plot is given in Figure 4.10. It is observed that residual APC ranged between -1.76 to 1.19 log CFU/g.

This empirical formula was compared statistically and it can be regarded as sufficient to describe the APC in sucuks during the storage periods. It was found that APC during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 1].

Table 4.1 The constants derived from the empirical formula with their standards errors and significance levels

Constant	Value	P values
a	7.4970±0.5872	<0.0001
b	-0.0380±0.0023	<0.0001
c	-0.1634±0.0369	<0.0001
d	-0.1819±0.0386	<0.0001
e	0.0258±0.0029	<0.0001
f	0.0031±0.0006	<0.0001

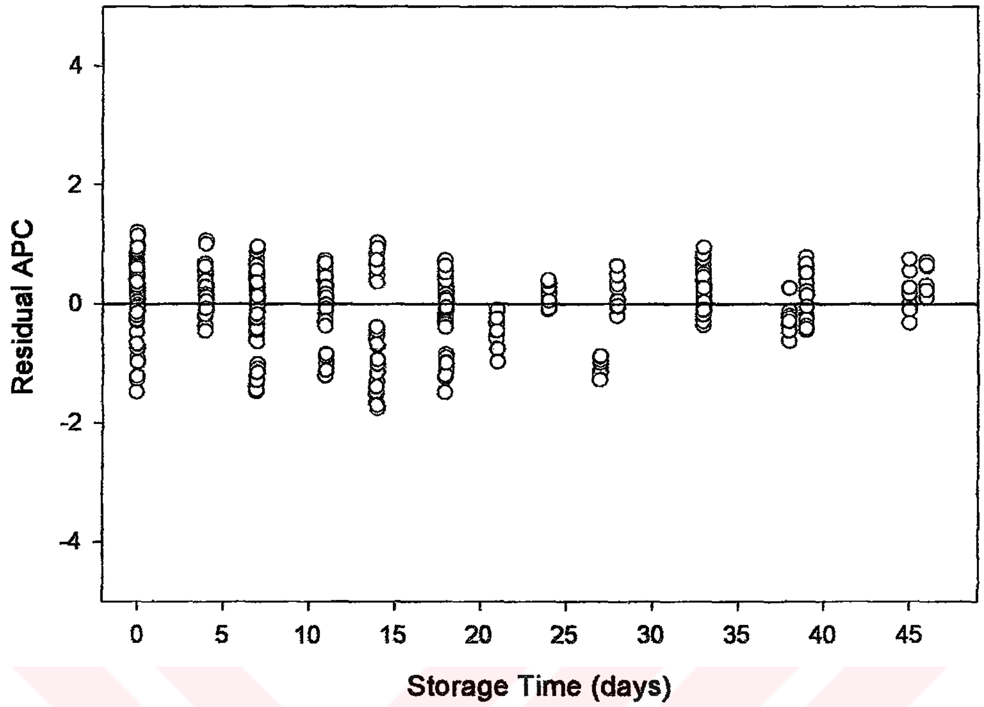


Figure 4.10 Residual plot of APC in sucuks with fitted empirical formula

4.1.1.3. Aerobic Plate Count (APC) in Retailed Sucuks

Aerobic plate counts (APC) in firm-made and traditional sucuks were found to be in the range of 3.48 to 7.68 log CFU/g (average 5.91 log CFU/g) and 4.70 to 7.82 log CFU/g (average 5.93 log CFU/g), respectively. Traditionally produced sucuks had higher APC than firm-made sucuks. TSE (1070; Anonymous, 1983) declared that APC should not be more than 6 log CFU/g in sucuks. 48 % of traditionally produced sucuks had higher APC than 6 log units per gram and 41 % of firm-made sucuks had higher APC. Traditional sucuks were produced and stored under non-hygenic conditions and raw material could contain contamination microorganism. However, firm-made sucuks were produced and stored under hygienic conditions using antimicrobial additives and heat treatment after ripening and were packed under vacuum. These treatments decreased the number of microorganism in the firm-made sucuks (Gökalp, 1988). Coşansu and Ayhan (2002) reported that APC were changed between 3.98 to 7.90 log CFU/g in retailed sucuks.

Statistical analyses indicate that there was no significant differences ($P>0.05$) between the APC of firm-made and traditional sucuks. APC counts in retailed sucuk types were higher ($P<0.05$) than firm-made sucuks (Figure 4.11). Firm-made sucuks had the lowest ($P<0.05$) APC count due to heat treatment after ripening which is quite short time indeed. Traditional sucuks ripened at climatic conditions (at about 10°C and at about 60 % RH), some microorganism does not grow well under these conditions. However, sucuk types (A1-A3 and B1-B9) fermented and ripened at higher temperatures (at 18-25°C) and % RH (60-90 %). These conditions are suitable for growth of most of the microorganisms.

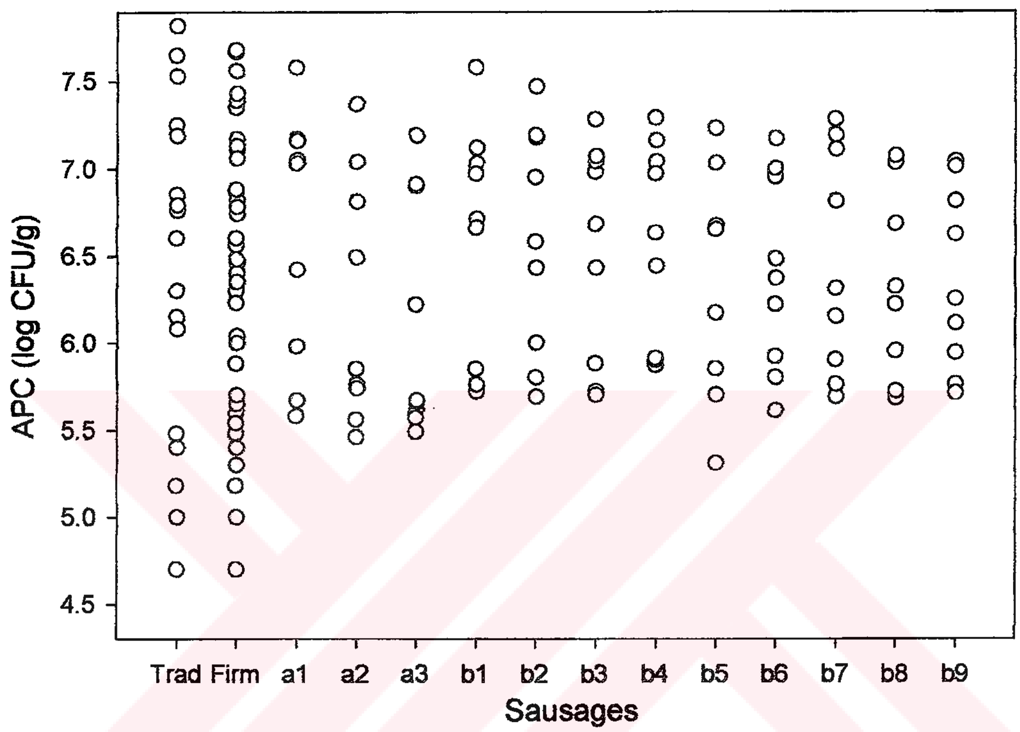


Figure 4.11 APC of sucuk types (A1-B9) and retailed sucuks (Trad: traditional; Firm: firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

4.1.2. Lactic Acid Bacteria Count

Lactic acid bacteria (LAB) counts were followed in only sucuk types A1, B1, B4, B7, B8 and B9 during the ripening and storage periods at 20, 30, and 40°C with 50, 65 and 80 % RH.

4.1.2.1 Lactic Acid Bacteria Count during the Ripening Period

LAB counts of sucuks are shown in Figures 4.12-4.20 for all batches. LAB count of sucuks increased ($P<0.05$) gradually during the first five or ten days of ripening periods. During the first days of ripening, temperature and % RH were adjusted to nearly the optimum conditions for growth of LAB (RH and temperature were 80-90 % and 22-25°C, respectively; Table 3.3).

The highest ($P<0.05$) level of LAB (about 6.0 log CFU/g) was detected in sucuk type B1 made with starter culture and without any additives. Sucuk type A1 made without starter culture and additives had the lowest ($P<0.05$) LAB count during ripening periods (about 5.4 log CFU/g). Increase in LAB count in sucuk type B1 was around 0.9 log CFU/g after ripening period (15 days) and it was about 0.6 log CFU/g in sucuk type A1. The reason of this could be due to the addition of starter culture into sucuk dough.

Sucuk types B4-B9 made with nitrite/nitrate had lower ($P<0.05$) LAB count than that of sucuk types B1-B3 made without nitrite/nitrate. The increment level of LAB in sucuk types B4-B9 was about 0.7 log CFU/g. Increasing the concentration of nitrite/nitrate decreased the LAB count of sucuks. These results showed that nitrite/nitrate could be used to control growth of LAB in sucuks during the ripening periods. LAB are important for aroma formation in sucuks, but high LAB reduces pH values of sucuks to unacceptable low level, and cause souring of sucuks (Gökalp *et al.*, 1999; Ordonez *et al.*, 1999).

4.1.2.1 Lactic Acid Bacteria Count during the Storage Period

The changes of LAB count in sucuks during storage periods at 20°C with 50, 65, and 80 % RH are given in Figures 4.12, 4.13 and 4.14, respectively. LAB decreased ($P<0.05$) during the storage periods at 50, 65 and 80 % RH. The lowest LAB was found in sucuk types A1, B8 and B9 after storage periods. LAB was found in the range from 4.39 to 4.71 log CFU/g, 4.64 to 4.75 log CFU/g, and 3.93 to 4.30 log CFU/g at 50, 65 and 80 % RH after 29 days, respectively.

Figures 4.15, 4.16 and 4.17 show the changes of LAB count of sucuks during the storage periods at 30°C with 50, 65, and 80 % RH, respectively. LAB decreased during the storage periods except 80 % RH storage. LAB count of sucuks stored at 80 % RH increased ($P<0.05$) during the storage period. It was observed that sucuk type A1 had the lowest ($P<0.05$) LAB count, and sucuk type B1 had the highest (Figures 4.15-4.17). LAB count after 29 days were about 2.49, 3.30; 4.16, 5.68; and 5.67, 6.16 log CFU/g in sucuk types A1 and B1 stored at 30°C with 50, 65 and 80 % RH, respectively. These results indicate that increasing the percent relative humidity increased ($P<0.05$) the number of LAB in sucuks.

LAB count of sucuks stored at 40°C with 50, 65, and 80 % RH are given in Figures 4.18, 4.19 and 4.20, respectively. LAB count of sucuks decreased ($P<0.05$) from initial LAB count of 3.83 to about 2.59-3.32, from 4.65 to 3.60-4.48, and from 5.49 to 5.10-5.98 log CFU/g during the storage period at 50, 65 and 80 % RH, respectively. These results indicate that increasing the % RH increased ($P<0.05$) the LAB. The lowest ($P<0.05$) LAB count was found in the A1 at all % RH. This can be due to the absence of starter culture.

Lactic acid bacteria counts were affected ($P<0.05$) from storage time. Statistical analyses show that LAB count of sucuks decreased significantly ($P<0.05$) during the storage period. This could be due to the low amount of available water in sucuks. During the storage periods, browning reactions (Maillard reactions; between carbohydrates and amino acids, proteins, etc.) can produce some antibacterial compounds such as 5- hydroxymethyl furfural (Frazier and Westhoff, 1988; Ordonez *et al.*, 1999).

ANOVA test indicates that storage temperatures affect ($P<0.05$) LAB in sucuks. Duncan's Multiple Range test was also carried out to determine the effect of temperatures on the LAB. It was found that LAB in sucuks was not different at 20°C and 30°C. At these temperatures, LAB counts of sucuks were higher than that at 40°C. This proved that low temperatures (20 and 30°C) were suitable for growth of the LAB.

Sucuks made with starter culture (B1) had higher ($P<0.05$) LAB count than that without starter culture. It can be observed from the Figures 4.12-4.20 that nitrite/nitrate decreased ($P<0.05$) the LAB count of sucuks. The results showed that addition of nitrite played major role in the reduction of LAB, however, the other additives had minor effect ($P>0.05$) on the reduction of LAB count of sucuks.

LAB count increased with increasing the storage temperature from 20°C to 40°C. Increasing the % RH of the storage decreased the LAB count significantly ($P<0.05$).

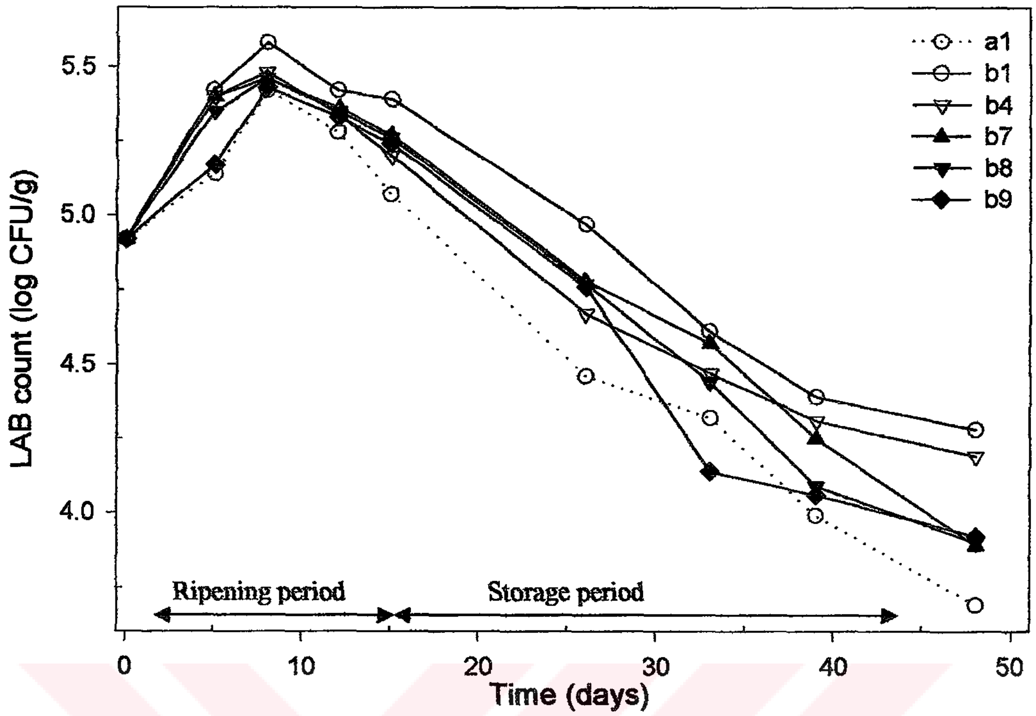


Figure 4.12 Changes of LAB count of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

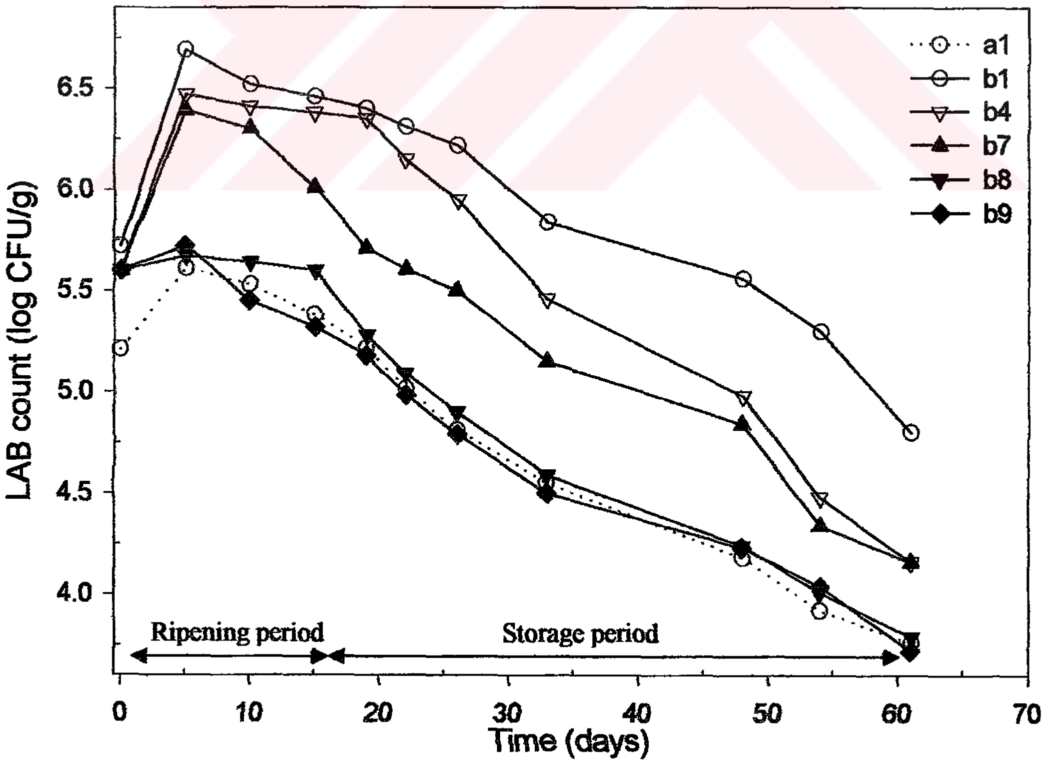


Figure 4.13 Changes of LAB count of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

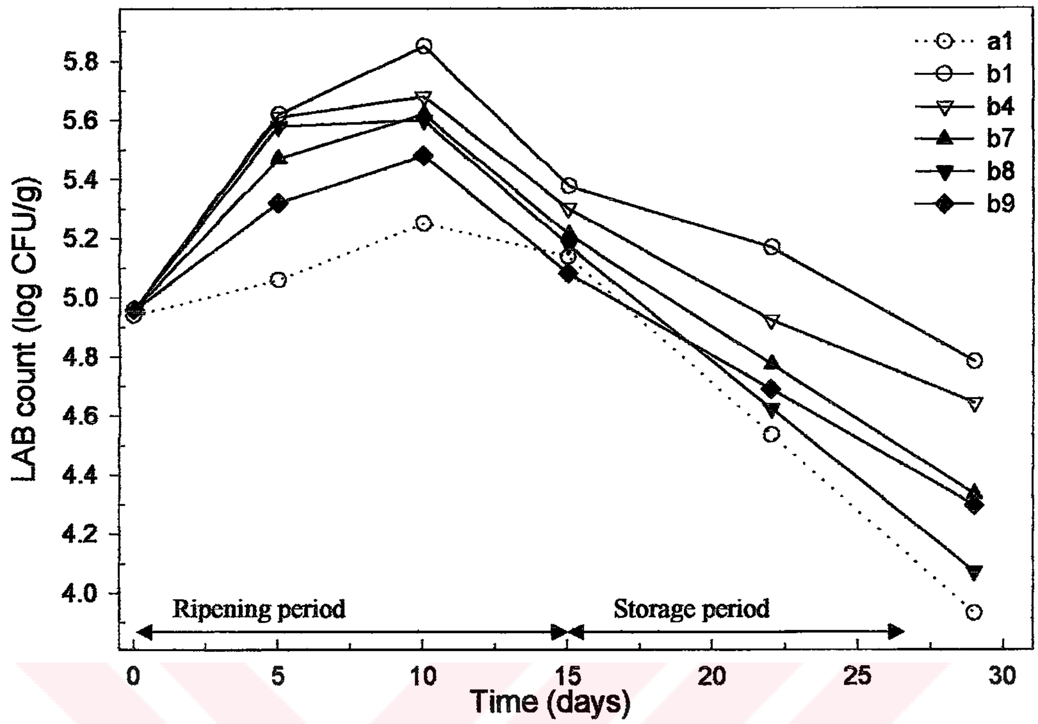


Figure 4.14 Changes of LAB count of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

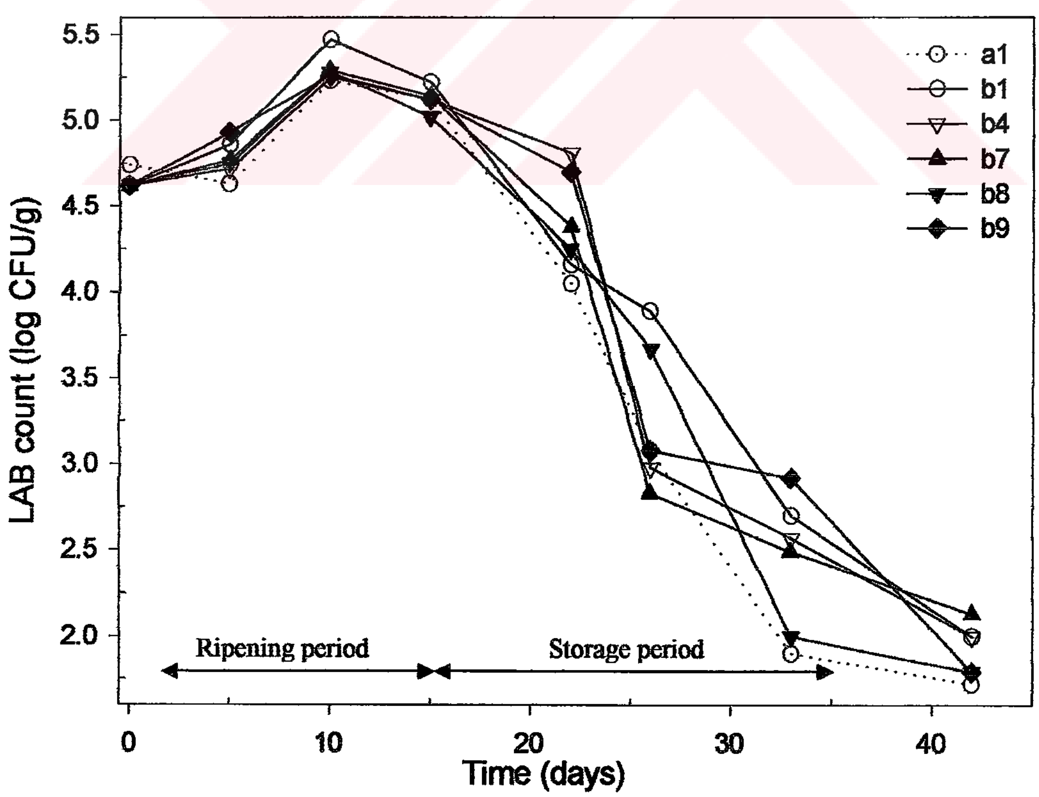


Figure 4.15 Changes of LAB count of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

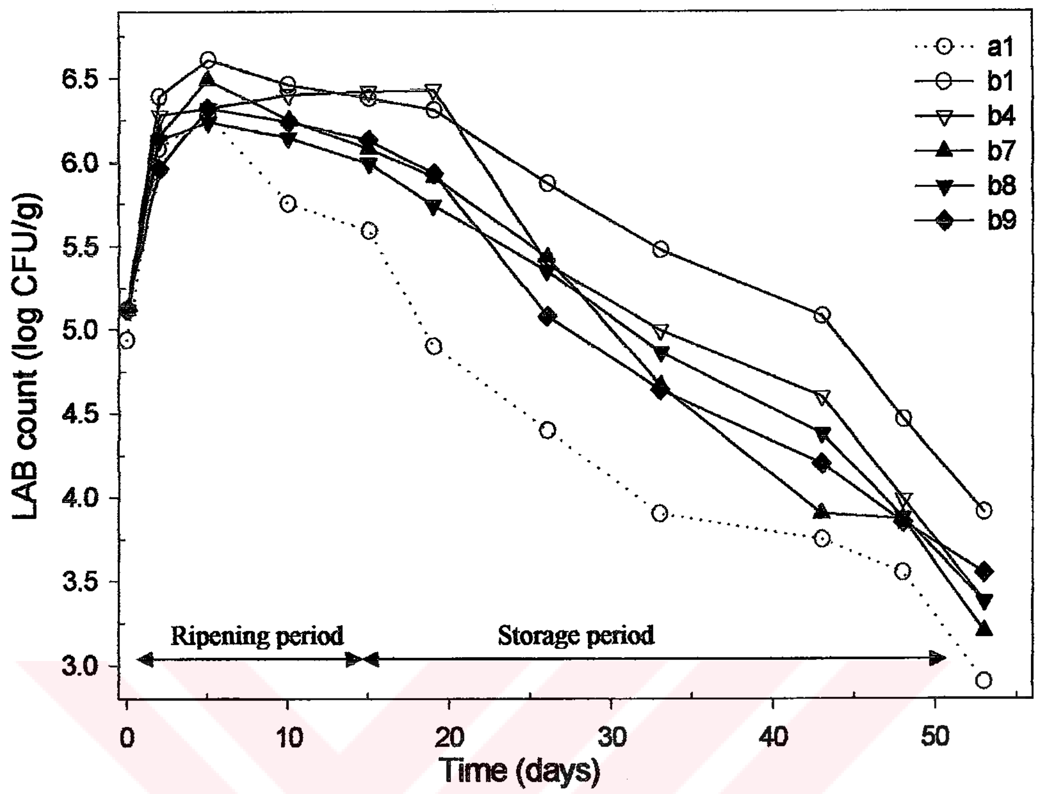


Figure 4.16 Changes of LAB count of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

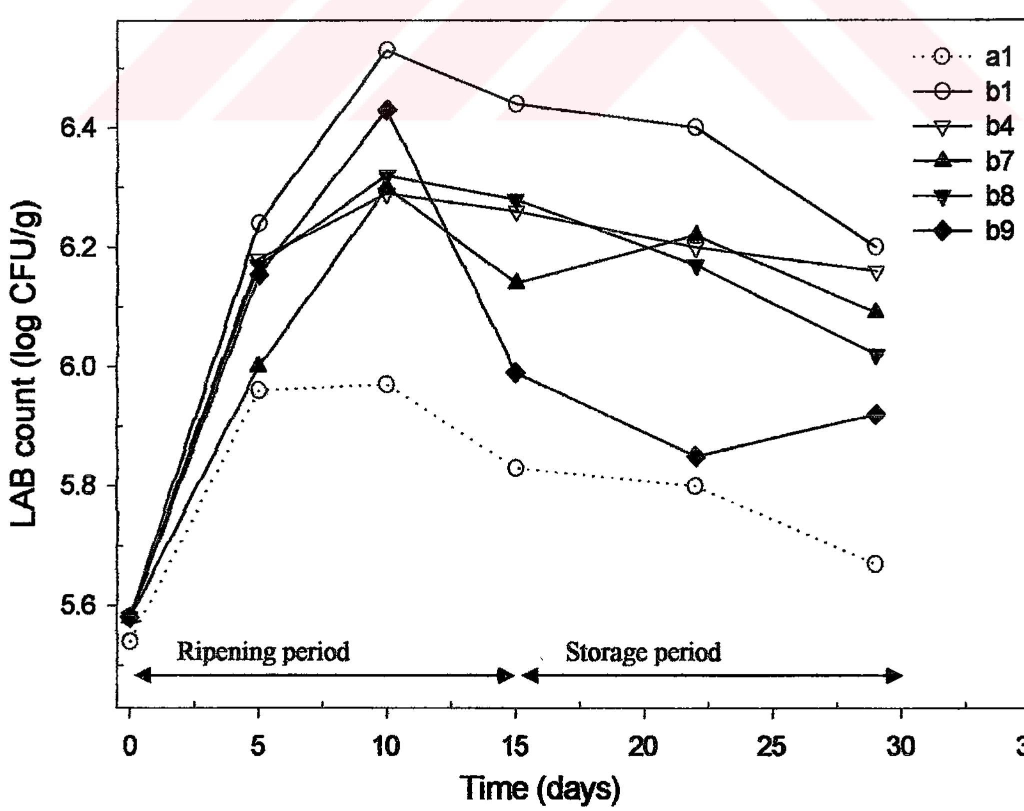


Figure 4.17 Changes of LAB count of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

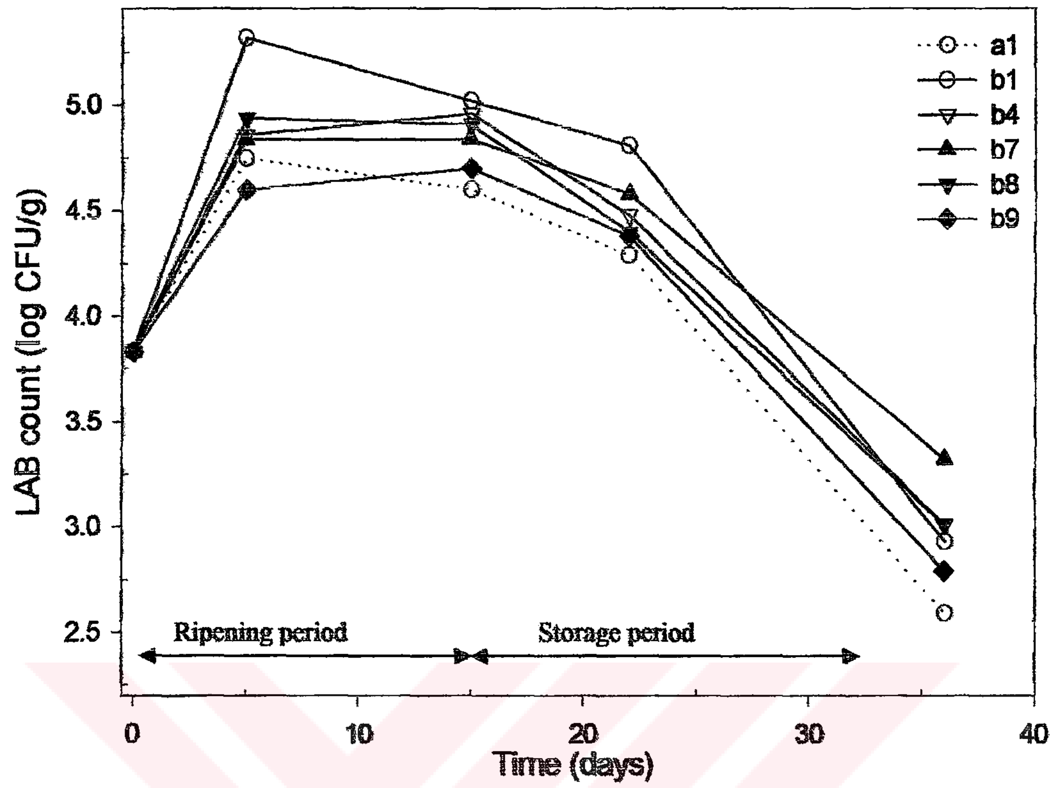


Figure 4.18 Changes of LAB count of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

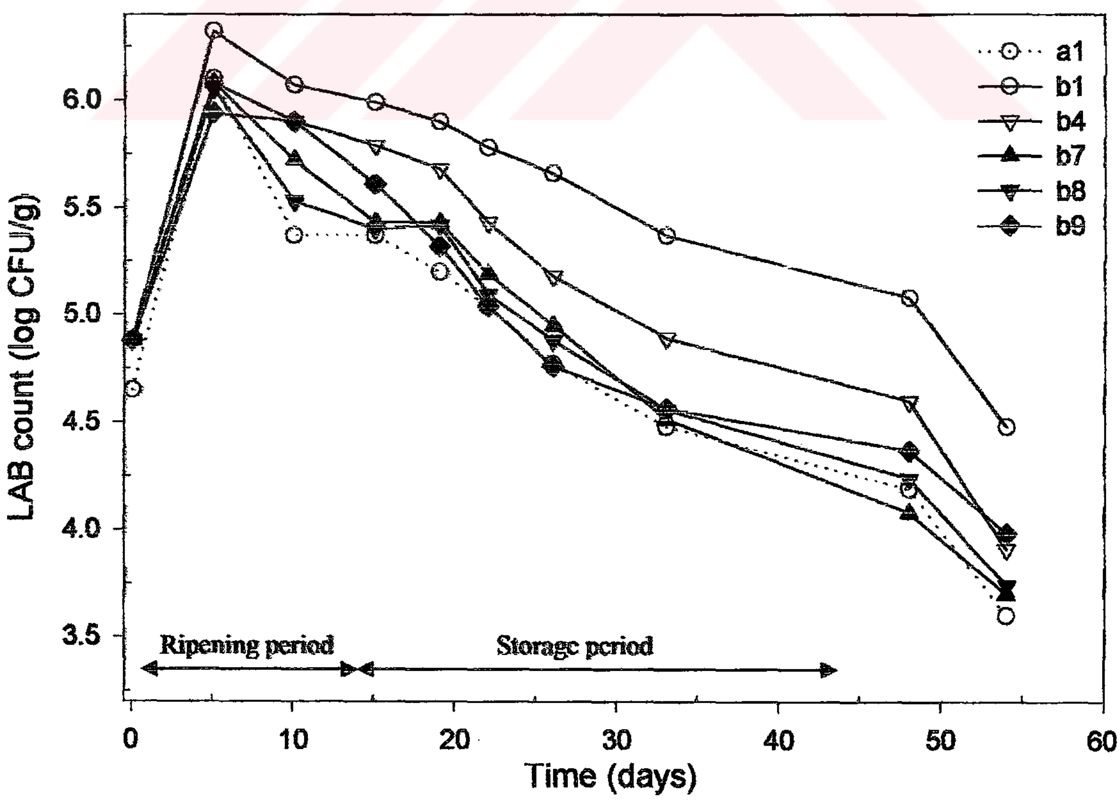


Figure 4.19 Changes of LAB count of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

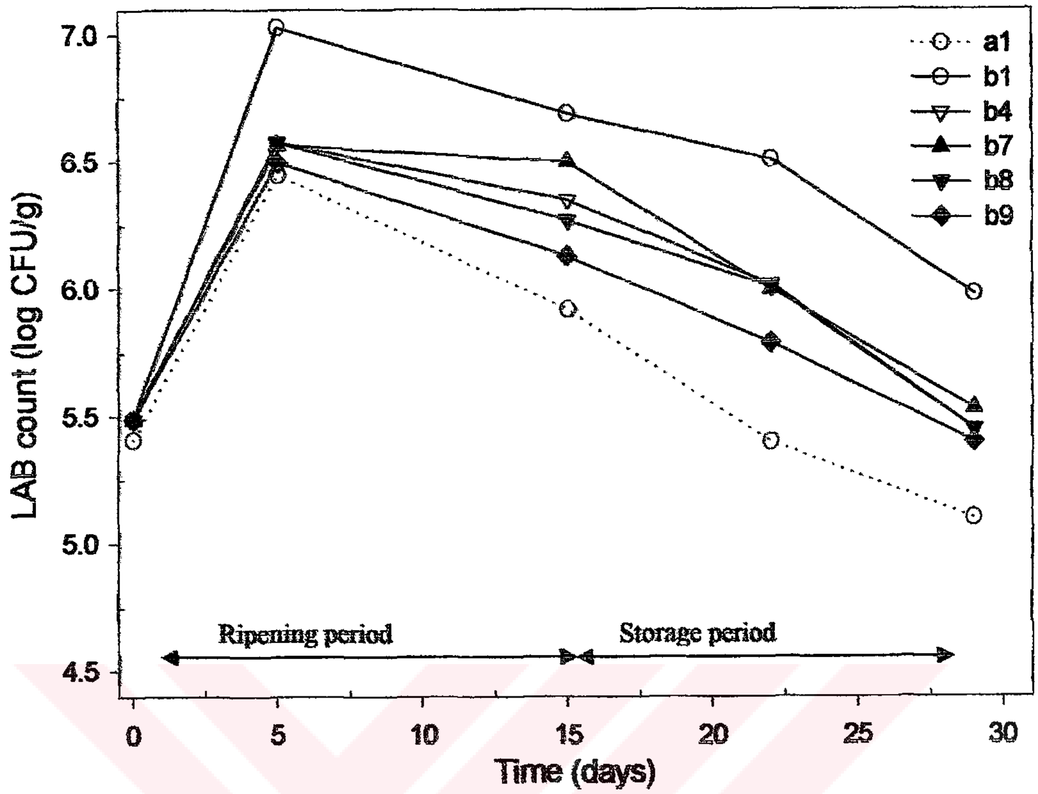


Figure 4.20 Changes of LAB of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

LAB changed linearly with mold and yeast counts, APC, overall sensory quality and putrescine concentration of sucuks and the simple positive correlation coefficients were between these parameters 0.321, 0.830, 0.270 and 0.124, respectively. Positive correlation means that the higher the LAB the higher the mold and yeast counts, APC, and overall sensory quality of sucuks. There was negative correlation between LAB and pH, residual nitrite levels and tyramine concentration in sucuks. Negative correlation means that lower LAB higher pH, residual nitrite levels and tyramine concentration in sucuks

4.1.2.2.1. Prediction of Lactic Acid Bacteria Count during the Storage

The following empirical formula was applied to describe the LAB in sucuks (B1-B9) during the storage periods. Statistical analyses was carried out in the residual data (difference between experimental and predicted data) by applying one-way ANOVA and significance level (P value) of regression is $P<0.0001$. This lowest level of significance indicates that empirical formula was well fitted to the experimental data.

$$LAB\ (\log CFU/g) = a + b\,t + c\,A + d\,T + e\,R + f\,T^2 \qquad [2]$$

where a, b, c, d, e and f are the constants derived from non-linear regression, t is the storage time in day, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significance of the constants are given in Table 4.2. Significance of the constants indicates that presence of this constant necessary or not; if significance level is lower than $P<0.0001$, it is necessary to put this constant into equation. Residual data versus time (days) plot was drawn and it is given in Figure 4. 21. It is observed that residual LAB ranged between -1.88 to 1.08 log CFU/g.

This empirical formula was compared statistically and it can be regarded as sufficient to describe the LAB in sucuks during the storage periods. LAB during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 2].

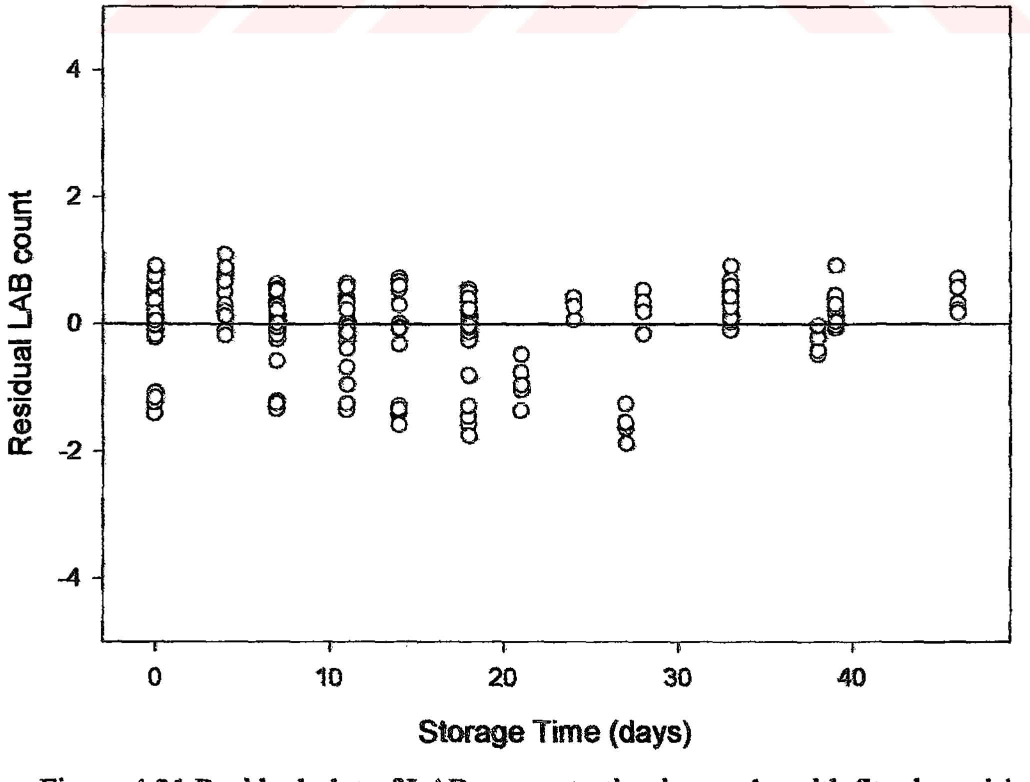


Figure 4.21 Residual plot of LAB concentration in sucuks with fitted empirical formula

Table 4.2 The constants derived from the empirical formula with their standards errors and significance levels

Constant	Value	P values
a	5.2750±0.8248	<0.0001
b	-0.0433±0.0034	<0.0001
c	-0.2458±0.0502	<0.0001
d	-0.1478±0.0539	<0.0001
e	0.0473±0.0041	<0.0001
f	0.0023±0.0009	0.0103

4.1.2.3. Lactic Acid Bacteria Count in Retailed Sucuks

ANOVA test was carried out to determine if any significant differences exist between LAB count of traditional and firm-made sucuks. It was found that there were no statistical differences existance between LAB count of traditional and firm-made sucuks. Traditional sucuks produced with chance inoculation, however, firm-made sucuks made up with suitable starter culture. It was expected that LAB count of firm-made sucuks could have higher number than the traditional sucuks. However, after the ripening period heat (at 60°C for 1 hr) could be applied to the firm-made sucuks. Some of LAB would be inhibited with this heat treatment.

LAB count of retailed sucuks (Firm-made and traditional) sucuks had higher ($P<0.05$) LAB count than sucuk types (A1, B1, B4, B7-B9; Figure 4.22). The lowest LAB count was found sucuk type A1.

4.1.3. Mold and Yeast Counts

Mold and yeast counts of sucuk types A1-A3 and B1-B9 were monitored during the ripening and storage periods at 20, 30 and 40°C with 50, 65, and 80 % RH, respectively. Their changes are given in Figures 4.23-4.31. Mold and yeast counts did not increase ($P>0.05$) during the ripening and storage periods in sucuks, which made with potassium sorbate. These results indicate that potassium sorbate should be used to decrease mold and yeast growth.

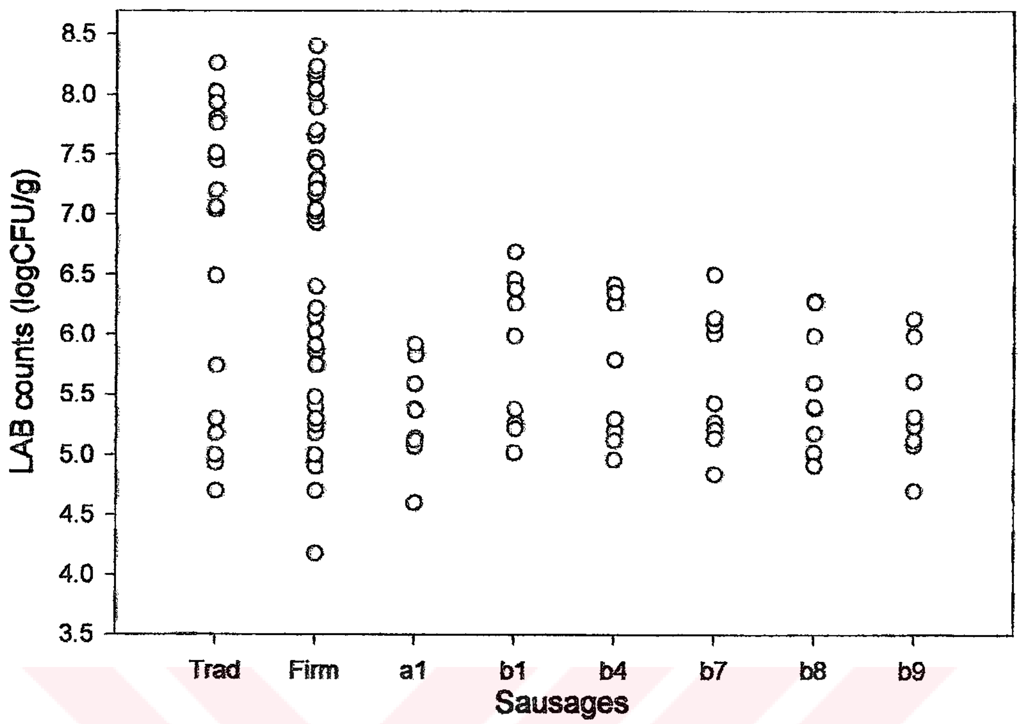


Figure 4.22 LAB count of sucuk types (A1, B1, B4, B7, B8 and B9) and retailed sucuks (Trad: traditional; Firm: firm-made sucuks) (Meanings of A1, and B1-B9 are given in Table 3.2; page 40)

4.1.3.1. Mold and Yeast Counts during the Ripening Periods

Mold and yeast counts of some sucuks (A1, B1, B4, and B7) increased (P<0.05) during the first five days of ripening periods after that they decreased during the further ripening periods. These sucuks made without potassium sorbate, phosphates (potassium pyrophosphate and di-potassium hydrogenphosphate) and antioxidants (ascorbic acids and α-tocopherol; Table 3.2).

The highest mold and yeast counts were observed in sucuk type A1 during the ripening periods (P<0.05), about 0.8 log CFU/g increments were observed from initial number. The order of mold and yeast counts in sucuks were A1>B1>B4>B7. Mold and yeasts were readily grown during the first five days in sucuk type A1 made without additives and starter culture inoculation (Table 3.2). Addition of starter culture and nitrite/nitrate could be reduced (P<0.05) the growth of mold and yeast, so B1, B4, and B7 had lower (P<0.05) mold and yeast counts than A1.

Potassium sorbate containing sucuks had lower (P<0.05) mold and yeast counts than sucuks made without potassium sorbate. Increasing the concentration of potassium sorbate decreased (P<0.05) mold and yeast counts. Since sucuk types A3, B3, B6 and

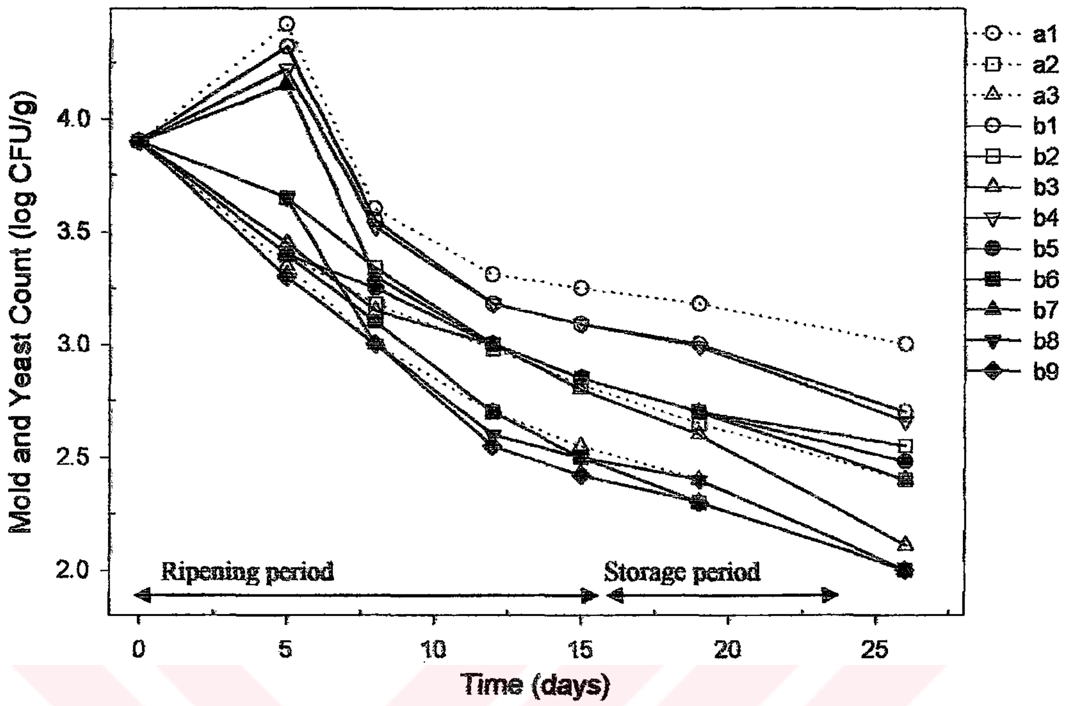


Figure 4.23 Changes of mold and yeast count of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

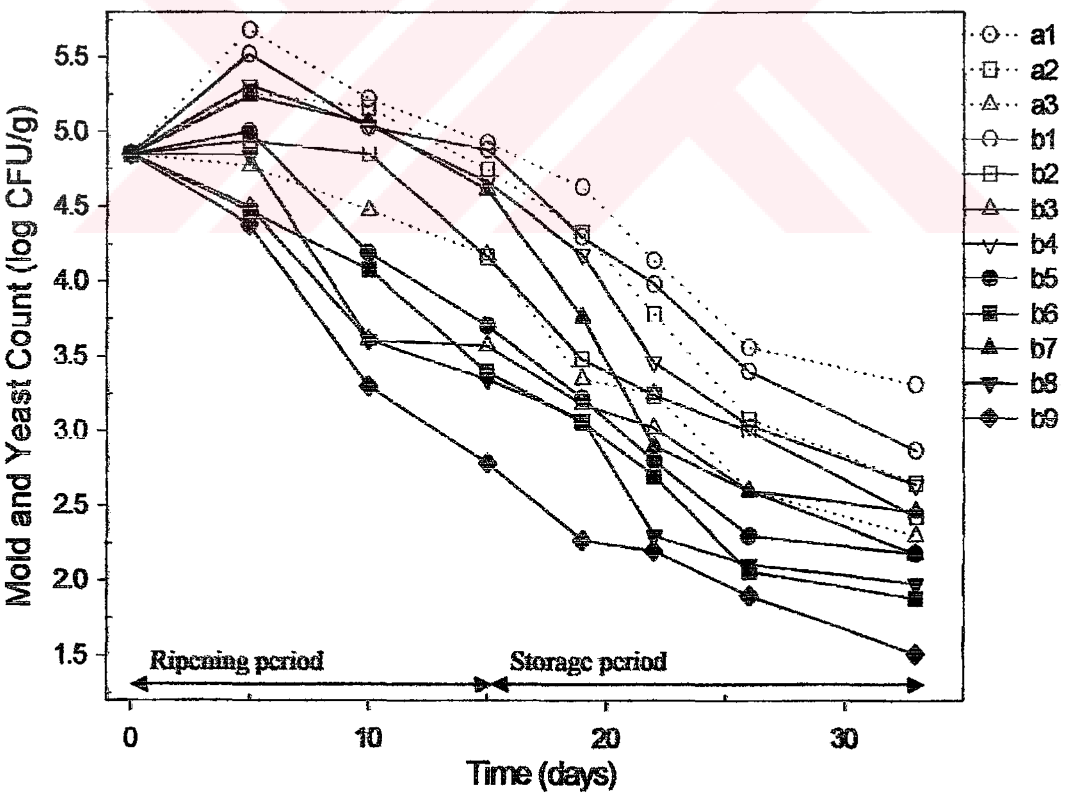


Figure 4.24 Changes of mold and yeast count of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

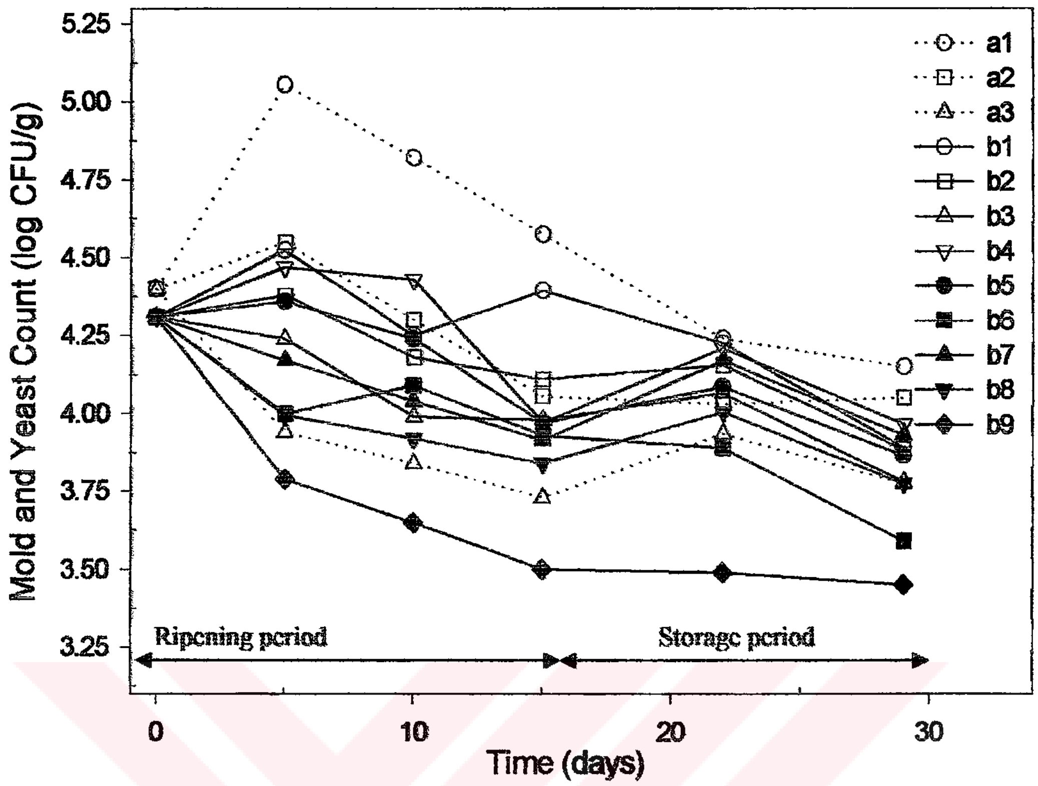


Figure 4.25 Changes of mold and yeast count of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

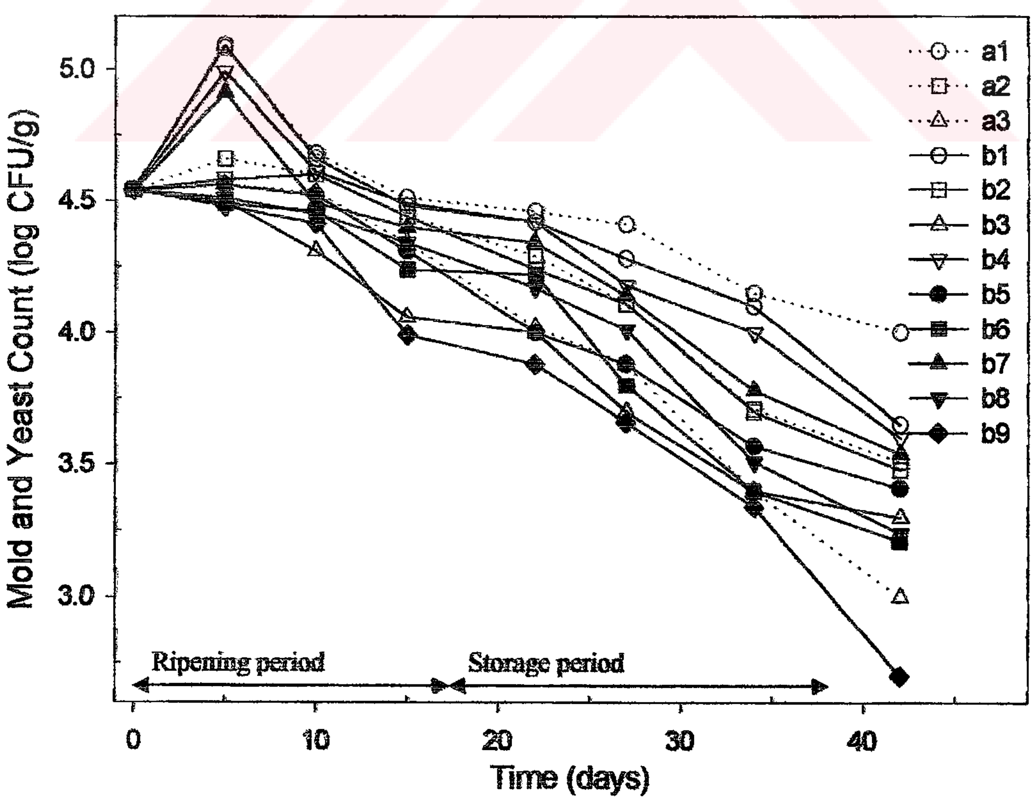


Figure 4.26 Changes of mold and yeast count of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

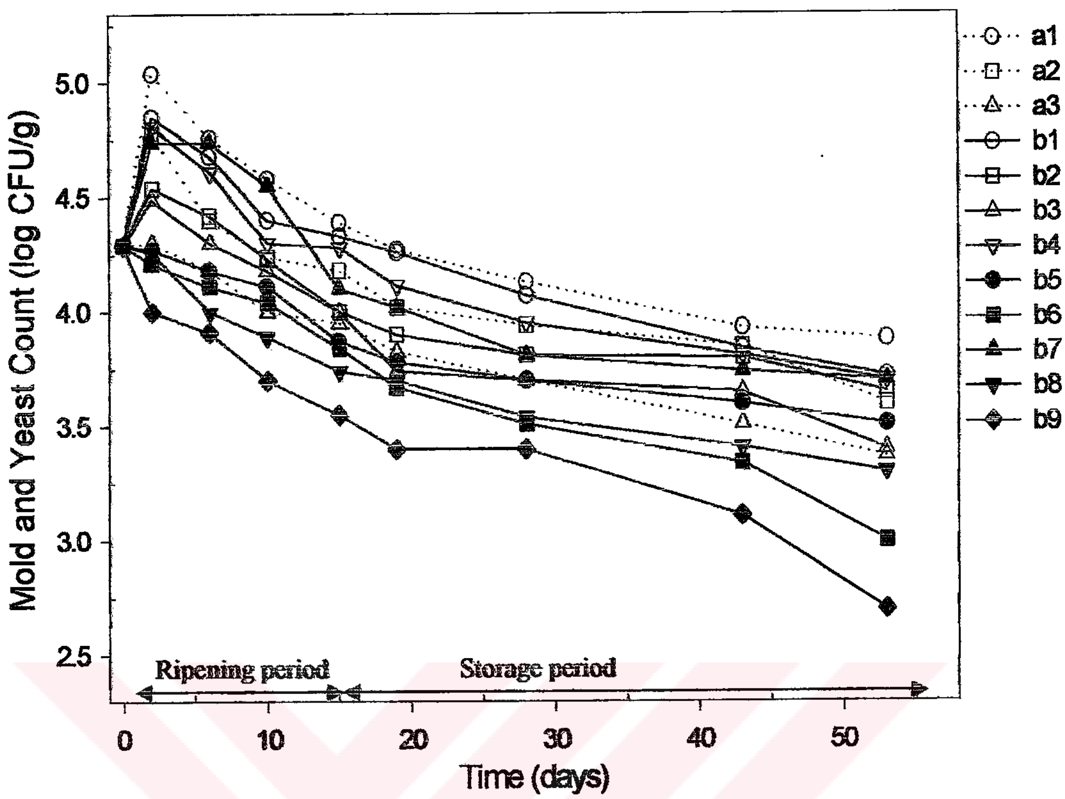


Figure 4.27 Changes of mold and yeast count of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

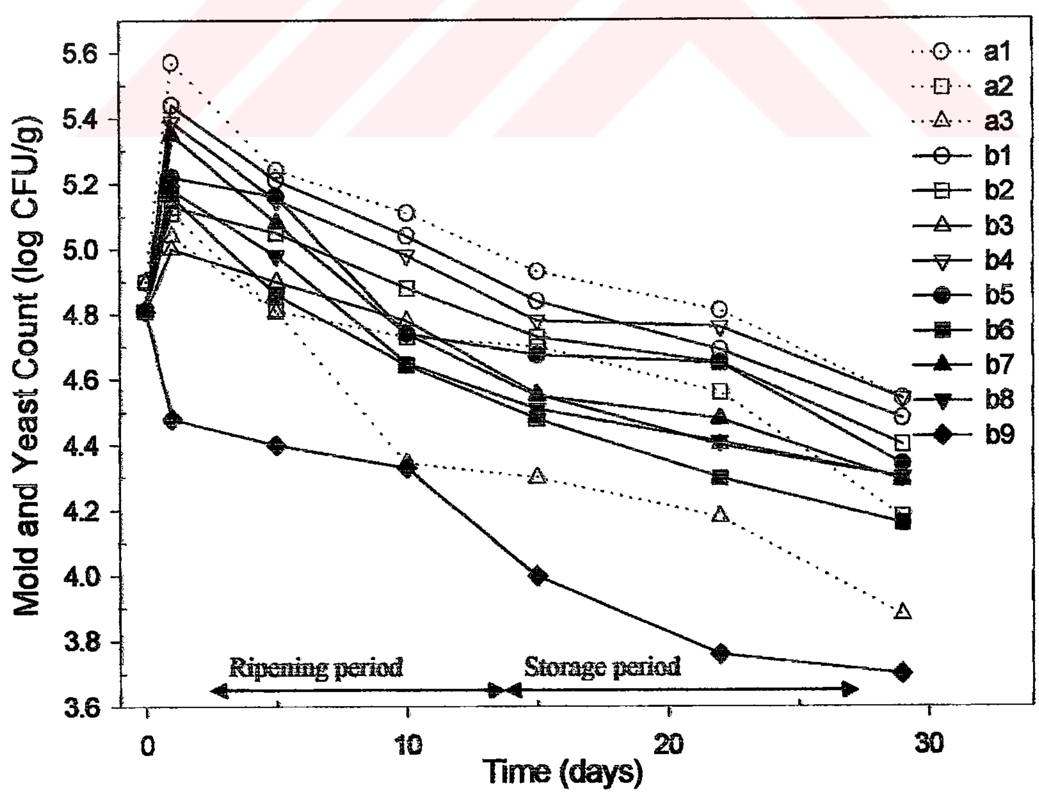


Figure 4.28 Changes of mold and yeast count of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

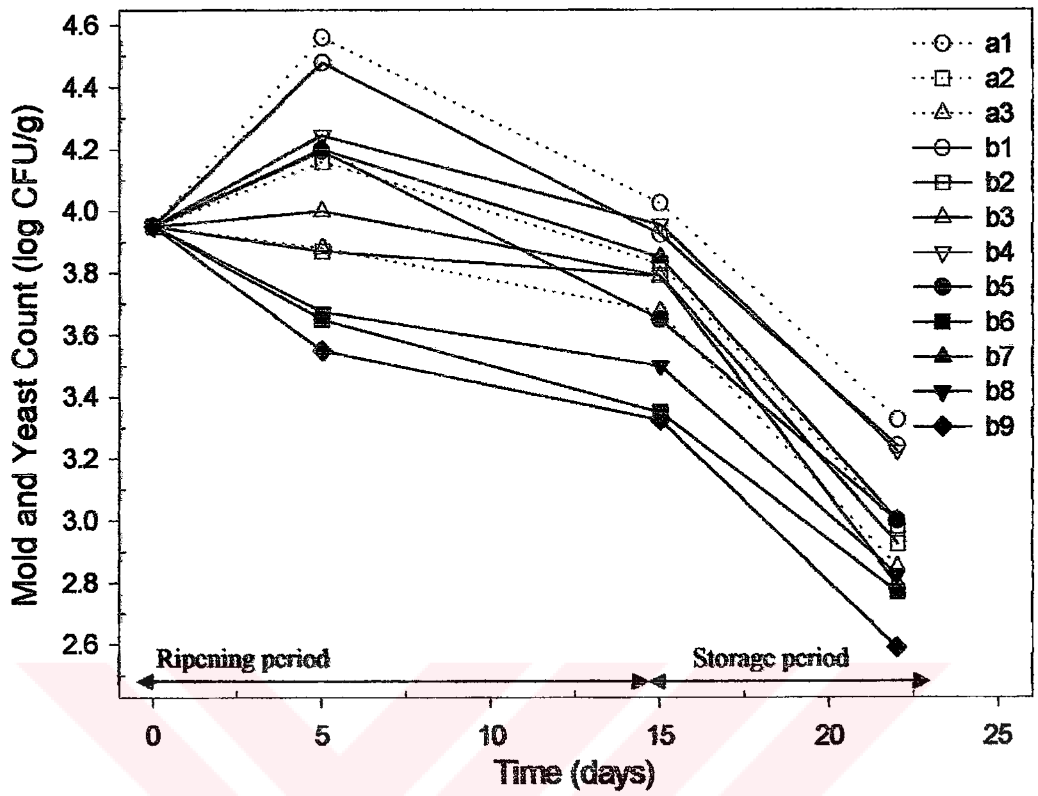


Figure 4.29 Changes of mold and yeast count of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

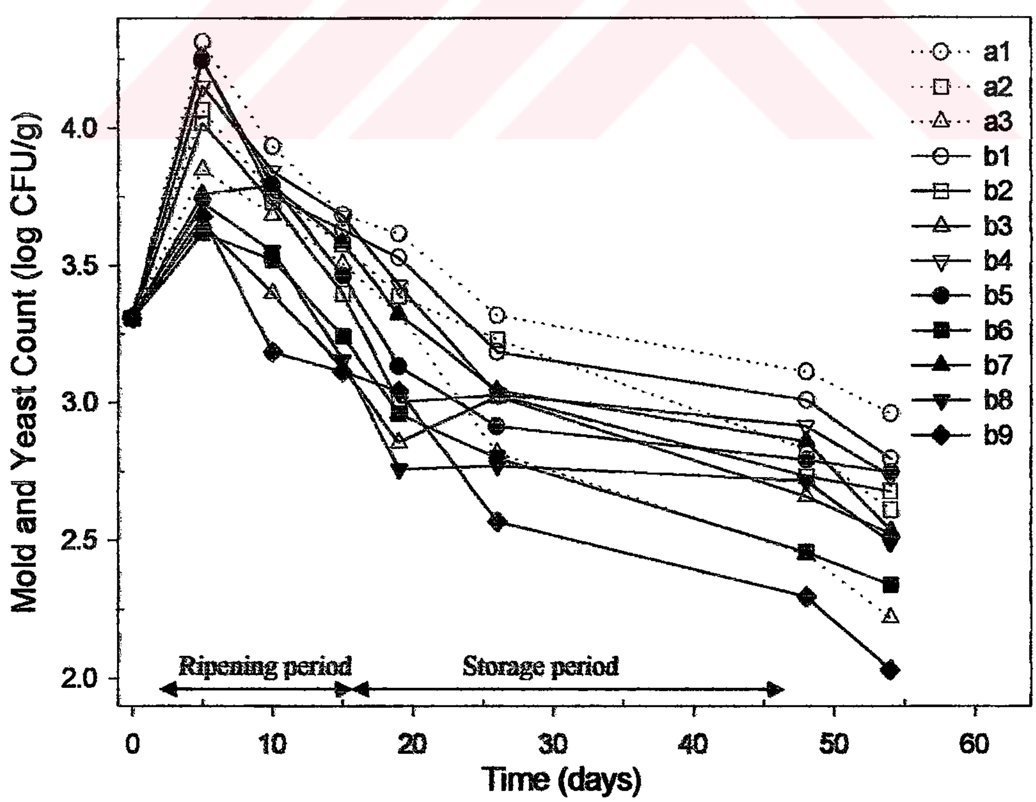


Figure 4.30 Changes of mold and yeast count of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

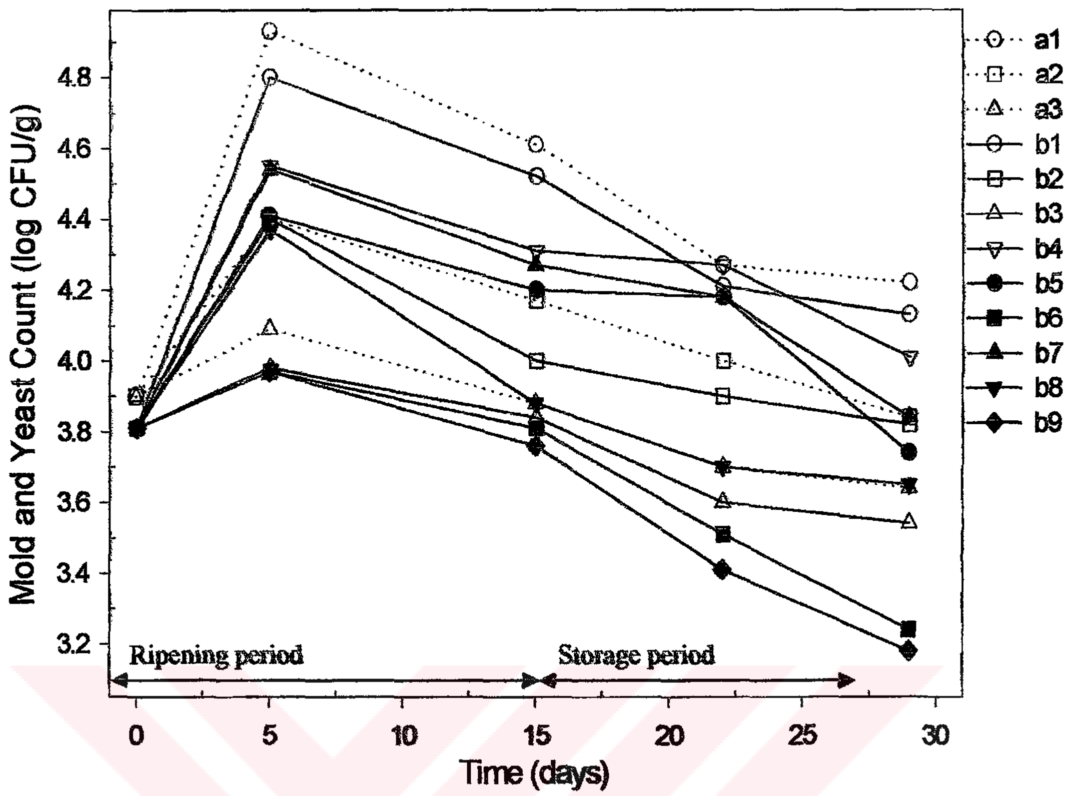


Figure 4.31 Changes of mold and yeast count of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

Statistical analysis indicated that increasing the storage temperature decreased the mold and yeast counts significantly ($P<0.05$). Microbial growth depends on the presence of available water in the food. If available water increases ($P<0.05$) growth of mold and yeast also increases. Sucuks stored at 80 % RH had higher ($P<0.0.5$) mold and yeast counts than 50 and 65 % RH (Figures 4.23-4.31). According to the TSE permissible number of mold and yeast in sucuk is 10^2 per gram (TS: 1070, Anonymous, 1983). Sucuks stored at 50 % RH, number of mold and yeast reduced to about 10^2 after 25 days, but sucuks stored at 65 % RH, this number could reach after 60 days. This clearly shows that reducing percent relative humidity decreased ($P<0.05$) mold and yeast counts (Figures 4.23-4.31).

Statistical analysis showed that additive - temperature, additives - % RH (two-way interactions) and additive - temperature - % RH (three-way interaction) were not significant ($P>0.05$), however, temperature - % RH two-way interaction was significant ($P<0.05$). Temperature - % RH two-way interaction shows that increasing both % RH and temperature decreased ($P<0.05$) mold and yeast much more than the increasing one factor (temperature or % RH) alone. As a result, to keep mold and yeast counts of sausages to safe level additives, temperature and % RH could be used as hurdle effect.

According to the TSE standards (TS 1070), sucuks of B5, B7, B8, B6 and B9 were found to be acceptable than the other sucuks. These results indicate that additives and starter culture should be added to obtain high quality of sucuks with respect to mold and yeast counts.

Positive significant correlation ($P<0.05$) was found from Pearson test and which coefficients were 0.321, 0.294, 0.140, and 0.237 between mold and yeast counts and lactic acid bacteria count (LAB), APC, residual nitrite level, and histamine concentration, respectively. This indicate that there was a linear relationship ($P<0.05$) between the mold and yeast counts and lactic acid bacteria count (LAB), APC, residual nitrite level and histamine content. This means the lower the mold and yeast counts the lower the LAB, APC, residual nitrite level, and histamine formation, and also higher the APC higher these parameters. Negative correlations were observed among mold and yeast counts and pH, nitrosomyoglobin formation, TBA values, overall sensory quality of sausages and tyramine concentration. Negative correlation mean higher pH, nitrosomyoglobin conversion, TBA, overall sensory quality of sausages and tyramine concentration lower the mold and yeast counts.

4.1.3.2.1. Prediction of Mold and Yeast Counts during the Storage

The following empirical formula was applied to describe the mold and yeast counts in sucuks (B1-B9) which were collected during the storage periods. Statistical analyses were carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is $P<0.0001$. This low level of significance indicates that empirical formula was well fitted to the experimental data.

$$\text{Mold and Yeast count (log CFU/g)} = a + b\,t + c\,A + d\,T + e\,R + f\,T^2 \qquad [3]$$

where a, b, c, d, e and f are the constants derived from non-linear regression, t is the storage time in day, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significance of the constants are given in Table 4.3. Significance of the constants indicates that presence of this constant necessary or not; if significance level is lower than P<0.0001, it is necessary to put this constant into equation. Residual data versus time (days) plot was drawn and it is given in Figure 4. 32. It is observed that residual mold and yeast count ranged between -1.76 to 1.19 log CFU/g.

Table 4.3 The constants derived from the empirical formula with their standards errors and significance levels

Constant	Value	P values
a	-5.4709±0.4548	<0.0001
b	-0.0277±0.0022	<0.0001
c	-0.1809±0.0283	<0.0001
d	0.5552±0.0294	<0.0001
e	0.0262±0.0021	<0.0001
f	-0.0091±0.0005	<0.0001

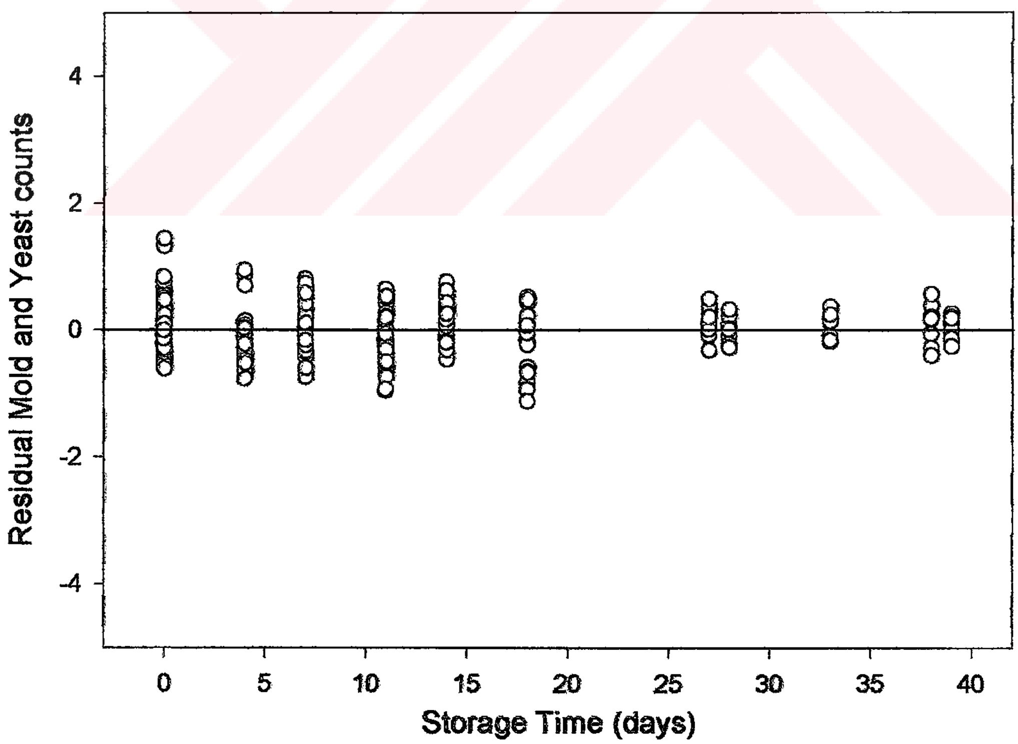


Figure 4.32 Residual plot of mold and yeast counts in sucuks with fitted empirical formula

This empirical formula was compared statistically and found that it can be regarded as sufficient to describe the mold and yeast counts in sucuks during the storage periods. It was found that mold and yeast counts during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 3].

4.1.3.3. Mold and Yeast Count in Retailed Sucuks

Mold and yeast count varied between 3.70-5.30 and 3.30-5.53 log CFU/g, respectively and average number of mold and yeast counts were 4.46 and 4.26 log CFU/g in the traditional and firm-made sucuks, respectively (Figure 4.33). Statistical analysis shows that traditional sucuks had higher ($P<0.05$) mold and yeast count than firm-made sucuks. Firm-made sucuks had antimicrobials especially potassium sorbate and this chemical has inhibitory effect on the mold and yeasts (Erkmen, 2000). Also, starter culture might be used in the firm-made sucuks, starter culture produce bacteriocins which have antimicrobial activity (Erkmen and Fadıloğlu, 2001). Thus, firm-made sucuks had lower mold and yeast count.

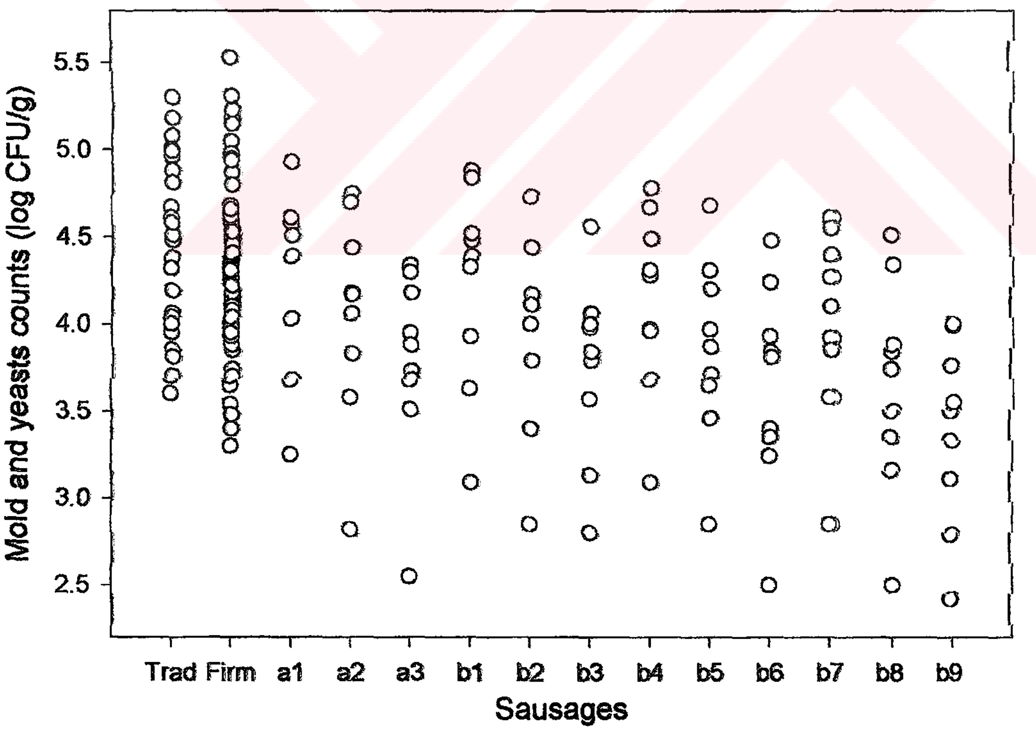


Figure 4.33 Mold and yeast count of sucuk types (A1-B9) and retailed sucuks (Trad: traditional; Firm: firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

TSE and Food Codex pointed out for sucuks that mold and yeast count should not be higher than 2 log cycle per g of sucuk for mold and yeast counts (TSE, 1070, Anonymous, 1983; Food Codex Anonymous, 2000/4). All of the traditional and firm-made sucuks had higher than this allowable limit. This result indicates that mold and yeast counts of both traditional and firm-made sucuks were made under unsanitary conditions, ingredients with higher amount of molds and yeasts, or stored under unsatisfactory conditions.

It was observed that traditional and sucuk type A1 (control sucuk, containing no additives and starter culture) had the highest ($P<0.05$) mold and yeast count and the lowest mold and yeast count was observed in sucuk type B9 (containing the high level of additives with starter culture). Sucuk type B9 had high level of potassium sorbate which have inhibitory effect on the mold and yeast, and it inhibited mold and yeasts (Figure 4.33).

4.2. Chemical Changes in Sucuks

In this research, pH, 2-thiobarbituric acid (TBA) values, nitrosomyoglobin conversion, residual nitrite level and biogenic amine formations were monitored for chemical changes in sucuk during the ripening and storage periods. These changes were also monitored for sucuks collected from local markets (firm-made) and butchers (traditional).

4.2.1. Changes of pH

pH values of sucuks A1-A3 and B1-B9 were followed during the ripening and storage periods and their changes are given in Figures 4.34–4.42. During the first days of- ripening (fermentation period), lactic acid bacteria produced organic acids under anaerobic condition and the pH values of sucuks were sharply decreased from an initial pH value about 5.60-5.80 to about 4.50-4.90 level. A decline in pH has a number of beneficial effects on the quality and shelf life of sucuks. A rapid and sharp decrease in pH has been reported to reduce the growth of amine-positive bacteria at the beginning of fermentation, accelerates nitrosomyoglobin conversion, increase in the drying processes and improves textural attributes of sucuks (gelation of meat protein) (Lücke, 1994; Bover-Cid *et al.*, 1999; Ordonez *et al.*, 1999; Bover-Cid *et al.*, 2001).

4.2.1.1. Changes of pH in Sucuks during the Ripening Periods

The changes of pH values during the ripening periods are given in Figures 4.34-4.42. It was observed that during the first three days of ripening, pH values of all sucuks were sharply decreased ($P<0.05$) to about 4.90 from an initial value about 5.70. After that, pH values were slowly increased ($P<0.05$) gradually (except at 80 % RH). During the first three days of ripening, lactic acid bacteria produced organic acids under anaerobic condition and the pH values of sucuks were decreased. Turantaş and Ünlütürk (1993) found that pH values of sucuks made with or without starter culture gradually decreased to about 4.90 levels from an initial value of 5.67. Vural and Öztan (1992b) found that pH values of sucuks were sharply decreased during the first three days of ripening. After three days only slight increase in pH were observed in sucuk samples. The similar results also were observed in different types of sausages by Hernandez-Jover *et al.* (1997a), Maijala *et al.* (1995), Roig-Sagues and Eerola (1997), Eerola *et al.* (1996). Reduction in pH values (to about 4.40) were higher in dry sausages produced with starter culture stored at 16°C at 70 % RH (Eerola *et al.*, 1996).

During the ripening period, the lowest ($P<0.05$) pH values were observed in sucuk type A1 (Figures 4.34-4.42) made without additives and starter culture. Addition of additives increased ($P<0.05$) pH values of sucuks during the ripening periods. Increasing the levels of additives increased ($P<0.05$) pH values of sucuks during the ripening periods. Sucuk types A3 and B9 made with high level of additives had higher ($P<0.05$) pH values than the other sucuks at the end of the ripening periods.

Reduction of pH values in sucuks were not influenced ($P>0.05$) from starter culture inoculation during the ripening periods compared to the control, because meat naturally contained a number of lactic acid bacteria. The lactic acid bacteria grow during the first three days of ripening period and reduce pH values of sucuks (Figures 4.12-4.20). Turantaş and Ünlütürk (1993) observed similar results that pH values of sucuks made with or without starter culture decreased ($P<0.05$) during the first days of the ripening period and this reduction did not depend on the starter culture inoculation.

4.2.1.2. Changes of pH values of Sucuks during the Storage Periods

pH values of sucuks become nearly constant ($P>0.05$) during the storage period. The increase ($P>0.05$) in pH values during the storage period might be due to decomposition of organic acids over time.

The changes of pH values of sucuks during the storage periods at 20°C with 50, 65 and 80 % RH are shown in Figures 4.34, 4.35 and 4.36, respectively. pH values of sucuks increased ($P<0.05$) gradually during the storage periods. pH values of sucuks stored at 80 % RH were lower than sucuks stored at 50 and 65 % RH. This would be due to higher growth rate of lactic acid bacteria (Figures 4.12-4.20). pH values of sucuks made with starter culture (B1-B9) were more stable ($P>0.05$) than made without starter culture (A2 and A3) during the storage periods. Sucuks made without starter culture, A2 and A3, had higher ($P<0.05$) pH values than sucuks B5 and B9 made with starter culture. Sucuk type A2 and B5 made with same amount of additives, also A3 and B9, too (Table 3.2).

pH values of sucuks stored at 30°C with 50, 65 and 80 % RH are given in Figures 4.37, 4.38 and 4.39, respectively. pH values of sucuks increased ($P<0.05$) during the storage periods, except stored at 80 % RH. Sucuks stored at 80 % RH, pH values increased and decreased during the storage period due to variation in the number of lactic acid bacteria and others (Figures 4.1-4.18).

The changes of pH values of sucuks stored at 40°C with 50, 65 and 80 % RH are shown in Figures 4.40, 4.41 and 4.42, respectively. pH values of sucuks increased ($P<0.05$) during the storage periods up to about 5.30 (except at 80 % RH). The lowest pH value ($P<0.05$) was observed in sucuk type A1.

Additives increased ($P<0.05$) pH values of sucuks. Sucuks made without additives (A1 and B1) had lower ($P<0.05$) pH values than the other sucuks. Sucuks (A3, B3, B6 and B9) made with higher levels of additives had higher level of pH than the other sucuks. However, pH values were not different ($P>0.05$) in sucuks made with only nitrite/nitrate (B1, B4 and B7). Moreover, pH values were not different ($P>0.05$) in sucuks made with low level of nitrite/nitrate. Some additives, especially potassium pyrophosphate, di-potassium hydrogen phosphate, nitrite, nitrate and salt, have buffering capacity in foods (Bozkurt and Erkmen, 2002a). Yetim *et al.* (1992) found that additives increased pH of in frankfurter-sausage. Additives would stabilize pH value of sucuk during storage periods. These results show that addition of α-tocopherol

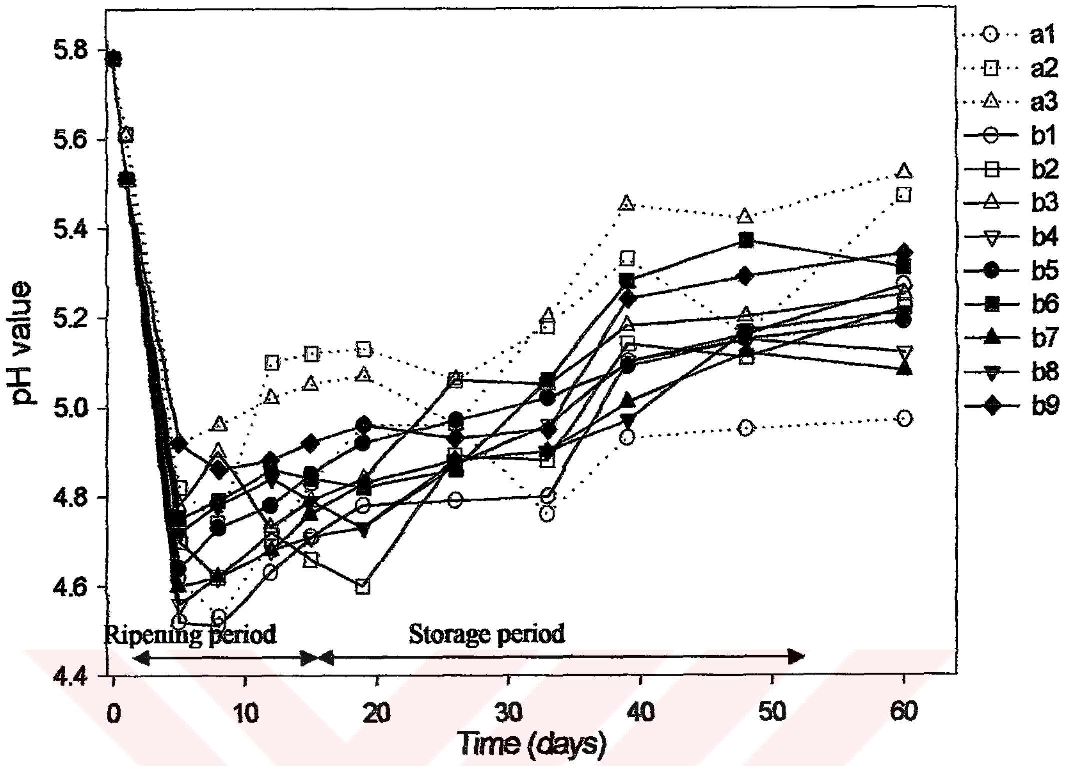


Figure 4.34 Changes of pH values of sausages during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

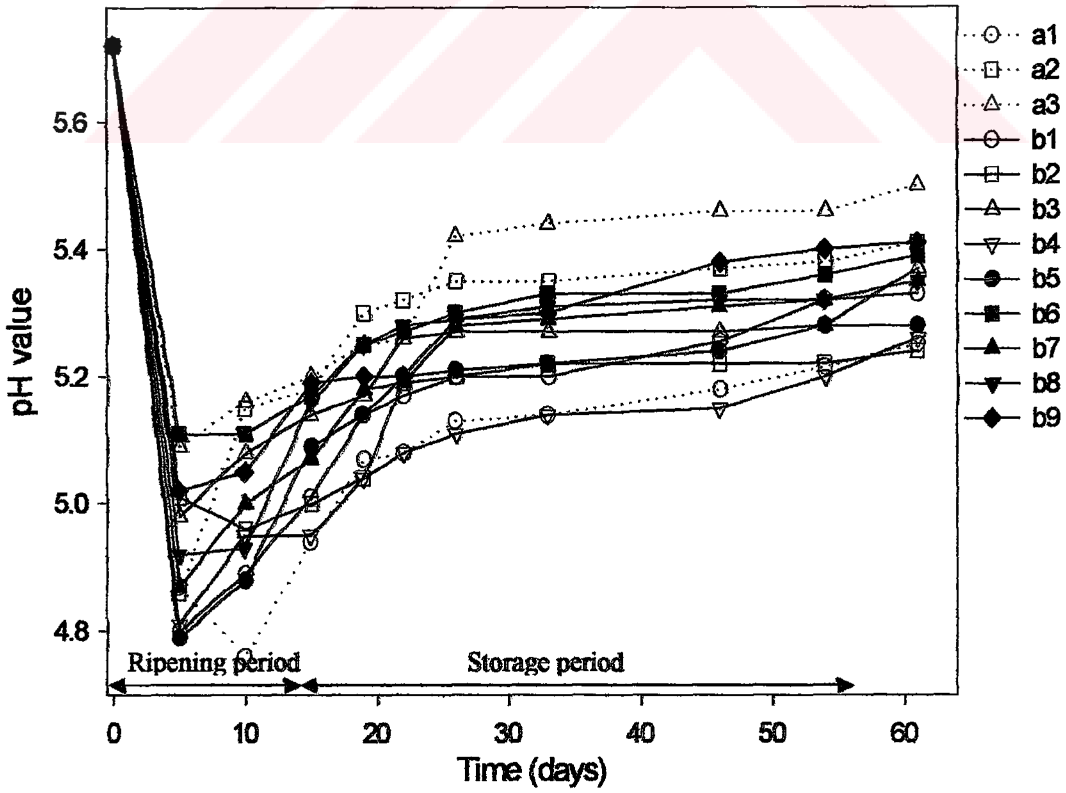


Figure 4.35 Changes of pH values of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

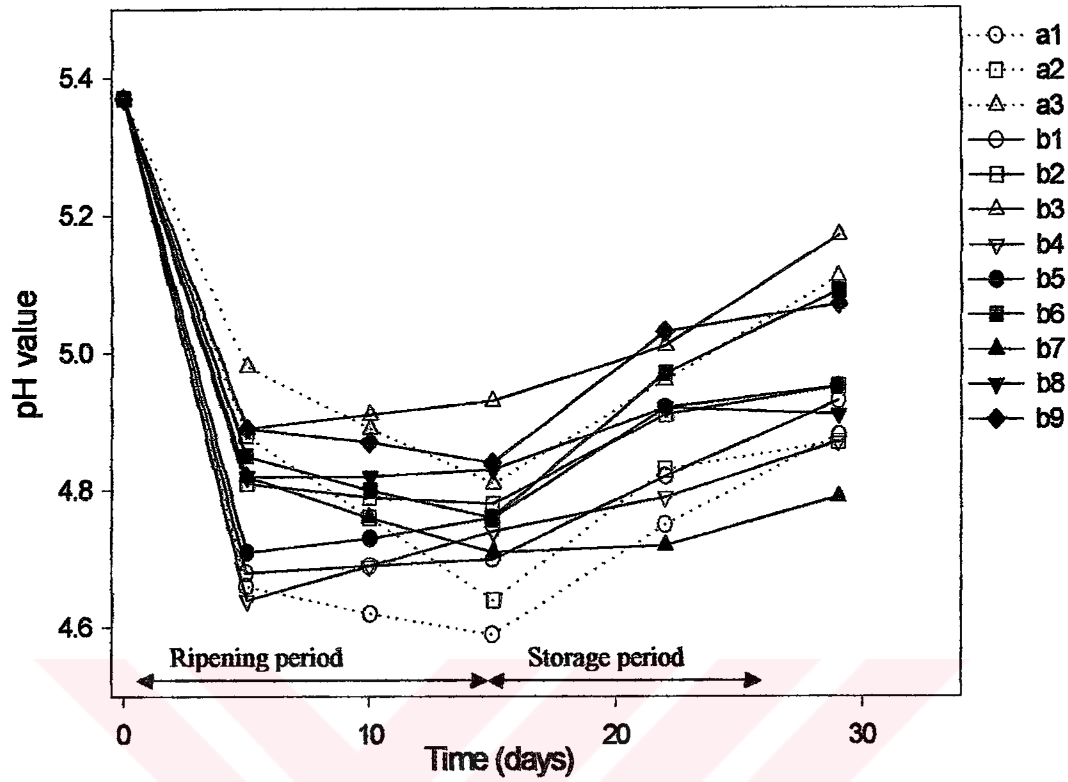


Figure 4.36 Changes of pH values of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

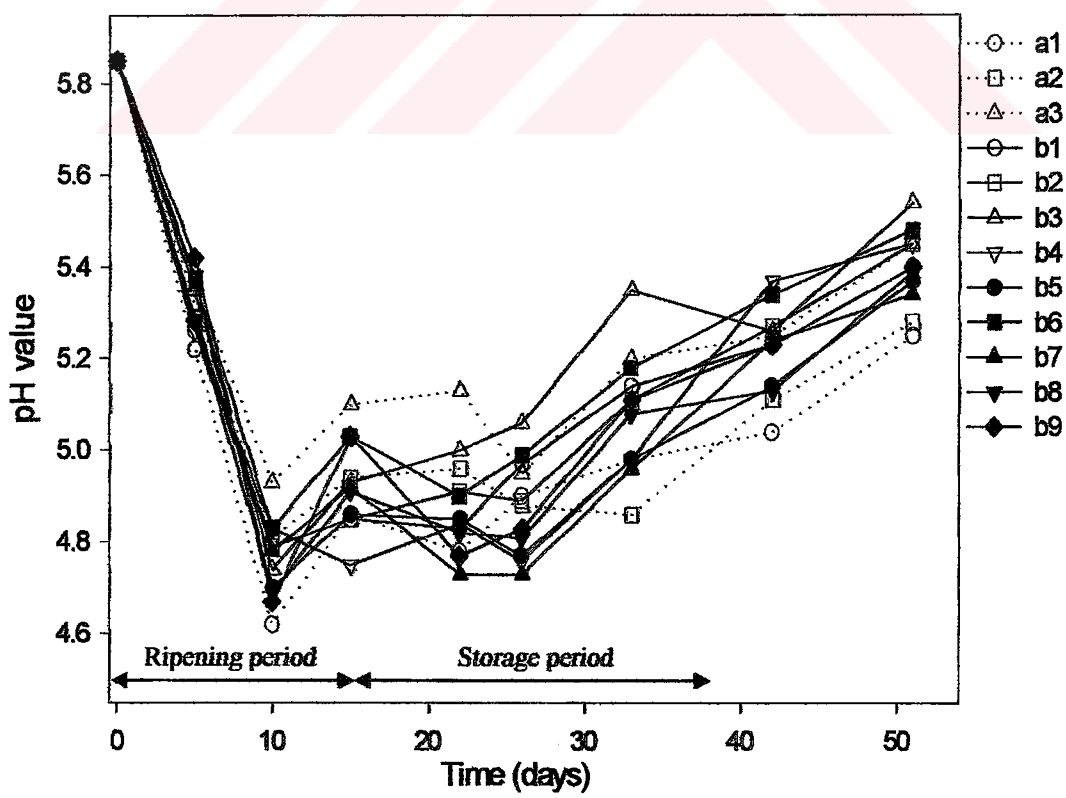


Figure 4.37 Changes of pH values of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

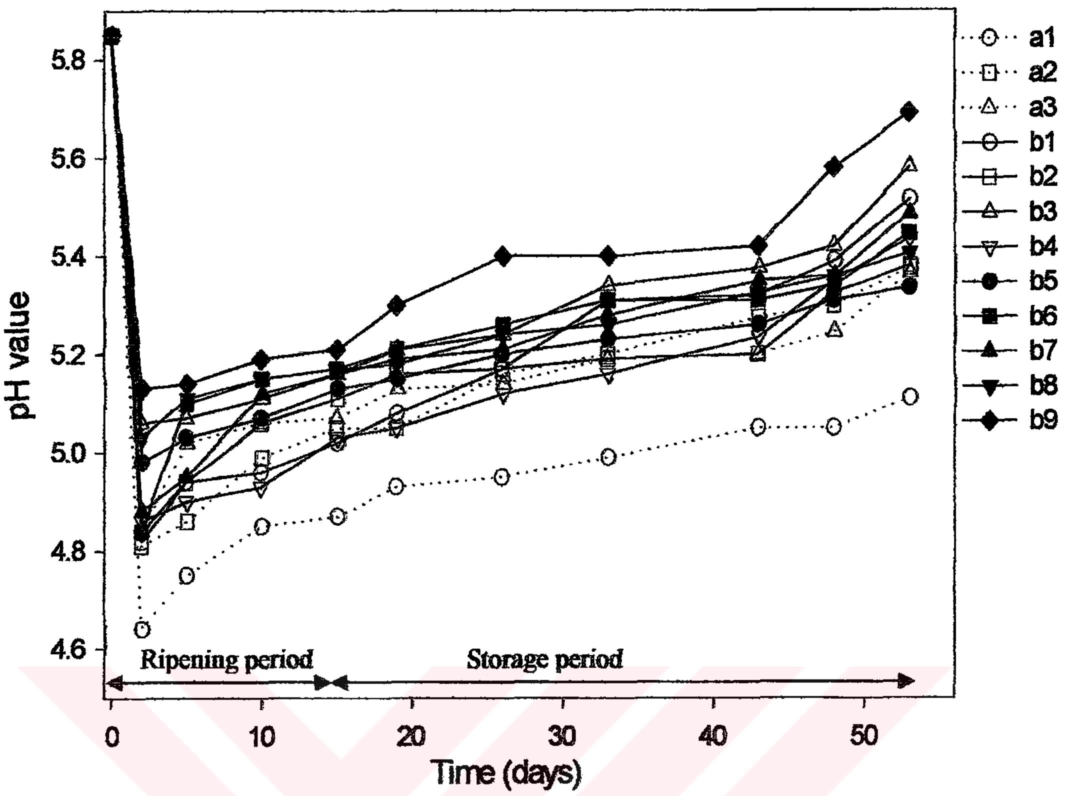


Figure 4.38 Changes of pH values of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

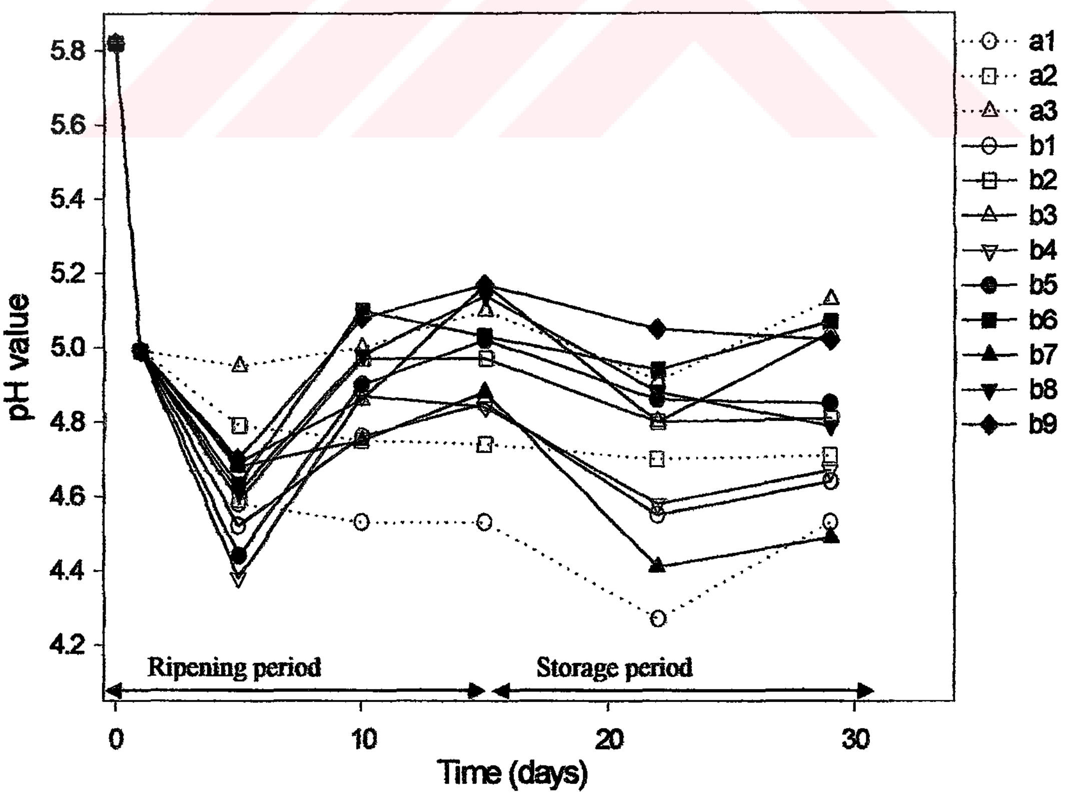


Figure 4.39 Changes of pH values of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

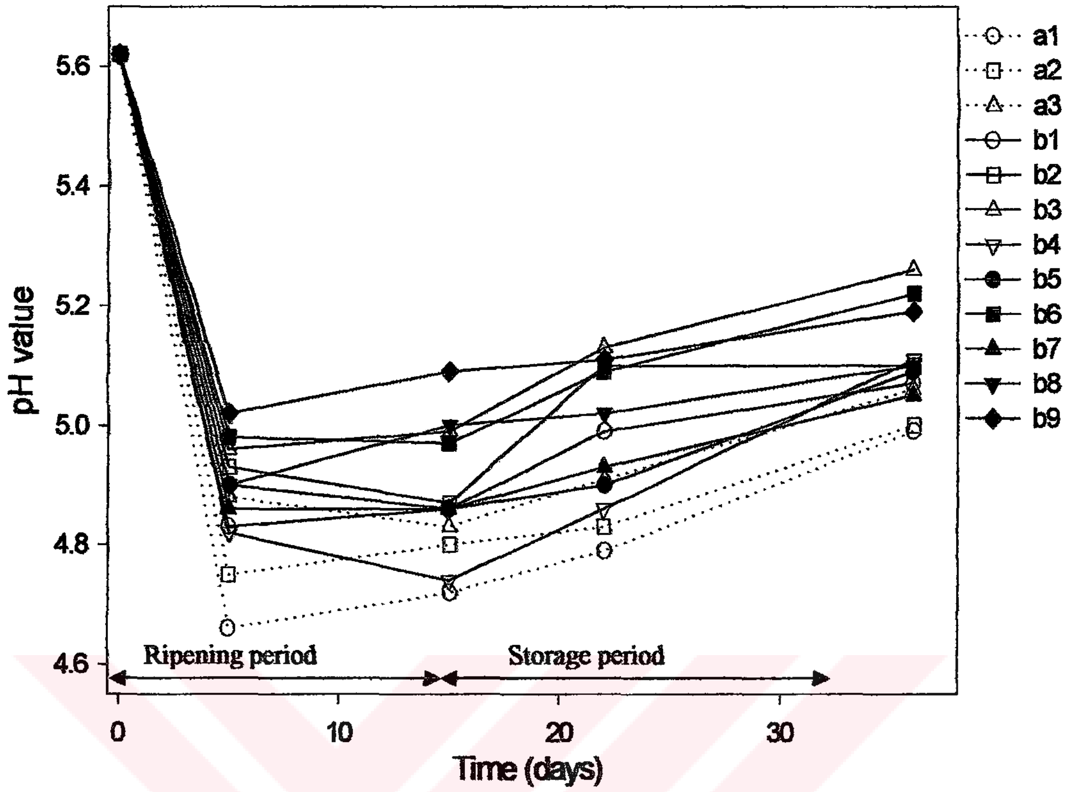


Figure 4.40 Changes of pH values of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

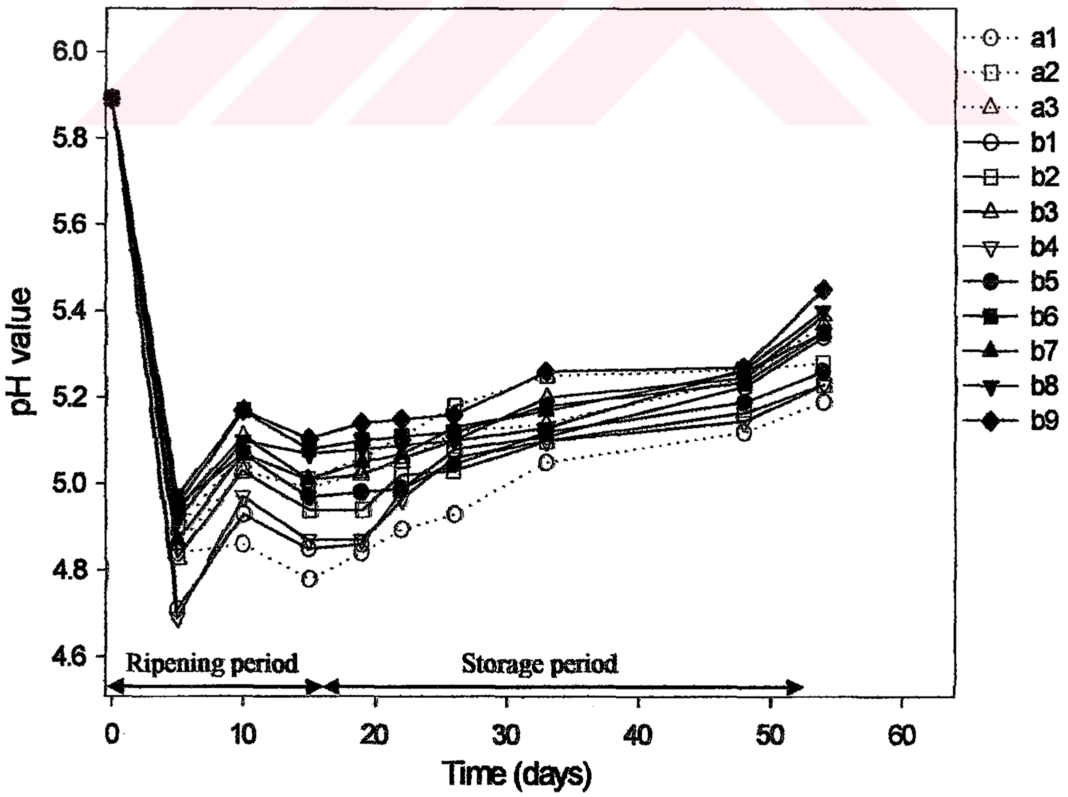


Figure 4.41 Changes of pH values of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

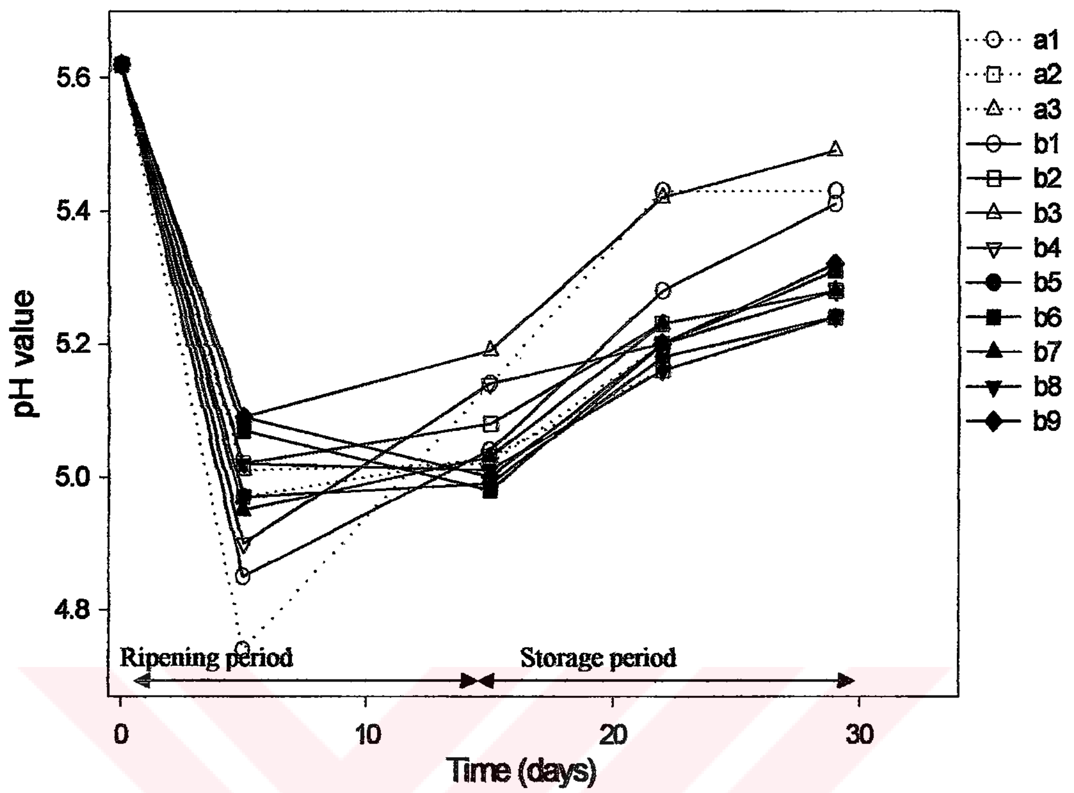


Figure 4.42 Changes of pH values of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

ascorbic acid, potassium sorbate, potassium pyrophosphate and di-potassium hydrogen phosphate into sucuks affected pH values more than ($P<0.05$) nitrite and nitrate. pH values of sucuks prepared without additives (control sample), A1, were not acceptable at the end of the storage because of its lower pH values than Turkish Food Codex standards (Anonymous, 2000). pH values of sucuks prepared with additives were found to be in an acceptable range.

Effect of temperature on the pH values of sucuks was found to be significant ($P<0.05$). Increasing or decreasing temperature from optimum temperature of lactic acid bacteria (about 30°C) decreased or increased ($P<0.05$) pH values. This would be due to the reduction in the growth rate of lactic acid bacteria below or over the optimum growth temperature.

Effect of % RH on the pH values was found to be significant ($P<0.05$). Sucuks stored at 80 % RH had the lower ($P<0.05$) pH values than that stored at 50 and 65 % RH. This could be due to growth of organic acid forming bacteria (Figures

4.12-4.20). Bacteria produced more organic acid at 80 % RH than at 50 % and 65 % RH and thus reduced pH values of sucuks more at 80 % RH.

Pearson correlation test was carried out to determine any correlation between pH and microbial, chemical and overall sensory quality changes of sucuks. Positive significant correlation ($P<0.05$) was found between pH and residual nitrite level and correlation coefficient was 0.565. It means that the lower the pH lowers the residual nitrite level in sucuks. Negative correlations were observed between pH and APC, mold and yeast counts, LAB count, TBA value, overall sensory quality, putrescine, histamine and tyramine concentrations; the correlation coefficients were -0.294, -0.076, -0.225, -0.483, -0.204, -0.210, -0.436 and -0.341 between pH and APC, mold and yeast counts, LAB count, TBA value, overall sensory quality, putrescine, histamine and tyramine concentrations. Negative correlation means lower the pH values of sucuks higher the APC, mold and yeast counts, LAB count, TBA value, overall sensory quality, putrescine, histamine and tyramine concentrations.

For high quality sucuks, pH value should be in the range of 5.1-5.2 according to Gökalp *et al.* (1999) and 5.4-5.8 according to the TSE (TS 1070; Anonymous, 1983) and maximum 5.4 for Turkish Food Codex (Anonymous, 2000). pH values of sucuks should not be greater than 5.40 (Gökalp *et al.*, 1999; Anonymous, 2000). Sucuks prepared without starter culture inoculation were not acceptable with respect to higher pH values than declared value. All these results indicate that, to obtain a good quality sucuk, it should be prepared with starter culture and additives, ripening temperature should be low (<20°C) and should be stored at low temperatures (20°C).

4.2.1.2.1. Prediction of pH values during the Storage

The following empirical formula was applied to describe the pH values in sucuks (B1-B9) which were collected during the storage periods. Statistical analyses were carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is $P<0.0001$. This lowest level of significance indicates that empirical formula was well fitted to the experimental data.

$$pH = a + b\,t + c\,e^{A} + d\,T + e\,R + f\,R^2 \qquad [4]$$

where a, b, c, d, e and f are the constants derived from non-linear regression, t is the storage time in day, e is the exponential term, A additives given in Table 3.2, T is temperature in °C and R is percentage the relative humidity of the storage.

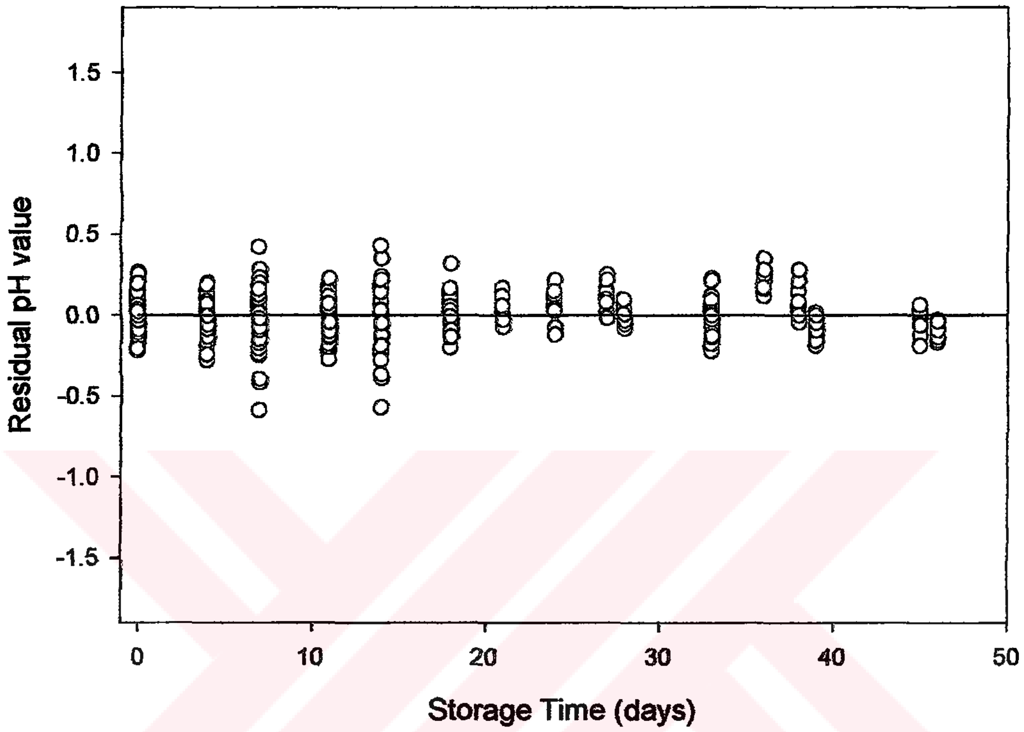


Figure 4.43 Residual plot of pH values in sucuks with fitted empirical formula

The constants with their standard errors and significance of the constants are given in Table 4.4. Significance of the constants indicate that presence of this constant necessary or not, if significance level is lower than P<0.0001 it is necessary to put this constant into equation. Residual data versus time (days) plot was drawn and it is given in Figure 4.43. It is observed that residual pH values ranged between -0.59 to 0.43.

Table 4.4 The constants derived from the empirical formula with their standards errors and significance levels

Constant	Value	P values
a	2.1144±0.2486	<0.0001
b	0.0088±0.0005	<0.0001
c	0.0019±0.0008	0.0212
d	0.0032±0.0008	0.0002
e	0.0858±0.0008	<0.0001
f	-0.0007±.00001	<0.0001

This empirical formula was compared statistically and found that it can be regarded as sufficient to describe the pH values in sucuks (B1-B9) during the storage periods. It was found that pH values during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 4].

4.2.1.3. pH of Retailed Sucuks

pH values of traditional sucuks were found to be in the range of 4.22 and 6.36 with average 5.27. pH values of firm-made sucuks were found to be in the range of 4.53 and 6.41 with average 5.48 (Figure 4.44). pH values of firm-made sucuks were higher ($P<0.05$) than that of traditional sucuks. Gökalp *et al.* (1999) noted that pH value for high quality sucuks should be in the range of 5.1-5.2 and not higher than 5.4. With respect to their pH values, 64 % of traditional sucuks were found to be in the acceptable range, but 41 % of firm-made sucuks were in the acceptable range. Gökalp (1986) observed that the mean pH values of sucuk decreased to around 5.10, but retailed firm-made sucuks had pH values around 5.70. He concluded that this high pH value for traditional sucuks (higher than 5.4) may be due to the use of poor quality of raw material (meat and tail-fat), the use of spoiled meat and/or the presence of non-hygienic conditions in sucuk production.

These results indicate that firm-made sucuks had low acidity due to their different manufacturing techniques (duration of ripening, heat application during the ripening) and the presence of additives, which were allowed by regulations for sucuks. Especially, using of nitrite and nitrate in sucuk dough inhibits acid producing bacteria, so nitrite and nitrate added sucuk showed high pH values. Wu *et al.* (1991) concluded that increasing the ripening temperature increased the pH values of sucuks. During the manufacturing of firm-made sucuks, ripening temperature could be adjusted to about 18°C or greater than 18°C for rapid ripening and after the ripening heat would be applied (at 60°C for 1 hr). Ripening of traditional sucuks is made on the climatic conditions. Traditional sucuks were usually produced at the end of fall, winter and beginning of the spring seasons, so temperature is usually around 10°C on these seasons. Therefore, observed pH values were higher in firm-made sucuks than that in traditional sucuks.

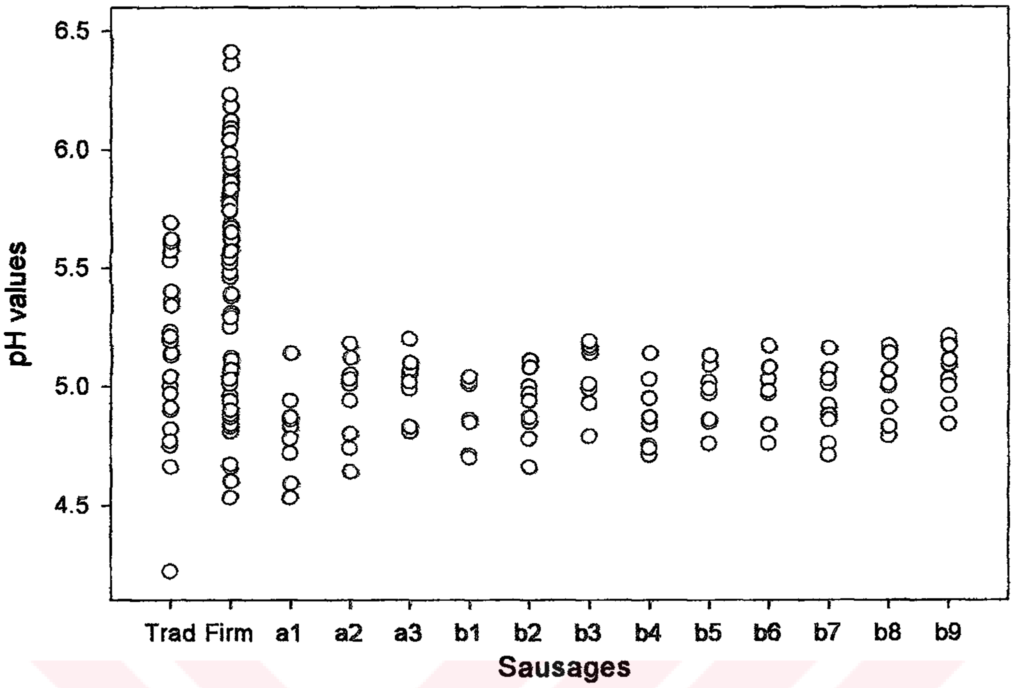


Figure 4.44 pH values of sucuk types and retailed sucuks (Trad: traditional; Firm: Firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

Sucuk type A1 (containing no starter culture and additives) had the lowest ($P<0.05$) pH value about 5.03. Addition of additives increased ($P<0.05$) pH values. The highest ($P<0.05$) pH value was obtained in the firm-made sucuks (Figure 4.44). Means of pH values were found to be in the increasing order of A1, B4, B1, B2, B7, B5, A2, B8, B6, A3, B3, traditional, B9 and firm-made sucuks. Ripening at high temperatures increased the pH values (Wu *et al.*, 1991). Gökalp (1986) and Turkish Food Codex (Anonymous, 2000) restricted that the pH value of good quality sucuk should not be grater than 5.40. Therefore, only firm-made sucuks were not in the acceptable range for their pH values (pH: 5.46). The other sucuks were stated as acceptable with respect to their pH values.

4.2.2. 2-Thiobarbituric Acid Value

The stability of fat often limits the shelf-life of dry sucuk and cured meat products (Eerola *et al.*, 1993). Peroxides, aldehydes, ketones and off-flavor are formed from oxidation of fats. Malonaldehyde is one of the by products of lipid oxidation, and it can be followed by measuring the 2-thiobarbituric acid (TBA) value (Raharjo *et al.*, 1993). Aguirrezaba *et al.* (2000) reported that the major modes of deterioration in meat products is lipid oxidation which affects nutritional quality; color, flavor, odor and texture.

TBA values of sucuks were followed during the ripening and storage periods at 20, 30 and 40°C with three % RH (50, 65 and 80 %) and their results are given in Figures 4.45-4.53. TBA values of sucuk samples increased ($P<0.05$) up to about 2.0 mg/kg during first five days of the ripening period, then they become nearly constant ($P>0.05$) during next ripening period. During the storage periods, TBA values of sucuks increased ($P<0.05$).

4.2.2.1. 2-Thiobarbituric Acid Value during the Ripening Periods

TBA values sharply increased ($P<0.05$) during the first five days of the ripening (Figures 4.45-4.53). After the further ripening periods their number was slightly decreased or became nearly constant ($P>0.05$).

Sucuks made without additives had higher ($P<0.05$) TBA values than that with additives. Especially, sucuks made with high level of additives (ascorbic acid, α-tocopherol, nitrite and nitrate) had the lowest ($P<0.05$) TBA values (Figures 4.45-4.53). It is well known that ascorbic acid, 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 additives in sausages may retard ($P<0.05$) the lipid oxidation during the ripening periods of sucuks.

In general, sucuks made with starter culture had lower ($P<0.05$) TBA values than that without starter culture. Sucuks B1, B5 and B9 made with starter culture had lower ($P<0.05$) TBA values than sucuks (A1, A2 and A3) made without starter culture, but had same amount of additives.

4.2.2.2. 2-Thiobarbituric Acid Value during the Storage Periods

TBA values of sucuks during the storage periods at 20°C with 50, 65 and 80 % RH are given in Figures 4.45, 4.46 and 4.47, respectively. The lowest TBA values were observed in sucuk type B9 and the highest in sucuk type A1 at all storage conditions (Figures 4.45-4.47). TBA values of sucuks became nearly constant ($P>0.05$) during the storage periods at 50 and 65 % RH and TBA values increased in sucuks stored at 80 % RH. The reasons behind this change could be due to the high water activity of sucuks. Malonaldehyde is the one of by-products of the lipid oxidation and it can be followed by changes of TBA value. Lipid oxidation increased with increasing the water activity, so formation of malonaldehyde increased in high water activity medium. Sucuks stored at 80 % RH had higher ($P<0.05$) TBA values than at 50 and 65 % RH. Aguirrezabal *et al.* (2000) have found that TBA values were increased up to 7 mg/kg for 30 days of storage. Lorenzo *et al.* (2000) studied two kinds of Spanish dry-cured sausages. They observed that TBA value increased up to 15 mg/kg during the ripening period.

Figures 4.48, 4.49 and 4.50 show the changes of TBA values in sucuks stored at 30°C with 50, 65 and 80 % RH, respectively. TBA values were in the range of 1.03-2.35 mg/kg, 1.18-2.28 mg/kg, 1.76-2.32 mg/kg in sucuks stored at 50, 65 and 80 % RH, respectively. The highest TBA values were observed in sucuk type A1, besides that the lowest TBA was found in sucuk type B9 at all relative humidities. Sucuks type A1 made without any additives and starter culture, although B9 made with high level of additives (ascorbic acid, α-tocopherol, nitrite and nitrate) and starter culture (Figures 4.48-4.50). These additives have antioxidative activity; therefore the lowest TBA values were observed in this sucuk type.

TBA values in sucuks stored at 40°C with 50, 65 and 80 % RH are given in Figures 4.51, 4.52 and 4.53, respectively. TBA values increased ($P<0.05$) during the storage periods. The highest increment ($P<0.05$) was found in sucuks stored at 80 % RH. Sucuk type A1 had the highest ($P<0.05$) TBA value. About 2.11 mg/kg, 2.00 mg/kg and 2.93 mg/kg TBA values were observed at the end of the 29 days in sucuk type A1 stored at 50, 65 and 80 % RH, respectively (Figures 4.51-4.53). Sucuk type B9 had the lowest ($P<0.05$) TBA values. Increasing % RH of sucuk increased the TBA values significantly ($P<0.05$).

It can be observed in Figures 4.45-4.53 that sucuks made without starter culture (A1) had higher ($P<0.05$) TBA values than sucuks prepared with starter culture inoculated. This can be attributed to the contamination of catalase negative, hetero-fermentative microflora to sucuk sample A1. Contamination of hetero-fermentative flora may accelerate peroxide formation and cause rancidity of fat present in sucuks (Ordonez *et al.*, 1999).

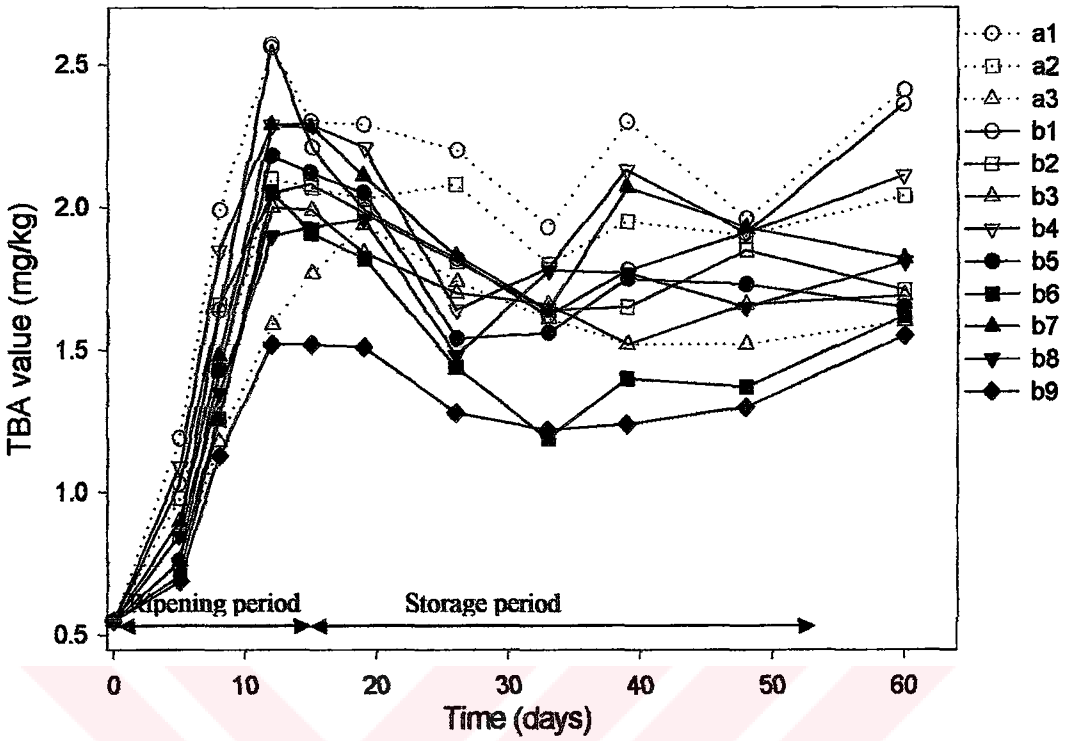


Figure 4.45 Changes of TBA values of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

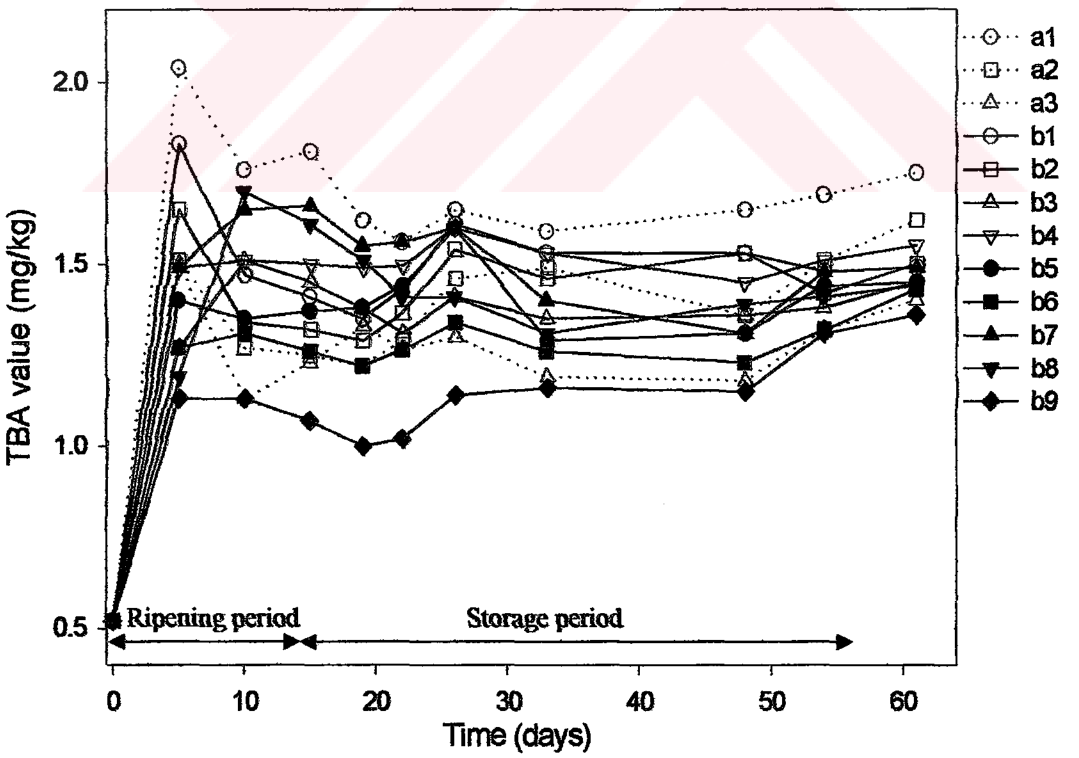


Figure 4.46 Changes of TBA values of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

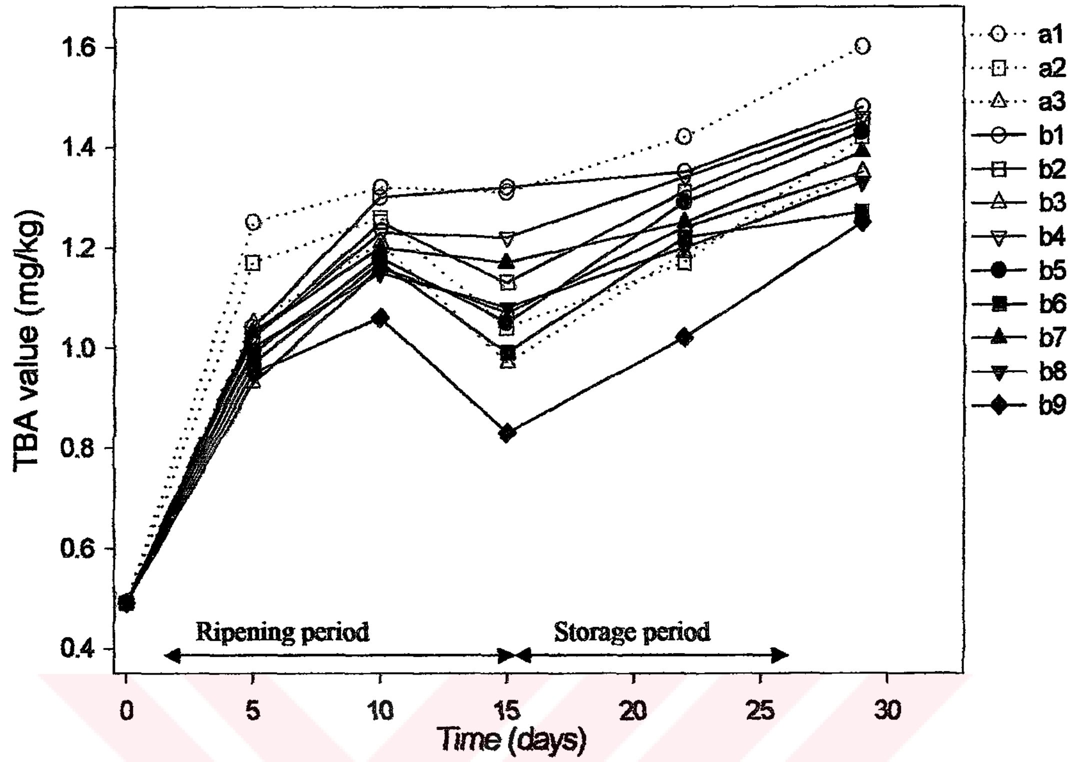


Figure 4.47 Changes of TBA values of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

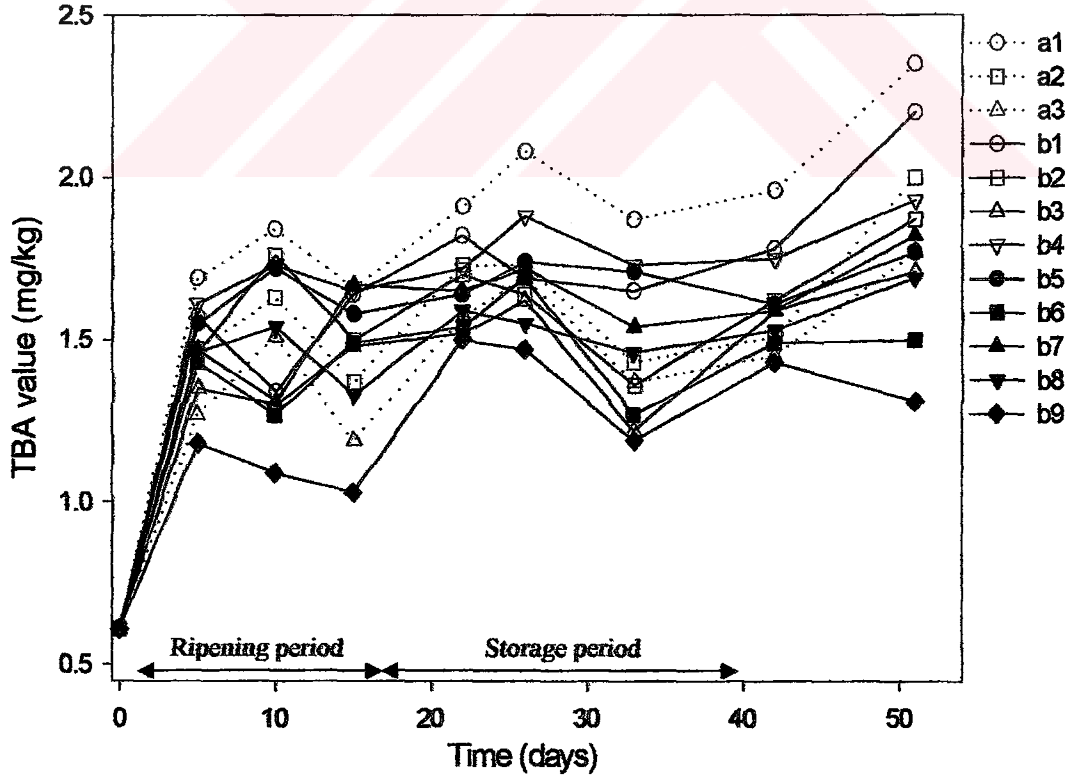


Figure 4.48 Changes of TBA values of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

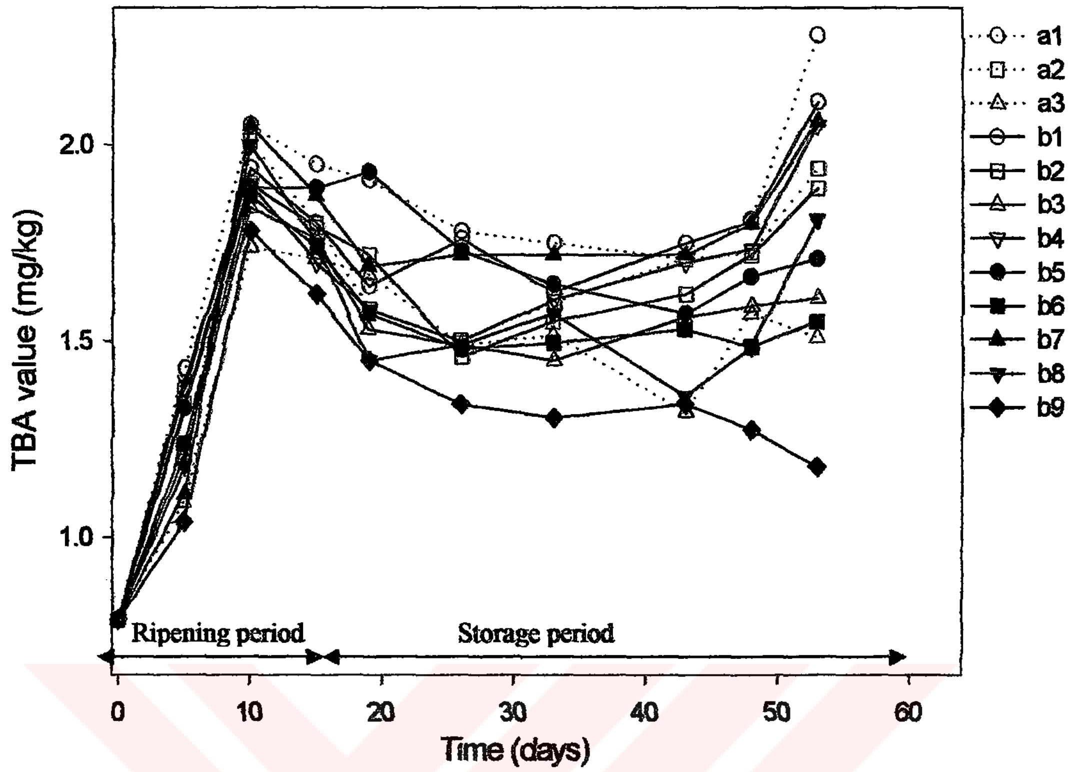


Figure 4.49 Changes of TBA values of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

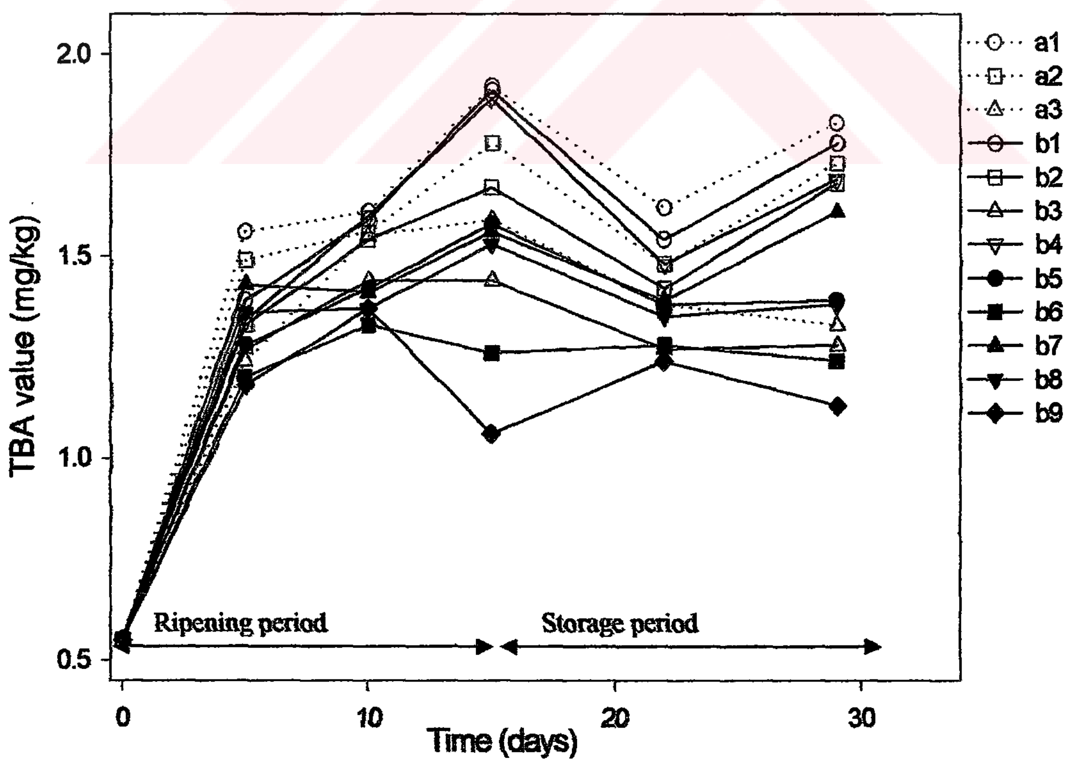


Figure 4.50 Changes of TBA values of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

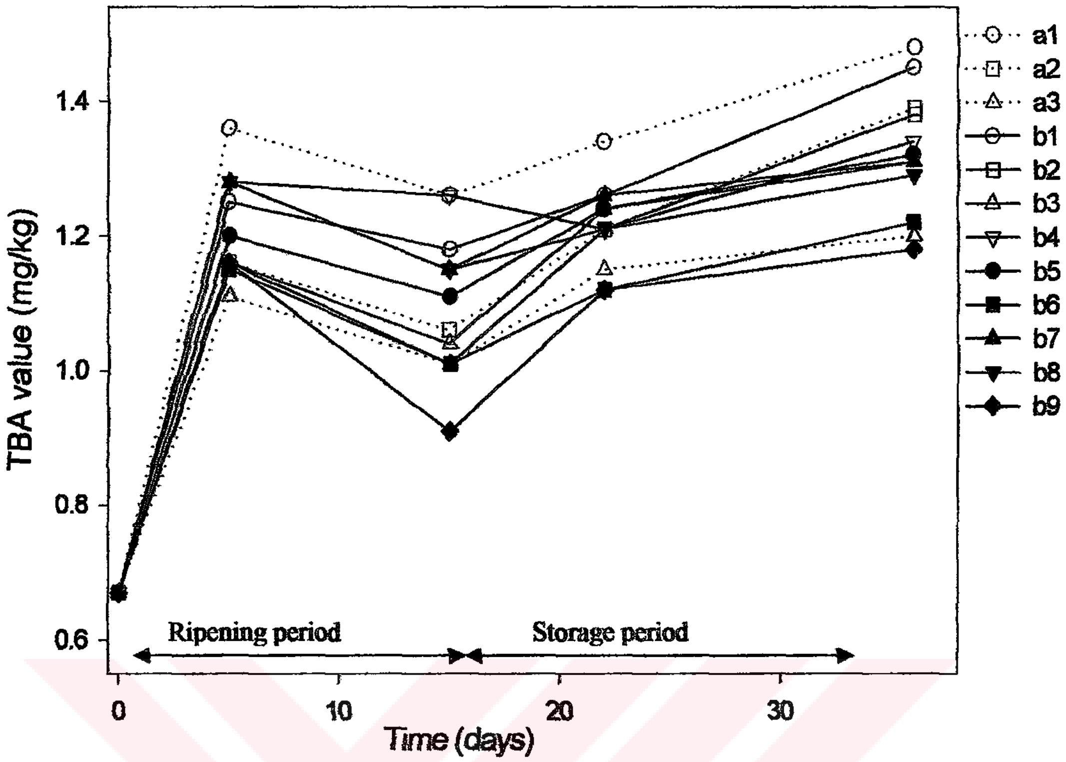


Figure 4.51 Changes of TBA values of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

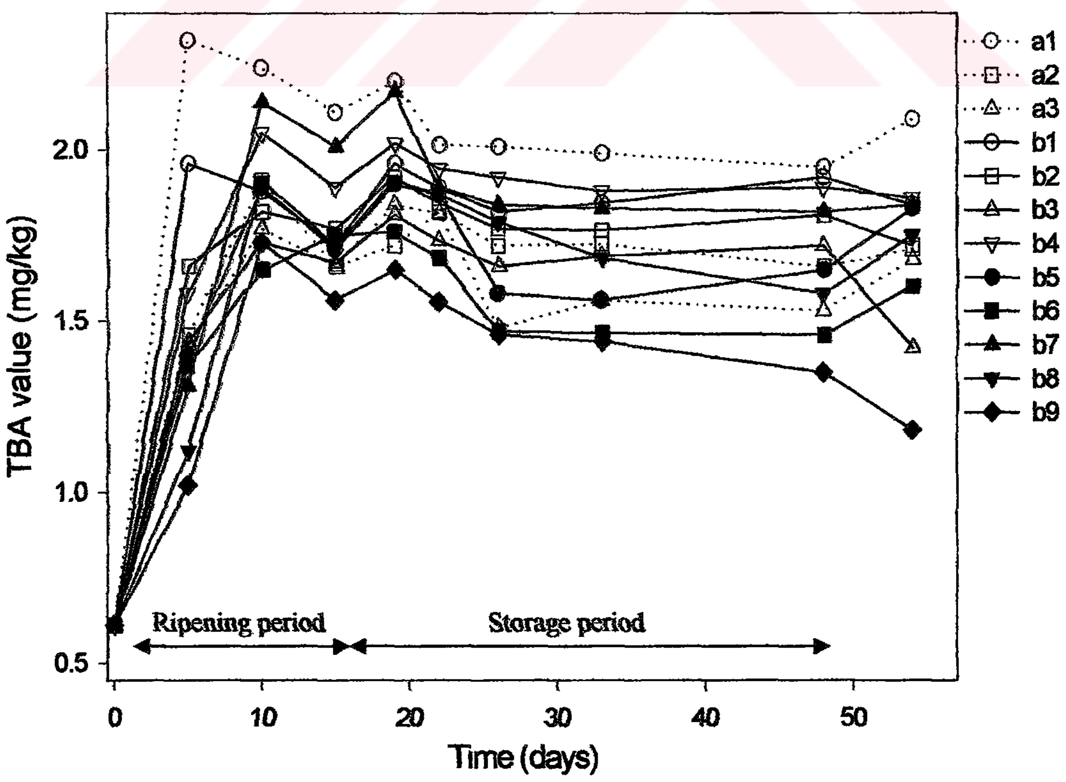


Figure 4.52 Changes of TBA values of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

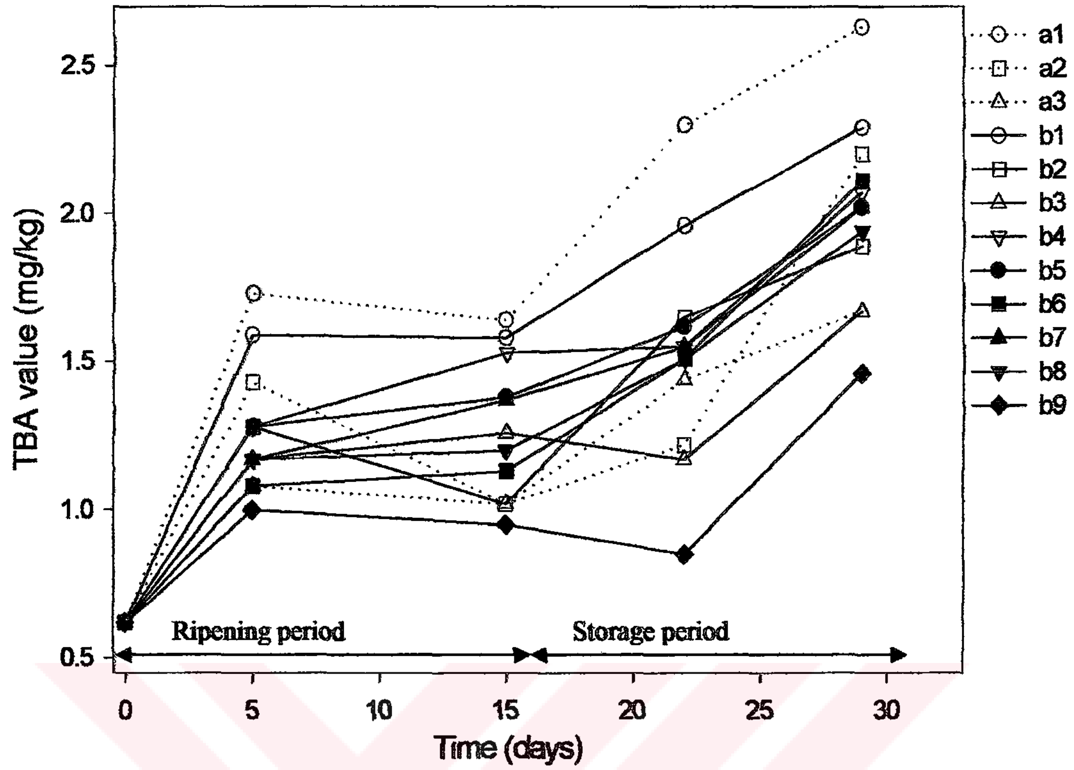


Figure 4.53 Changes of TBA values of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

Additives decreased ($P<0.05$) the TBA values of sucuks as would be expected (Figures 4.45-4.53). In sucuks made without additives (A1 and B1), TBA values were higher ($P<0.05$) than that of the others. To observe the effect of additives on TBA values of sucuks multiple range test were performed. TBA levels were found to be inversely related with the concentration of additives. Increasing the additive levels decreased ($P<0.05$) the TBA values. The lowest TBA values were observed in sucuks B3, B6 and B9 those had high levels of additives (Table 3.2) because α-tocopherol and ascorbic acid håve antioxidant properties. Statistical analysis indicate that α-tocopherol and ascorbic acid had higher ($P<0.05$) reducing properties on the TBA than nitrite/nitrate. Faustman *et al.* (1989) studied the effect of α-tocopherol on the lipid oxidation in beef samples. They found that α-tocopherols capable of quenching free radicals and thus, protect phospholipids and cholesterols against oxidation and subsequent breakdown to potentially harmful, chemically reactive products. They also observed that α-tocopherol reduced the TBA level from about 1.75 mg/kg to about 1.5 mg/kg. Brewer *et al.* (1992) indicated that phosphate retard lipid oxidation and preserve color in Bologna type sausage during the storage periods. Frederick *et al.* (1994) found in low-fat Beef German sausage with different formulation (added water and phosphate changing) at the end of the 4 weeks storage, control samples become more rancid than the other samples. Nitrite is an effective antioxidant and has been found to retard oxidation in cooked beef when used at 50 ppm (Ordonez *et al.*, 1999). However, Ockerman and Sun (2002) found that addition of higher than 75 ppm nitrite did not affect TBA values. They pointed out that 75 ppm of nitrite is enough to retard lipid oxidation.

To determine the effect of storage temperature on the TBA values of sucuk, ANOVA test was carried out. Results show that temperature has a significant ($P<0.05$) effect on the TBA values. Increasing temperature increased ($P<0.05$) the TBA values. The reasons of those could be due to the higher rate of lipid oxidation at high temperature than low temperatures.

The effect of % RH of storage on the TBA values of sucuks was found to be statistically significant ($P<0.05$). Multiple range test was carried out to determine the changing of TBA values with respect to changes of % RH of storage. At lower % RH of storage water activity of sucuks were lower. Water activity is one of the parameters that affect the rancidity either oxidative or hydrolytic rancidity. Rancidity (malonaldehyde formation) was lower in sucuks stored at 50 % RH than that at 65% and 80 % RH.

Positive significant correlation ($P<0.05$) was found between TBA and nitrosomyoglobin conversion, putrescine, histamine and tyramine formations. It means that higher the TBA values higher nitrosomyoglobin conversion, putrescine, histamine and tyramine formations in sucuks. Negative correlations were observed between TBA and mold and yeast counts and overall sensory quality. Negative correlation means lower TBA values of sucuks higher the mold and yeast counts and overall sensory quality.

As a conclusion, reduction of TBA value in sucuks is important for human health and also for sucuk quality (color, flavor and texture). Figures 4.37-4.45 indicate that TBA mainly produced during the ripening periods and after the ripening period only small changes were observed. Therefore, to reduce the TBA formation the ripening periods of sucuks should be strictly controlled. All of the results show that additives (ascorbic acid, α-tocopherol and nitrite/nitrate) and starter culture should be used and sucuks stored at low temperature (20°C) and low % RH (50 % RH).

4.2.2.2.1. Prediction of TBA values during the Storage

The following empirical formula was applied to describe the TBA values in sucuks (B1-B9) which were collected during the storage periods. Statistical analyses were carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is P<0.0001. This low level of significance indicates that empirical formula was well fitted to the experimental data.

$$\text{TBA (mg/kg)} = a + b\,(t + t^2) + c\,A + d\,T + e\,R \qquad [5]$$

where a, b, c, d and e are the constants derived from non-linear regression, t is the storage time in day, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significant level of constants are given in Table 4.5. Significance of the constants indicates that presence of this constant necessary or not, if significance level is lower than P<0.0001 it is necessary to put this constant into equation. Residual data versus time (days) plot was drawn and it is given in Figure 4.54. It is found that residual TBA values (mg/kg) ranged between -0.67 to 0.78 mg/kg.

This empirical formula was compared statistically and it can be regarded as sufficient to describe the TBA values in sucuks (B1-B9) during the storage periods. It was found that TBA values (mg/kg) during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 5].

4.2.2.3. TBA values of Retailed Sucuks

TBA values of traditional and firm-made sucuks ranged between 0.49 – 2.87 mg/kg and 0.40 – 3.34 mg/kg, respectively. The means of TBA values were found to be 1.27 mg/kg and 1.49 mg/kg for traditional and firm-made sucuks, respectively. Wu *et al.* (1991) concluded that when TBA values in sausages are greater than 1.0 mg/kg, off-odors generally appear and it is considered as the beginning of the lipid oxidation. Ertaş (1982) reported the TBA values in retailed sucuks sold in Ankara varied between 0.048

and 0.913 mg/kg.

Table 4.5 The constants derived from the empirical formula with their standards errors and significance levels

Constant	Value	P values
a	1.3585±0.0969	<0.0001
b	-0.0001±0.0000	0.0227
c	-0.0698±0.0158	<0.0001
d	0.0074±0.0016	<0.0001
e	0.0049±0.0012	<0.0001

Statistical analyses indicated that there were no statistical differences (P>0.05) exist between the TBA values of traditional and firm-made sucuks; however, firm-made sucuks had higher TBA values than that of traditional sucuks. This could be due to differences between production steps; especially due to use of high RH and temperature during ripening of sucuks to firm-made sucuks. Moreover, application of heat treatment after the ripening period (at 60°C for 1 hr) or poor quality tail fat might increase the TBA value of firm-made sucuks. Antioxidants were also used in the production of firm-made sucuks but antioxidative activity decreases lipid oxidation.

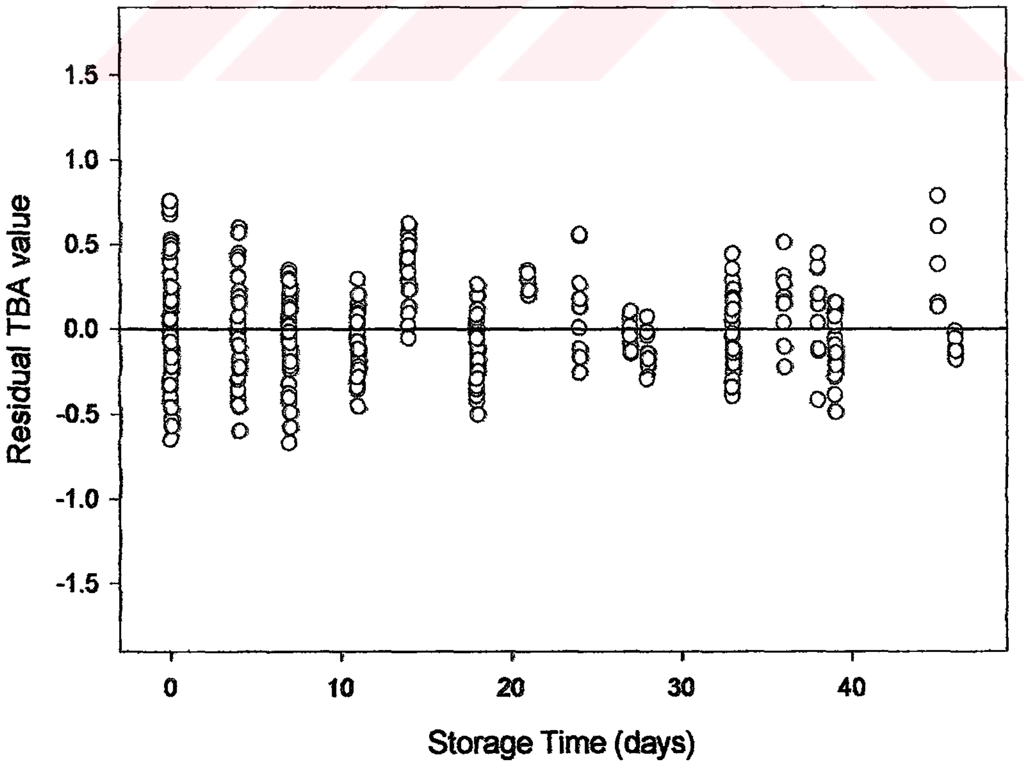


Figure 4.54 Residual plot of TBA values (mg/kg) in sucuks with fitted empirical formula

The highest ($P<0.05$) TBA values were observed in the firm-made sucuks and sucuk type A3 had the lowest ($P<0.05$) TBA value (Figure 4.55). These results could be due to; high ripening temperature, application of heat treatment after the ripening period (at 60°C for 1 hr) or use of poor quality tail fat during the manufacturing of firm-made sucuks. Antioxidants (e.g. ascorbic acids, ascorbates, α-tocopherols, etc.) were used in the production of firm-made sucuks but when lipid oxidation is started antioxidative activity of these additives decrease. Sucuk type A3 had the high level of additives (ascorbic acid, α-tocopehrol and nitrite/nitrate). These additives had antioxidant activity; therefore TBA value could be lowest in this sucuk.

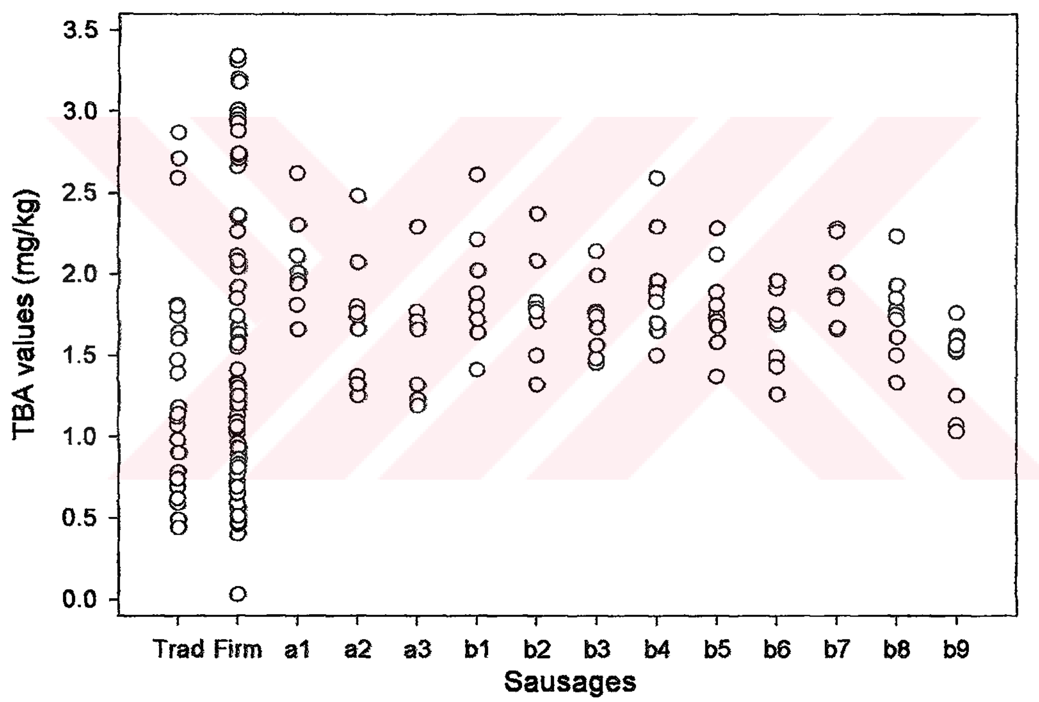


Figure 4.55 Means of TBA values of sucuk types and retailed sucuks (Trad: traditional; Firm: Firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

4.2.3. Nitrosomyoglobin Conversion

Color is one of the most important quality attributes of sucuk. The characteristic color of fermented sucuks is the pink-red color, which is formed when nitric oxide combined with meat pigment (myoglobin) to produce nitrosomyoglobin (Figure 2.2). Nitric oxide is produced from nitrite or nitrate when nitrate reductase enzyme produced by microorganisms such as *Micrococcaceae* species, under the acidic condition (Wirth, 1986). Nitrates itself does not form the cured color, it must be reduced to nitrite first. This reaction only takes place in the presence of enzyme nitrate reductase produced by microorganisms (Ordonez *et al.*, 1999).

Nitrosomyoglobin conversions during the ripening and storage periods in sucuks (A1-A3and B1-B9) were followed and results are given in Figures 4.46-4.54. Nitrosomyoglobin conversions in sucuks increased ($P<0.05$) about 80 % during the ripening period which is parallel to the reduction of pH. Later, during the storage period, nitrosomyoglobin conversion decreased ($P<0.05$) as pH values increased. These results were in good agreement with Vural and Öztan (1992a) results. They noted that nitrosomyoglobin conversion increased with decreasing the pH.

4.2.3.1. Nitrosomyoglobin Conversion during the Ripening Periods

Nitrosomyoglobin conversions in sucuks during the ripening periods are given in Figures 4.56-4.64. Nitrosomyoglobin conversion increased ($P<0.05$) sharply during the first five or ten days of the ripening periods. After that they decreased ($P<0.05$) during the further ripening periods (up to 15 days).

Nitrosomyoglobin conversion is formed by the interaction of myoglobin with nitric oxide produced from nitrite and/or nitrate. Nitrite/nitrate added sucuks had higher ($P<0.05$) nitrosomyoglobin conversion rate than no nitrite/nitrate containing sucuks. Sucuk type B7, B8 and B9 made with high level of nitrite/nitrate and they had the higher ($P<0.05$) nitrosomyoglobin conversion than the others. However, sucuk type B7 had the highest ($P<0.05$) nitrosomyoglobin conversion. It could be concluded that presence of additives other than nitrite/nitrate reduced ($P<0.05$) nitrosomyoglobin conversion in sucuks types B8 and B9.

Starter culture inoculation increased ($P<0.05$) the nitrosomyoglobin conversion. Sucuk type B1 made with starter culture had higher ($P<0.05$) nitrosomyoglobin conversion than sucuk type A1 made without starter culture. In a similar way, sucuk type A2 and sucuk type B5 had same additives with same concentration, also sucuk type A3 and B9, too. Starter culture containing sucuks B5 and B9 had higher nitrosomyoglobin conversion than the respective sucuks made without starter culture (A2 and A3). Similar pattern was also observed by Vural and Öztan (1992a, b). They reported that nitrosomyoglobin conversions in starter inoculated sucuks were about 70 %, but in control sucuk (made without starter culture) only about 32 %

nitrosomyoglobin conversion was observed. In this research these conversions were about 80 and 65 % for sucuks made with and without starter culture, respectively. These results clearly showed that to obtain a desired nitrosomyoglobin conversion, starter culture should be used in sucuk production.

4.2.3.2. Nitrosomyoglobin Conversion during the Storage Periods

Nitrosomyoglobin conversion in sucuks during the storage periods at 20°C with 50, 65 and 80 % RH are given in Figures 4.56, 4.57 and 4.58, respectively. Nitrosomyoglobin conversion in sucuks decreased ($P<0.05$) during the storage periods except sucuks stored at 80 % RH. At low RH, sucuks dried rapidly and color changes were higher than ($P<0.05$) sucuks stored at high RH. The highest ($P<0.05$) nitrosomyoglobin conversion was observed in sucuk type B7 and the lowest ($P<0.05$) in sucuk type A1. After 50 days, sucuk type B7 had about 55 % nitrosomyoglobin conversion at 50 % RH and sucuk type A1 had about 25 % conversion. Nitrosomyoglobin conversion in sucuks stored at 80 % RH, became nearly constant ($P>0.05$) during the storage period, about 60 % nitrosomyoglobin conversion was observed.

The changes of nitrosomyoglobin conversion in sucuks stored at 30°C with 50, 65 and 80 % RH are given in Figures 4.59, 4.60 and 4.61, respectively. Figures 4.62, 4.63 and 4.64 show the changes of nitrosomyoglobin conversion in sucuks stored at 40°C with 50, 65 and 80 % RH, respectively. Nitrosomyoglobin conversion decreased ($P<0.05$) during the storage periods and the lowest ($P<0.05$) nitrosomyoglobin conversion was observed in sucuk type A1.

Nitrosomyoglobin conversion was lower ($P<0.05$) in sucuks made without starter culture than sucuks made with starter culture. Addition of starter culture into sucuks, desired color formation (higher nitrosomyoglobin conversion) was observed in sucuks. Nitrosomyoglobin conversion in sucuks made without starter culture decreased to about 20 % from about 70 % during the storage periods, however, in sucuks made with starter culture it decreased to about 40 % from about 80 %.

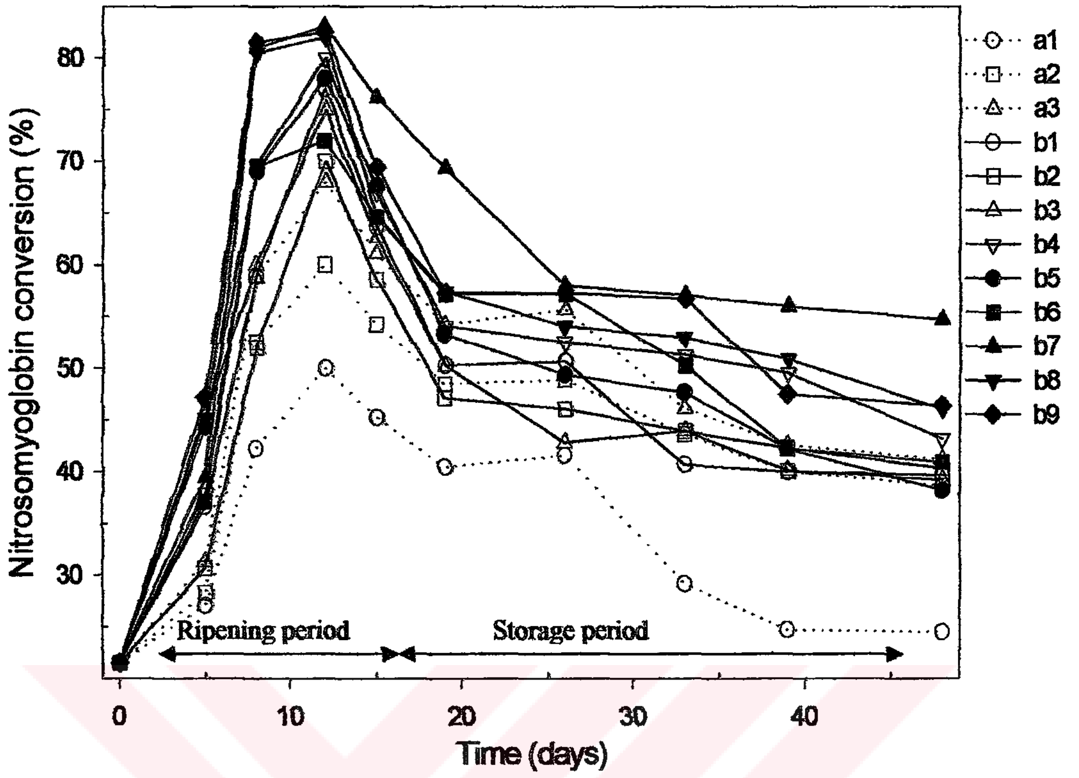


Figure 4.56 Changes of nitrosomyoglobin conversion of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

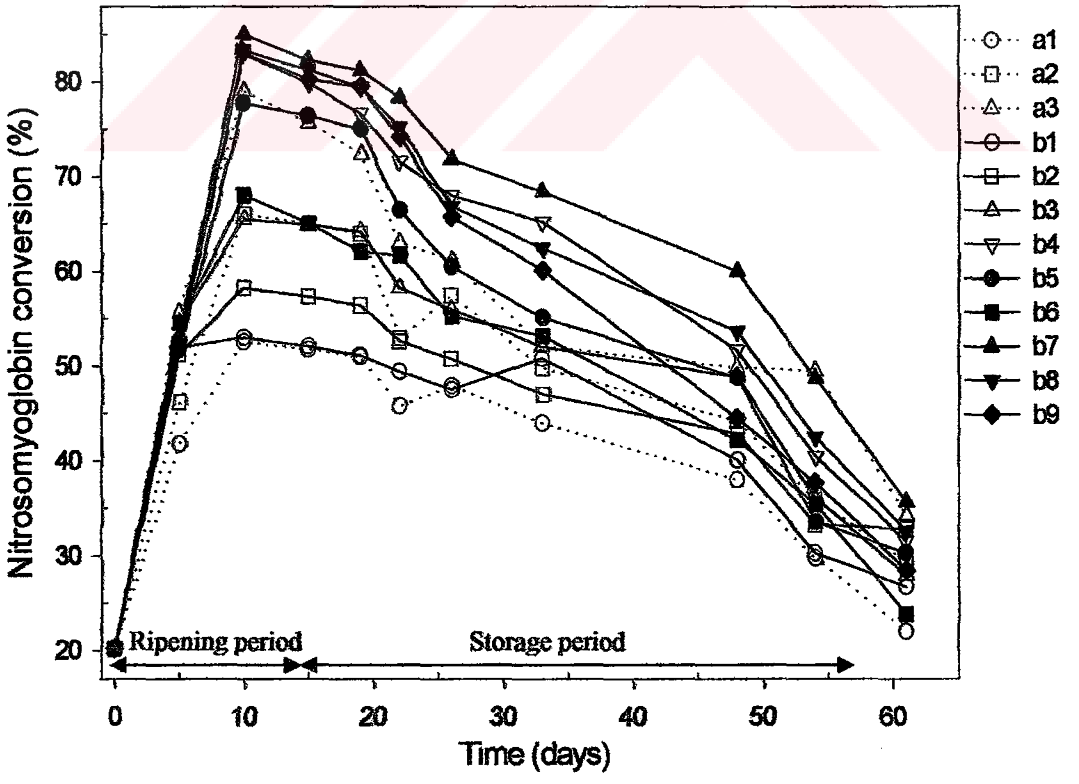


Figure 4.57 Changes of nitrosomyoglobin conversion of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

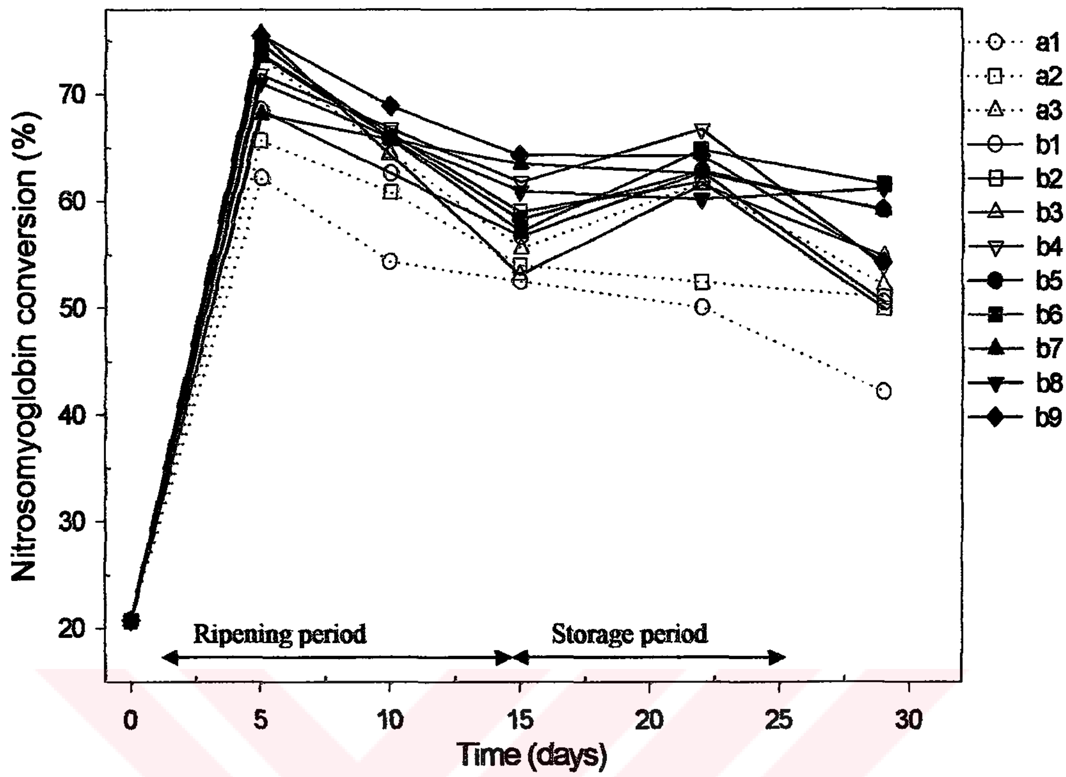


Figure 4.58 Changes of nitrosomyoglobin conversion of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

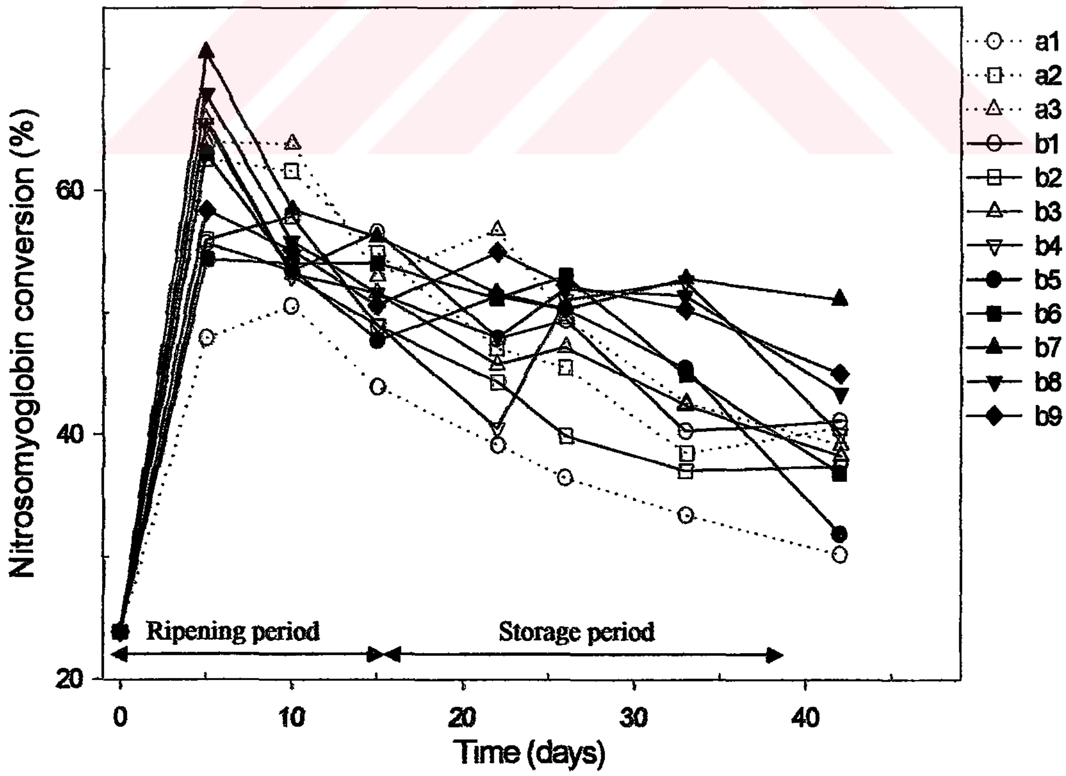


Figure 4.59 Changes of nitrosomyoglobin conversion of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

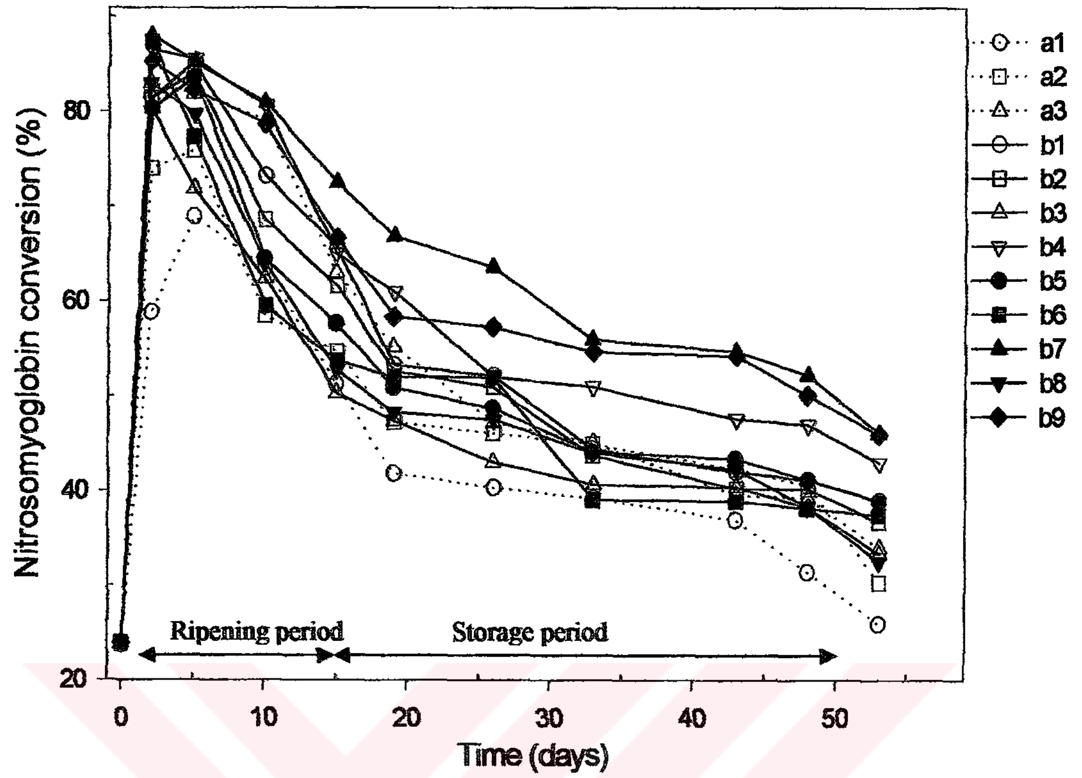


Figure 4.60 Changes of nitrosomyoglobin conversion of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

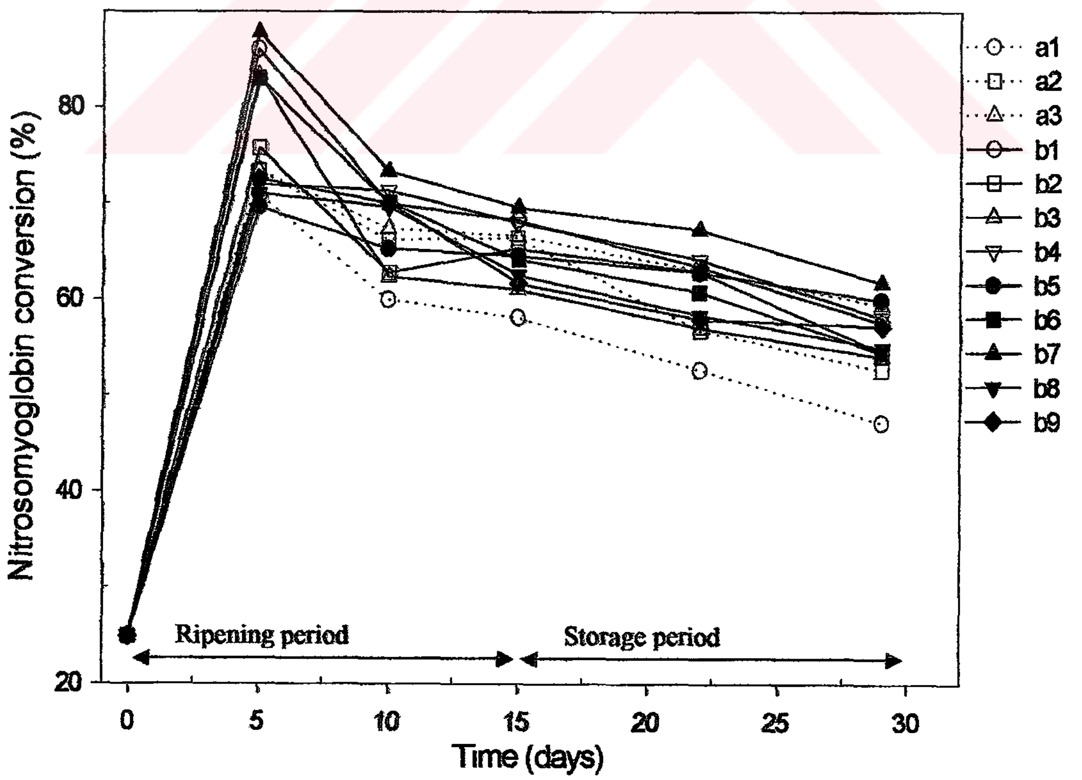


Figure 4.61 Changes of nitrosomyoglobin conversion of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

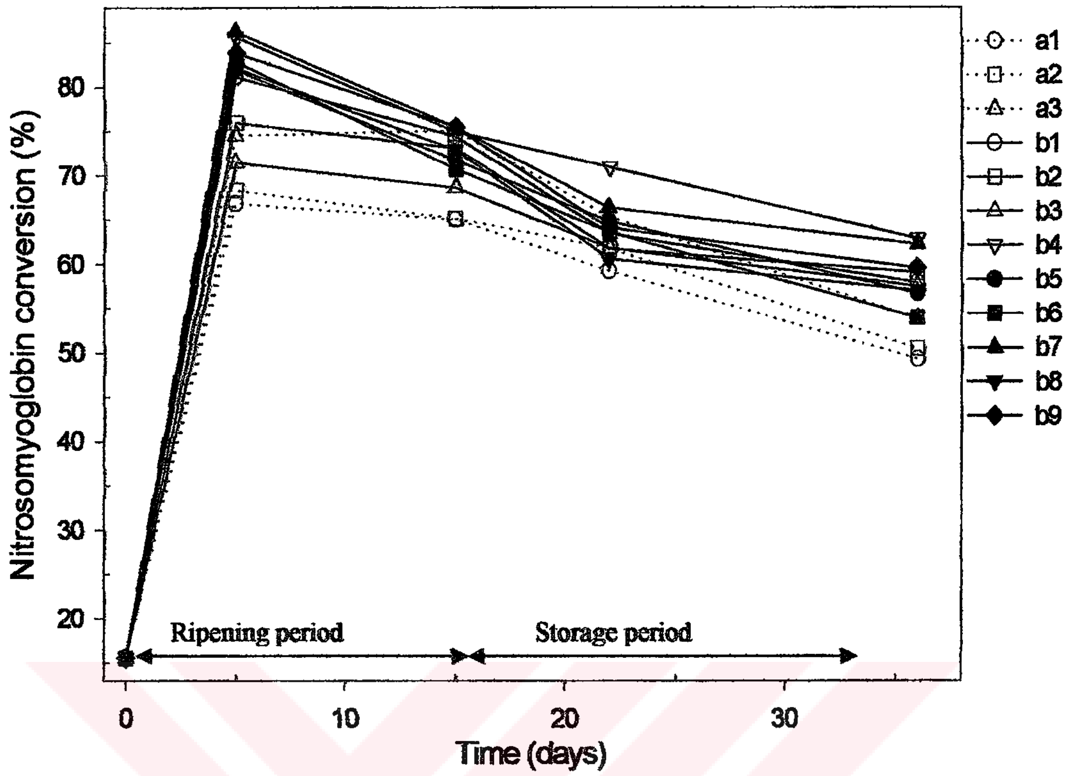


Figure 4.62 Changes of nitrosomyoglobin conversion of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

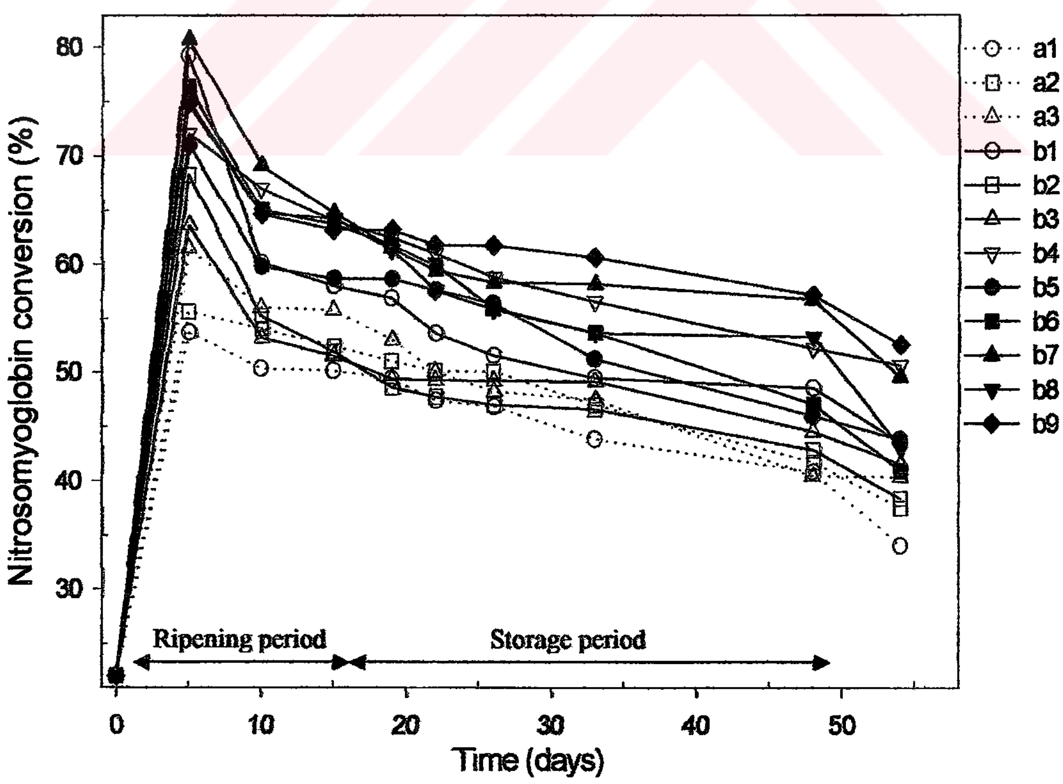


Figure 4.63 Changes of nitrosomyoglobin conversion of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

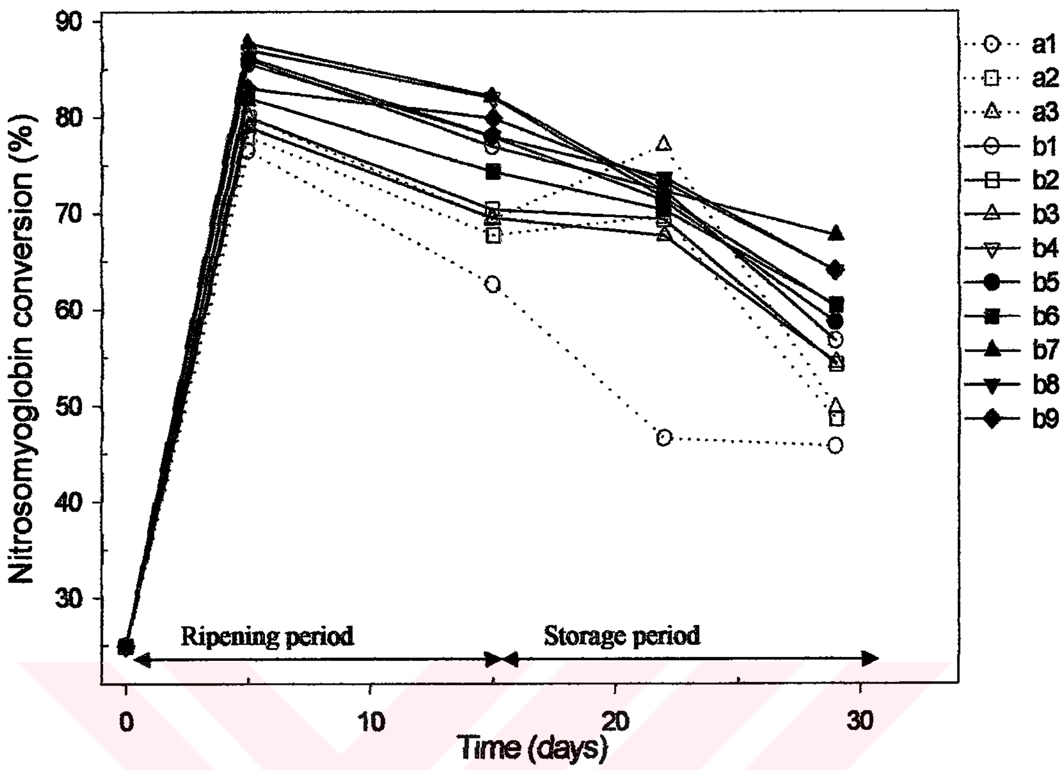


Figure 4.64 Changes of nitrosomyoglobin conversion of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

Additives increased ($P<0.05$) the nitrosomyoglobin conversion in sucuks. During the ripening period, maximum nitrosomyoglobin conversion (about 85 %) was observed in sucuk type B9, made with high levels of nitrite/nitrate level. Additives rather than nitrite/nitrate decreased ($P<0.05$) the nitrosomyoglobin conversion. Öztan and Vural (1991) observed that nitrosomyoglobin conversion was changed by changing the nitrite level in sucuk. They found that nitrosomyoglobin conversions were about 45 % in sucuks made with starter culture and 100 ppm of nitrite taken and this conversion increased to about 60 % with 125 ppm nitrite.

Statistical analyses were performed to determine the effect of storage temperature on the nitrosomyoglobin conversion of sucuks. It was found that increasing the storage temperature increased ($P<0.05$) the nitrosomyoglobin conversion. Changes of % RH of storage had significantly ($P<0.05$) effected the nitrosomyoglobin conversion in sucuks. Increasing the % RH of the storage increased ($P<0.05$) the nitrosomyoglobin conversion.

Statistical analyses indicated that there were additives-temperature, temperature-% RH interactions ($P<0.05$). This means that increasing the concentration of additives and temperature increased ($P<0.05$) the nitrosomyoglobin conversion in sucuks. Especially addition of high concentration of nitrite/nitrate with high temperatures increased ($P<0.05$) nitrosomyoglobin conversion much more than the other cases. Also, increasing the temperature and % RH of storage increased ($P<0.05$) the nitrosomyoglobin conversion more than that increasing temperature or % RH alone. From that it could be concluded that to get high nitrosomyoglobin conversion nitrite/nitrate should be added in high concentration, high storage temperature and % RH should be applied to sucuks.

Positive significant correlation ($P<0.05$) was found among nitrosomyoglobin conversion and TBA, overall sensory quality, putrescine, histamine and tyramine formations. It means that higher nitrosomyoglobin conversion in sucuks higher TBA values, overall sensory quality, putrescine, histamine and tyramine formations in sucuks. Negative correlations were observed between nitrosomyoglobin conversion and pH values, mold and yeast counts of sucuks. Negative correlations mean lower nitrosomyoglobin conversion of sucuks higher the mold and yeast counts and overall sensory quality.

From the point of nitrosomyoglobin conversion, sucuks B7, B8 and B9 were more acceptable than the other sucuks. In other words, to get a high quality (to get desired color attributes) of sucuks starter culture, and nitrite/nitrate should be added to the formulation.

4.2.3.2.1. Prediction of Nitrosomyoglobin Conversion during the Storage

The following empirical formula [Eqn. 6] was applied to describe the nitrosomyoglobin conversion values in sucuks (B1-B9) which were collected during the storage periods. Statistical analyses were carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is $P<0.0001$. This lowest level of significance indicated lower case (P) that empirical formula was well fitted to the experimental data.

$$\text{Nitrosomyoglobin Conversion (\%)} = a + b\,(t) + c\,A + d\,T + e\,R + f\,T^2 \qquad [6]$$

where a, b, c, d, e and f are the constants derived from a non-linear regression, t is the storage time in day, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significance of the constants are given in Table 4.6. Significance of the constants indicates that presence of this constant necessary or not, if significance level is lower than $P<0.0001$ it is necessary to put this constant into equation. Residual data versus time (days) plot was drawn and it is given in Figure 4.65. It was observed that residual nitrosomyoglobin conversion (%) ranged between -13.28 to 15.42 %.

This empirical formula was compared statistically and found that it can be regarded as sufficient to describe the nitrosomyoglobin conversion (%) in sucuks (B1-B9) during the storage periods. It was found that nitrosomyoglobin conversion (%) during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 6].

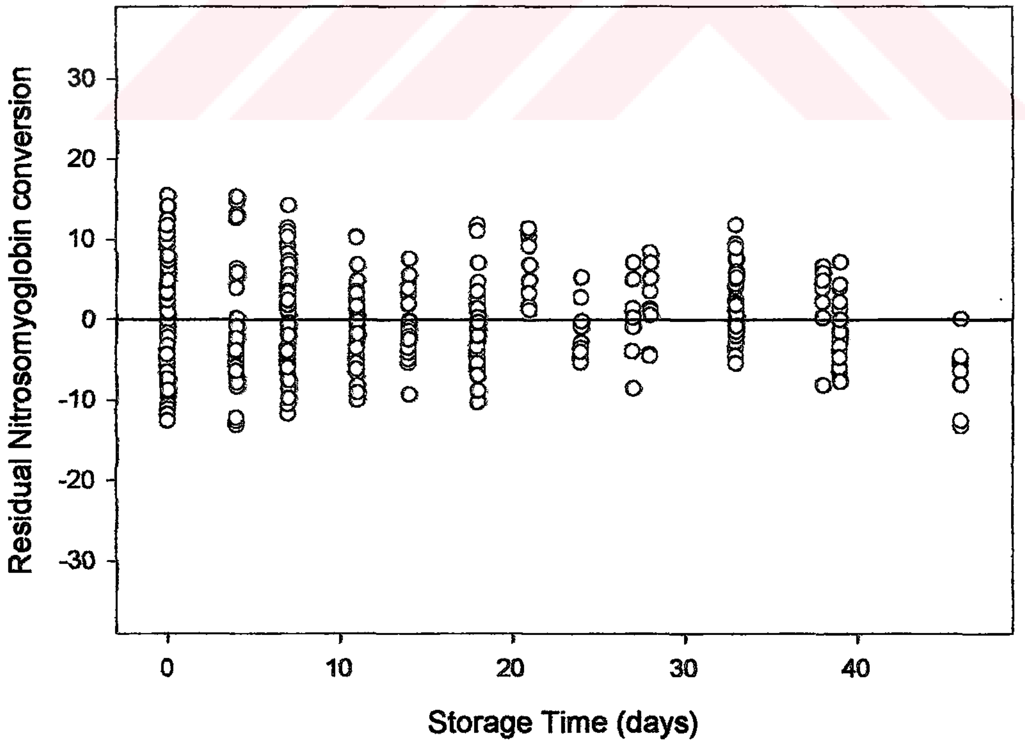


Figure 4.65 Residual plot of nitrosomyoglobin conversion (%) in sucuks with fitted empirical formula

Table 4.6 The constants derived from the empirical formula with their standards errors and significance levels

Constant	Value	P values
a	91.6266±5.7959	<0.0001
b	-0.6108±0.0238	<0.0001
c	3.9438±0.3687	<0.0001
d	-3.6731±0.3852	<0.0001
e	0.1992±0.0289	<0.0001
f	0.0636±0.0064	<0.0001

4.2.3.3. Nitrosomyoglobin Conversion of Retailed Sucuks

One of the quality parameters for sucuks evaluated by consumer is appearance. For good quality sucuk, cut surface should have; marbling view, and pink-red color with homogeneously distributed fat particles. Appropriate color (pink-red) is attained by conversion of myoglobin with nitric oxide to nitrosomyoglobin during the ripening period. Therefore, nitrosomyoglobin conversion is related to the consumer preferences.

Nitrosomyoglobin conversion in traditional sucuks ranged between 20.59 % and 78.78 % (average 46.61 %) while it ranged between 26.28 % and 95.69 % (average 57.70 %) in the firm-made sucuks (Figure 4.66). Nitrosomyoglobin conversion in traditional sucuks was significantly different ($P<0.05$) than the firm-made sucuks. Nitrite/nitrate is generally used in the firm-made sucuks for special purposes. However, in the traditional sucuks (butchers-made) there was no added nitrite/nitrate, therefore low amount nitrosomyoglobin conversion is attained when presence of low amount of nitric oxide. Application of heat in the fermentation stage increase the nitrosomyoglobin conversion (Lücke, 1994). Ripening of firm-made sucuks is attained at high temperature and relative humidity (Gökalp *et al*, 1999), but traditional sucuks are ripened under the climatic conditions. Wirth (1986) reported that use of ascorbic acid or sodium ascorbate, nitrite, nitrate and nitrogen in the vacuum package gave good color properties to sucuks and cured meat products. Thus, application of high temperature, using ascorbic acid and nitrate/nitrite in the firm-made sucuks gave higher nitrosomyoglobin conversion than that in traditional sucuks.

Nitrosomyoglobin conversion was the lowest ($P<0.05$) in traditional and sucuk type A1 (without starter culture and additive) sucuks, about 46.61 and 50.54 %, respectively (Figure 4.66). Addition of additives and starter culture increased ($P<0.05$) the nitrosomyoglobin conversion. The highest nitrosomyoglobin conversion was observed in B4, B7 and B9 sucuk types as 60.61, 59.06 and 57.90 %, respectively. Increasing the nitrosomyoglobin conversion increased the consumer preferences.

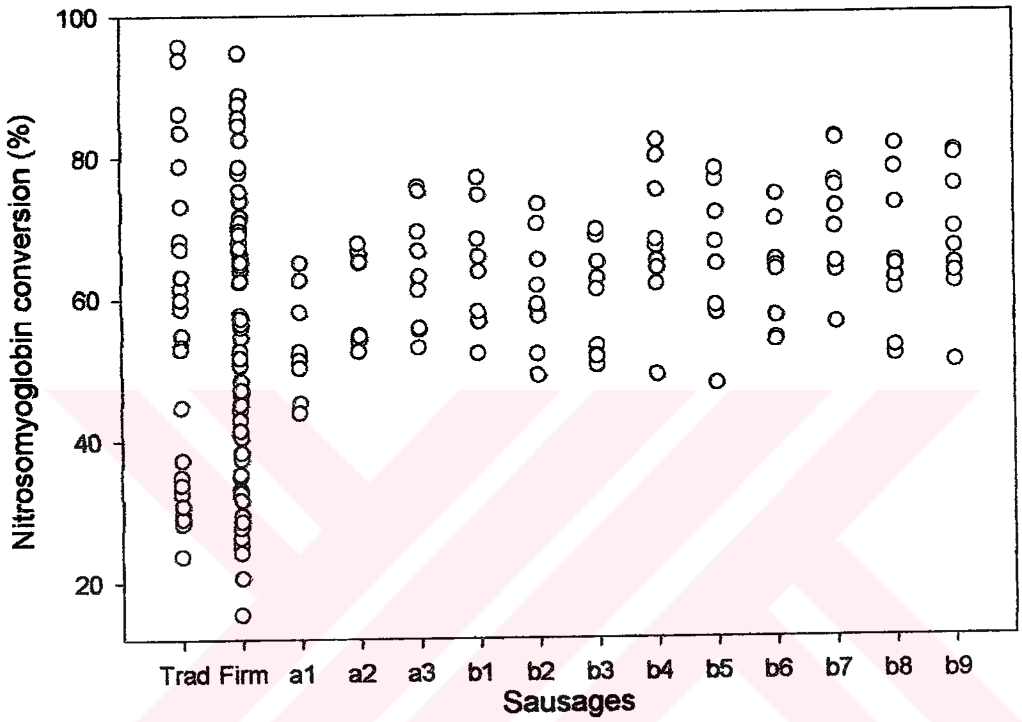


Figure 4.66 Nitrosomyoglobin conversion (%) of sucuk types and retailed sucuks (Trad: traditional; Firm: Firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

4.2.4. Residual Nitrite

Nitrate decomposed to nitrite and then nitrite is converted into nitric oxide by nitrate reductase enzymes produced by *Staphylococcus carnosus* or similar microorganisms. Nitrate/nitrite has an important preserving effect on meat products and relatively low doses of nitrate/nitrite inhibit the growth of some bacteria species (Ordonez *et al.*, 1999). Presence of high level of residual nitrite may cause some adverse health effect to humans (Jo *et al.*, 2002).

Residual nitrite levels during the ripening and storage periods in sucuks are given in Figures 4.67-4.75. The residual nitrite level of sucuk sharply decreased ($P<0.05$) in sucuks made with nitrate/nitrite during the first five days of ripening periods. After the further ripening and storage periods residual nitrite level of sucuks became nearly constant ($P<0.05$).

4.2.4.1. Residual Nitrite Level during the Ripening Periods

Residual nitrite level in sucuks during the ripening periods is shown in Figures 4.67-4.75. Residual nitrite level in sucuks made with nitrite/nitrate decreased ($P<0.05$) sharply during the first five days of ripening periods from 75 or 150 ppm to about 10 ppm. In sucuk types A1, B1, B2 and B3 made without nitrite/nitrate, residual nitrite level increased ($P<0.05$) during the ripening periods.

Residual nitrite levels of sucuks were sharply decreased ($P<0.05$) during the first 3 days of ripening periods. Sucuks made with starter culture and 75 or 150 ppm nitrite had approximately 5-10 ppm of residual nitrite after the first 3 days of ripening (Figures 4.67-4.75). Öztan and Vural (1991) observed that residual nitrite level was found to be 40 ppm when 100 ppm nitrite used in sucuk prepared without starter culture, when 150 ppm nitrite used residual nitrite level increased to 56.5 ppm. They also observed that when starter culture used for sucuk made with 100 ppm and 150 ppm nitrite, the levels decreased to about 20 and 30 ppm, respectively. Zaika *et al.* (1976) studied in Lebanon Bologna fermented sausage made with starter culture and different levels of nitrite (as high as 1000 ppm). They found that residual nitrite level was reduced to as low as 10 ppm during the fermentation after 4 days at 35°C. Lizaso *et al.* (1999) indicate that rapid ripened sausage made with *S. carnosus* residual nitrite level decreased below 30 ppm within a week. Residual nitrite level in sucuk type A3 made with high level of nitrite/nitrate was the highest ($P<0.05$) during the ripening periods. Starter culture produce nitrate reductase enzymes into sucuks. So, nitrite and nitrate decompose to nitric oxide in sucuks made with starter culture.

Residual nitrite level in sucuks decreased ($P<0.05$) in sucuks with addition of additives. Sucuk types B4, B5 and B6 made with same concentration of nitrite but they contain different concentration of other additives and residual nitrite level were found in the order of B5 > B4 > B6.

Üren and Babayiğit (1997) reported that the residual nitrite level in sucuk changed between 4.00 and 11.25 mg/kg. Samelis *et al.* (1998) found that nitrite was rapidly depleted (<10 ppm) within 3 days when 250 ppm nitrite and 250 ppm nitrate were added. The rapid decreasing in nitrite level was observed in dry sucuks is well documented (Alley *et al.*, 1992; Johansson *et al.*, 1994). Houng and Lin (1993) found that residual nitrite decreased rapidly with time in Chinese style sausage. They observed that 100 ppm nitrite reduced to 25-38 ppm during the 12 hr of ripening period.

4.2.4.2. Residual Nitrite Level during the Storage Periods

Residual nitrite level in sucuks during the storage periods at 20°C with 50, 65 and 80 % RH are given in Figures 4.67, 4.68 and 4.69, respectively. Residual nitrite level in sucuks slightly increased during the storage periods. The highest residual nitrite level was observed in sucuk type A3 made with nitrate/nitrite and without starter culture. Residual nitrite level in sucuks changed with the activity of microorganisms. Sucuks stored at high % RH had lower residual nitrite level due to the higher microbial activity. Residual nitrite level after 23 days was found to be about 14 ppm, 12 ppm and 10 ppm in sucuks stored at 50, 65 and 80 % RH, respectively.

The changes of residual nitrite level in sucuks stored at 30°C with 50, 65 and 80 % RH are shown in Figures 4.70, 4.71 and 4.72, respectively. Residual nitrite level in sucuks became nearly constant or reduced slightly ($P>0.05$) stored at 30°C. The microbial activity in sucuks stored at 30°C was higher than that in sucuks stored at 20°C, thus, residual nitrite level was continued to decrease during the storage. The lowest ($P<0.05$) residual nitrite level was observed in sucuk types A1, B1, B2 and B3. These sucuks made without nitrite/nitrate, therefore they had lower ($P<0.05$) residual nitrite than the others.

Residual nitrite level are given in Figures 4.73, 4.74 and 4.75 in sucuks stored at 40°C with 50, 65 and 80 % RH, respectively. Residual nitrite level did not change ($P>0.05$) during the storage periods in sucuks stored at 40°C with 50, 65 and 80 % RH (except sucuk type A3 stored at 50 % RH). Decreasing residual nitrite level could be explained by activity of microorganisms, as microbial activity increased with increasing the % RH of storage conditions. Addition of 150/300 ppm of nitrite/nitrate could be reduced to 10 ppm in sucuks stored at high % RH.

It was observed that residual nitrite level changed ($P<0.05$) from the presence of additives. Sucuk types prepared with same amount of nitrite/nitrate had different level of residual nitrite.

Residual nitrite level was found to be low (P<0.05) in sucuks stored at 30°C. ANOVA test was carried out to determine any significant affect of % RH of storage on the residual nitrite. Results of ANOVA indicate that changes of % RH of storage were not affecting the residual nitrite levels of sucuks. There were no two-way and three way interactions exist among additives, temperature and % RH of storage. It means that increasing or decreasing of two or three of the factors (additives, temperature and % RH of the storage) together did not change (P>0.05) residual nitrite level rather than the changing of these one of the factor.

Positive significant correlation (P<0.05) was found between residual nitrite level and pH values and mold and yeast counts of sucuks. It means that lower residual nitrite level in sucuks lowers pH and mold and yeast counts of sucuks. Negative correlations were observed between residual nitrite level and APC, LAB count, nitrosomyoglobin conversion, TBA values, putrescine, histamine and tyramine formations in sucuks. Negative correlations mean lower residual nitrite level of sucuks higher APC, LAB count, nitrosomyoglobin conversion, TBA values, putrescine, histamine and tyramine formations in sucuks.

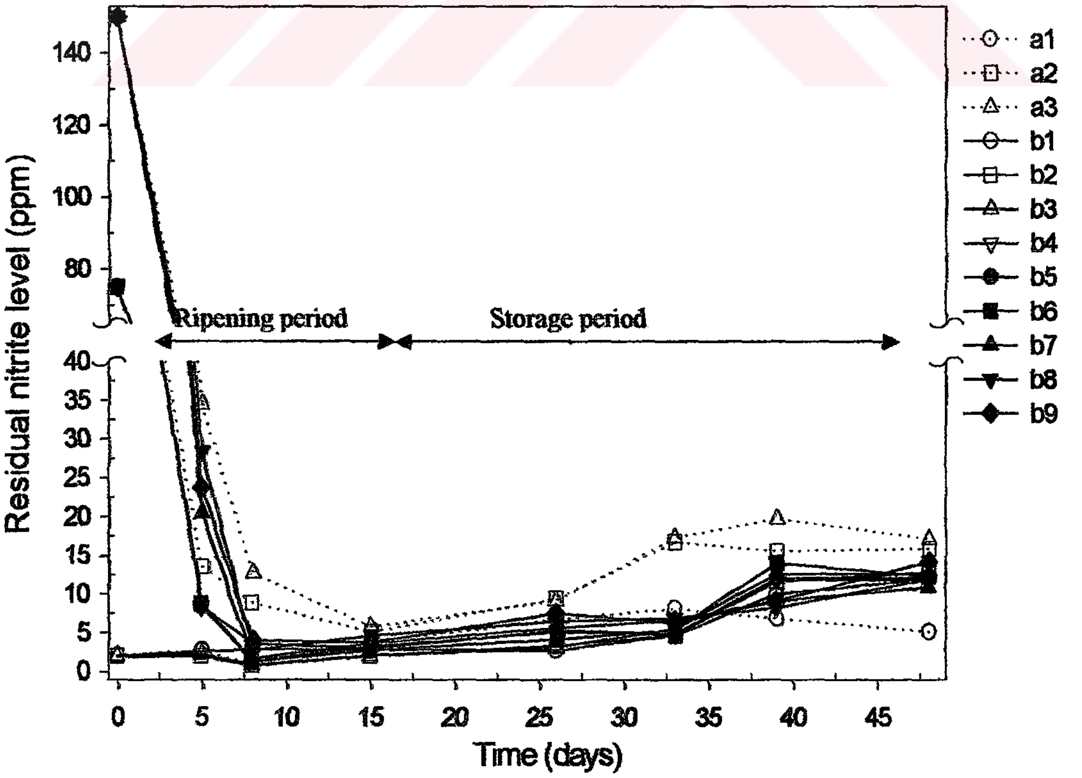


Figure 4.67 Changes of residual nitrite level of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

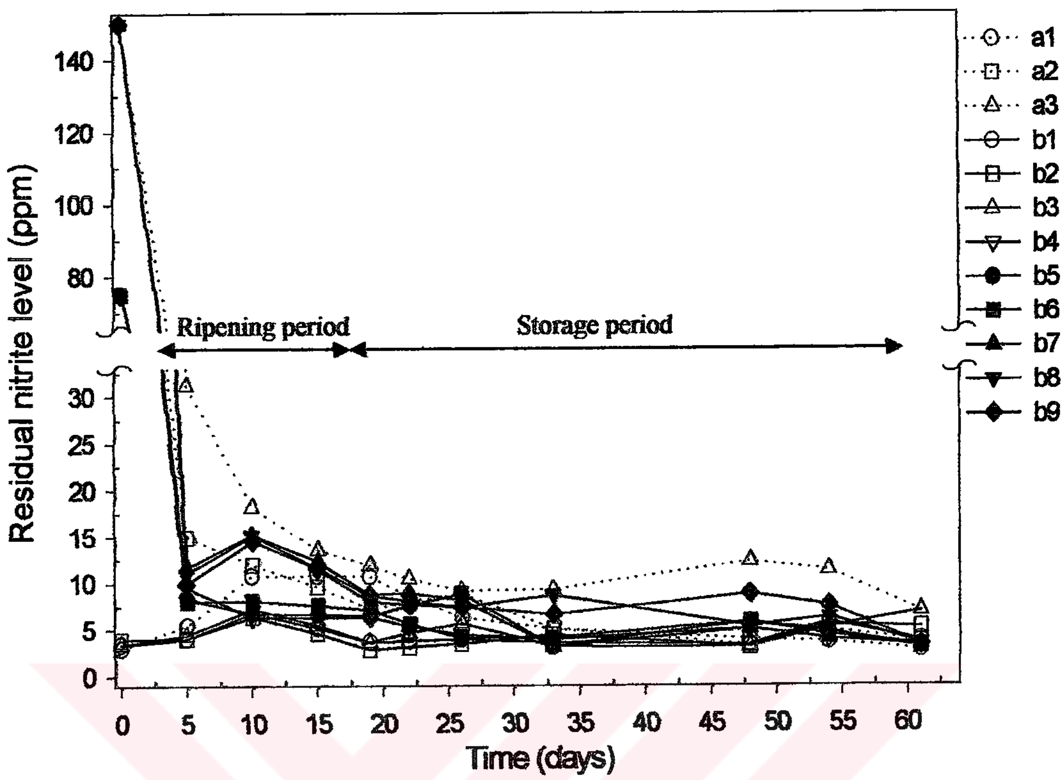


Figure 4.68 Changes of residual nitrite level of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

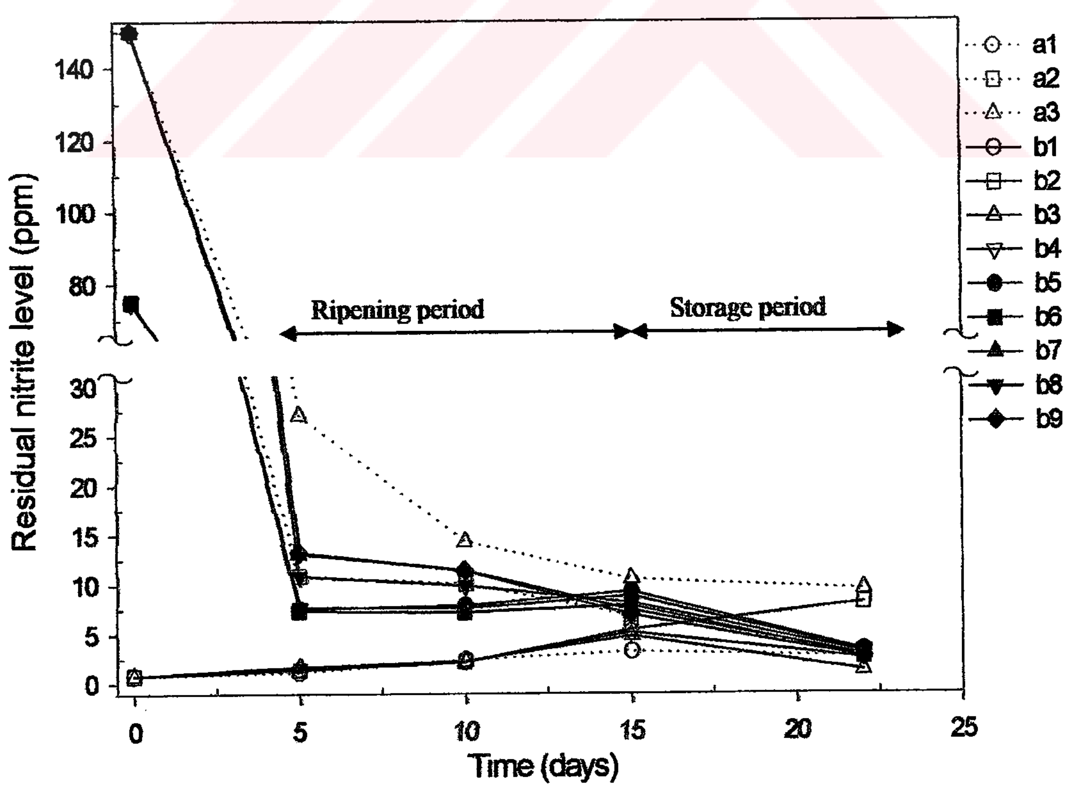


Figure 4.69 Changes of residual nitrite level of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

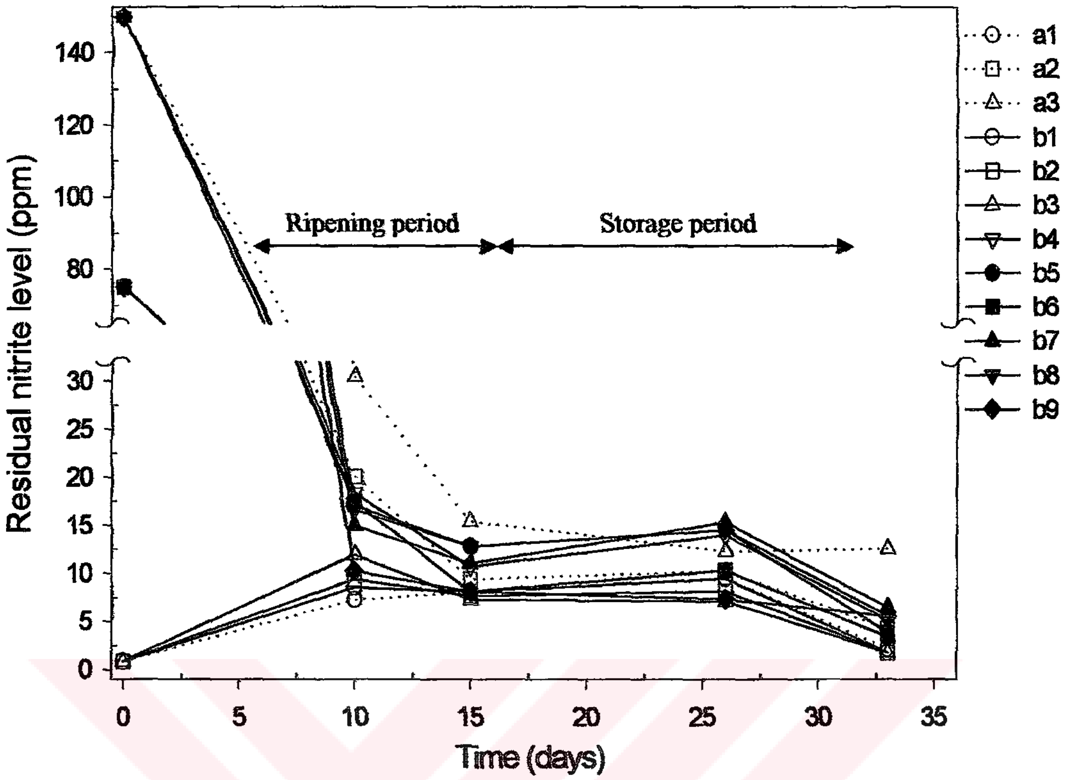


Figure 4.70 Changes of residual nitrite level of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

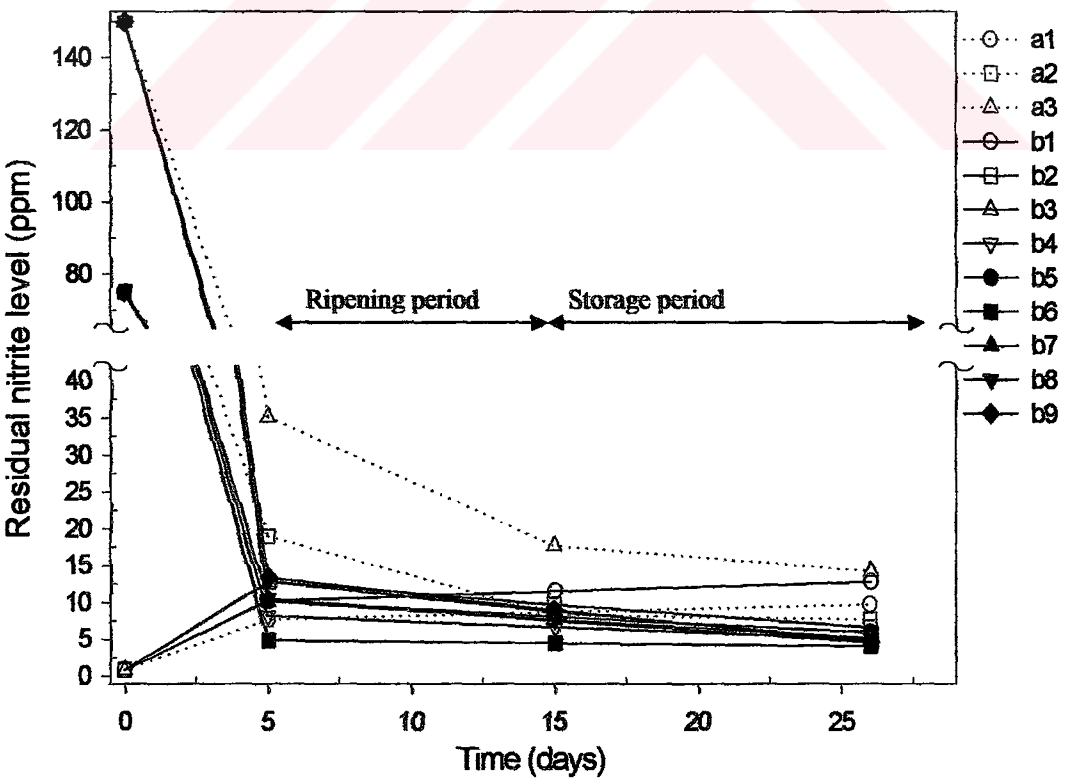


Figure 4.71 Changes of residual nitrite level of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

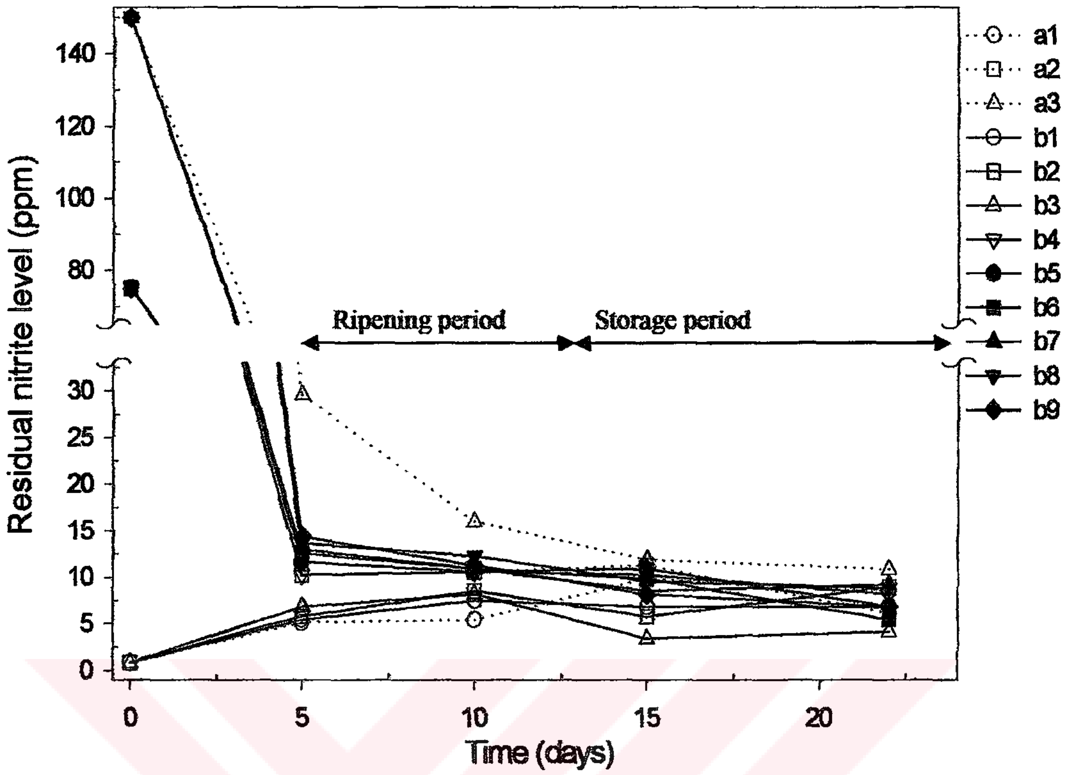


Figure 4.72 Changes of residual nitrite level of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

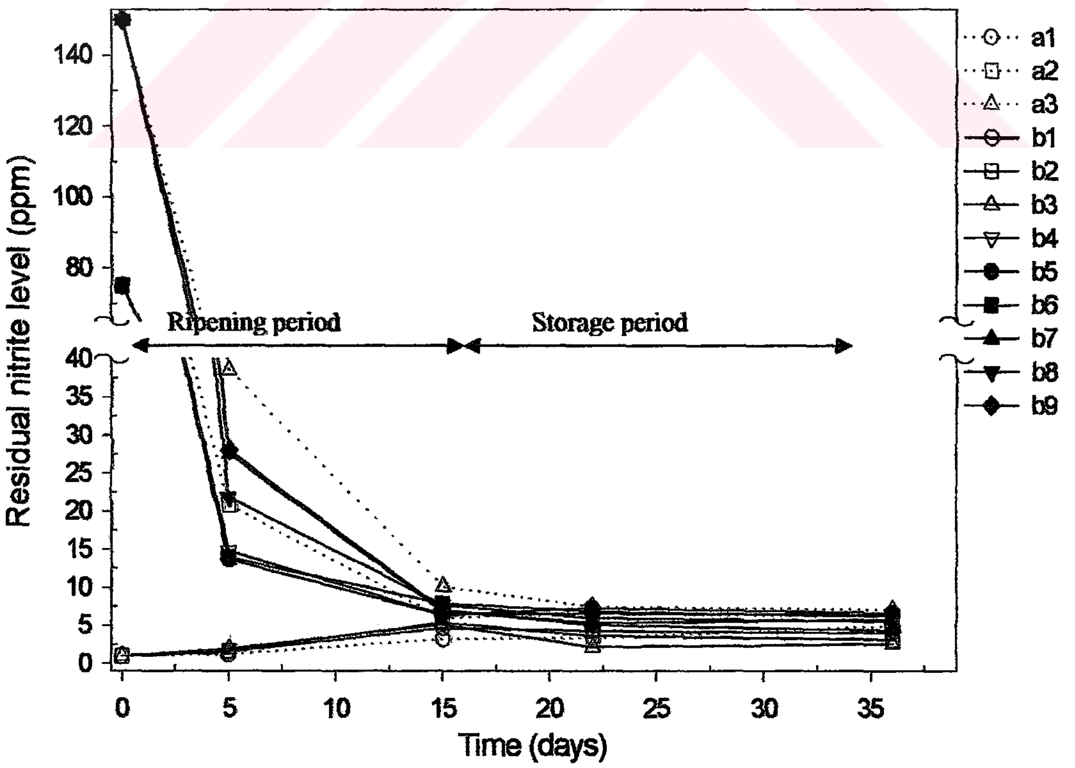


Figure 4.73 Changes of residual nitrite level of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

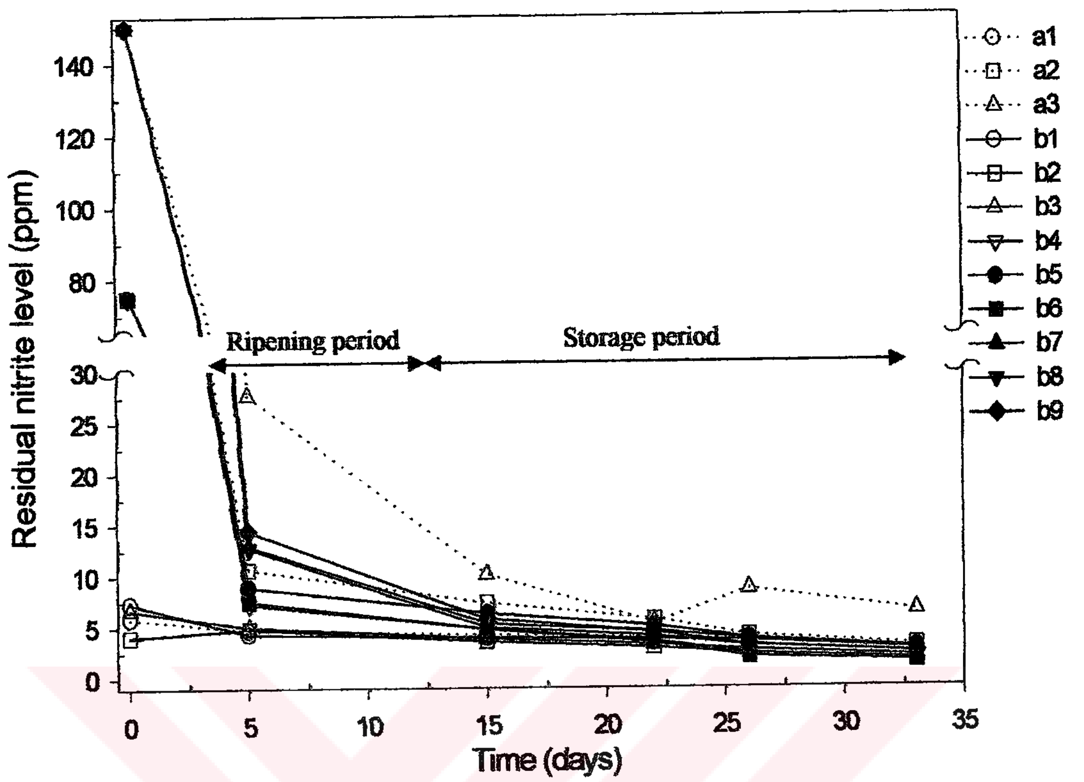


Figure 4.74 Changes of residual nitrite level of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

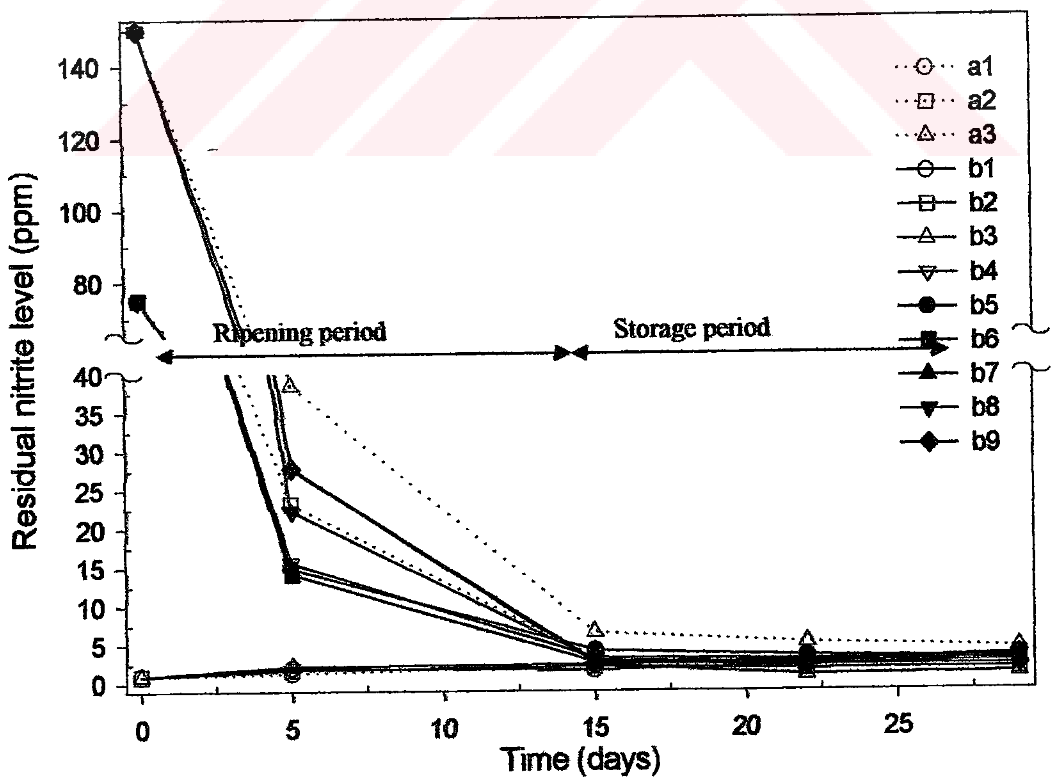


Figure 4.75 Changes of residual nitrite level of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

In Turkey residual nitrite level in sucuks is limited as maximum as 50 ppm according to the Turkish Food Codex (Anonymous, 1997), but in European countries limit residual nitrite level in sausages should be less than 15 ppm in the product. Thus, sucuks could be acceptable because of their low residual nitrite levels. All the results indicate that residual nitrite level of sucuks was affected from starter cultures only. Addition of as high as 150 ppm nitrite, which is the maximum allowable amount, could be reduced to as low as 5 ppm. It is safe for human consumption.

4.2.4.2.1. Prediction of Residual Nitrite Level during the Storage

The following empirical formula was applied to describe the residual nitrite level in sucuks (B1-B9) which were collected during the storage periods. Statistical analyses were carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is $P<0.0001$. This lowest level of significance indicates that empirical formula was well fitted to the experimental data.

$$\text{Residual Nitrite Level (ppm)} = a + b\,(t) + c\,A + d\,T + e\,R + f\,T^2 \qquad [7]$$

where a, b, c, d, e and f are the constants derived from non-linear regression, t is the storage time in day, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significance of the constants are given in Table 4.7. Significance of the constants indicate that presence of this constant necessary or not, if significance level is lower than $P<0.0001$ it is necessary to put this constant into equation. Residual data versus time (days) plot was shown in Figure 4.76. It was observed that residual of residual nitrite level (ppm) ranged between -6.21 to 7.29 ppm.

This empirical formula was compared statistically and found that it can be regarded as sufficient to describe the residual nitrite level in sucuks (B1-B9) during the storage periods. It was found that residual nitrite level (ppm) during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 7].

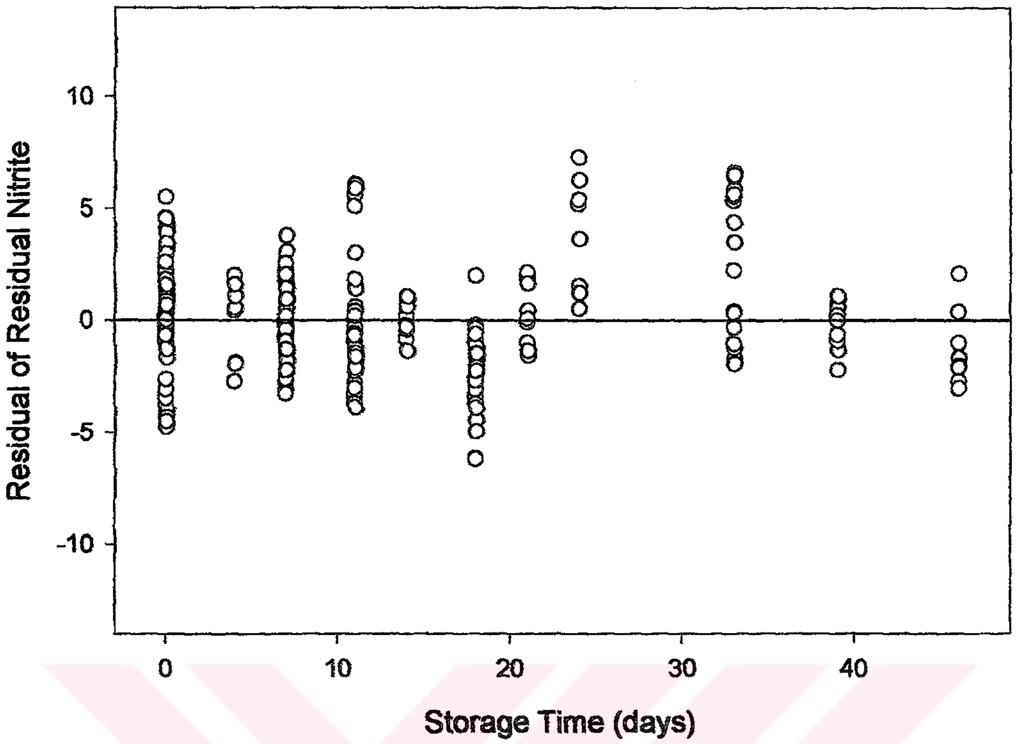


Figure 4.76 Residual plot of residual nitrite level (ppm) in sucuks with fitted empirical formula

Table 4.7 The constants derived from the empirical formula with their standards errors and significance levels

Constant	Value	P values
a	13.6646±1.1709	<0.0001
b	-0.0385±0.0144	<0.0001
c	0.0708±0.0197	<0.0001
d	-0.1104±0.0197	<0.0001
e	-0.0807±0.0150	<0.0001

4.2.4.3. Residual Nitrite Level of Retailed Sucuks

Nitrite is added into sucuk for attaining color, stabilizing the color during the storage period, limiting the oxidation (for antioxidant purpose), inhibiting unwanted microorganisms (especially *Clostridium botolinium, Salmonella spp.*, etc.). Maximum allowable level for addition of nitrate and nitrite into sucuk dough is 300 and 150 ppm and residual nitrite level is 50 ppm according to the Turkish Food Codex (Anonymous, 1997). However, Jo *et al.* (2002) pointed out that if the concentration of sodium nitrite is high enough, it oxidizes hemoglobin, resulting in various adverse health effects on humans.

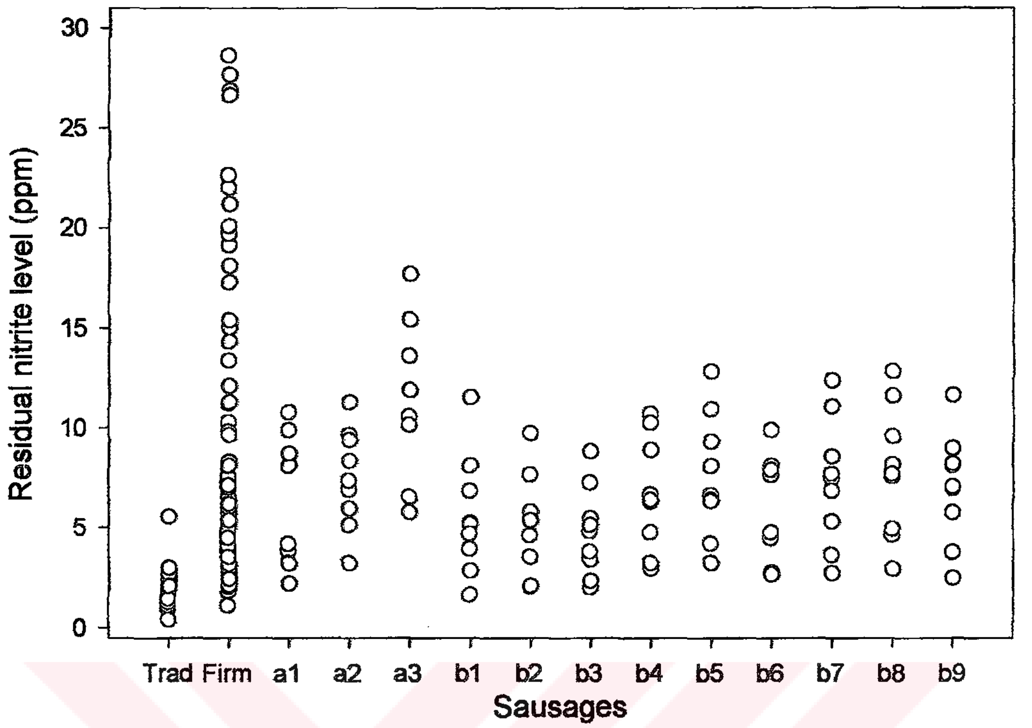


Figure 4.77 Residual nitrite level (ppm) of sucuk types and retailed sucuks (Trad: traditional; Firm: Firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

Residual nitrite level in traditional sucuks ranged between 0.4 and 5.54 ppm and in firm-made sucuks varied between 1.17 and 27.65 ppm. Firm-made sucuks had higher ($P<0.05$) residual nitrite levels than that in traditional sucuks, because during the manufacturing of the firm-made sucuks nitrite and nitrate were added into sucuk dough for production of high quality sucuks. Therefore, high amount of residual nitrite level was observed in firm-made sucuks. Özdemir *et al.* (1984) found that residual nitrite level in retailed sucuks were between 1.3 and 33.1 ppm.

Rapid pH decline (low pH) and low ripening temperature reduce residual nitrite level in sucuks (Huang and Lin, 1993; Gökalp, 1986). Firm-made sucuks had higher pH values and ripening temperature, therefore added nitrite or nitrate could not be reduced.

Residual nitrite level was found to be the highest ($P<0.05$) in firm-made sucuks, as about 9.09 ppm (Figure 4.77). Added nitrite/nitrate into sucuk dough was reduced by bacteria which have nitrite reductase/nitrate reductase. Sucuk type B1-B9 had *Staphylococcus carnosus* which have nitrite/nitrate reductase activity. Added nitrite/nitrate could be readily converted to nitric oxide during the first days of ripening period at low temperature (about 10-20°C) and thus nitrite/nitrate could be played an important role in the formation of desired color (nitrosomyoglobin conversion from myoglobin), inhibition of pathogenic bacteria and formation of desired aroma. Therefore, sucuk types B1-B9 had lower ($P<0.05$) amount of residual nitrite level than that those in firm-made sucuks.

4.2.5. Biogenic Amine Formation

Biogenic amines are toxic nitrogenous compounds formed by decarboxylation of amino acids by the action of living organisms (Shalaby, 1996; Maijala *et al.*, 1995). They can be found in meat products due to the decarboxylase activity of microorganisms during the ripening and storage periods (Shalaby, 1996). The formation of biogenic amines depends on the ripening and storage conditions of product, presence of amine positive microorganisms, free amino acids required for amine formation, quality of raw materials and hygienic conditions of processing environment. They have been proposed as possible indicators of poor hygienic quality of raw materials and poor process conditions of some foods (Vidal-Carou *et al.*, 1990; Maijala *et al.*, 1995; Shalaby 1996; Gonzalez-de-Liano *et al.*, 1998; Ordonez *et al.*, 1999; Bover-Cid *et al.*, 2001). High amount of certain amines may be found in food as a consequence of the use of poor quality raw materials, contamination and in appropriate conditions during food processing and storage (Bover-Cid *et al.*, 2001; Gardini *et al.*, 2002).

Some symptoms of biogenic amines reported as; they could be cause nausea, respiratory distress, hot flushes, sweating; heart palpitation, bright red rash, oral burning and hyper– or hypotension (Gonzales-de-Liano *et al.*, 1998). Concentrations of putrescine and cadaverine correlate with microbial, physical and organoleptic quality attributes in pork and beef (Sayem-El-Daher *et al.*, 1984). Putrescine is important for its pharmacological effects in humans which lead to hypotension, bradycardia, lockjaw, paresis of extremities and potentiate the toxicity of the other amines (Shalaby, 1996). Also, putrescine is important for their capability of pyrrolidine, which is a one of the carcinogenic nitrosamine, is formed when sucuks are heated.

Cadaverine was not detected during the ripening and storage periods of all sucuk types.

Starter cultures (*Pediococcus acidilactici, Lactobacillus plantarum* and *Staphylococcus carnosus*) used in this study for preparation of sucuk types B1-B9 did not produce any biogenic amines.

4.2.5.1. Histamine Formation

Histamine intake of 8-40 mg, 40-100 mg and higher than 100 mg may cause slight, intermediate and intensive poisoning, respectively (Parente *et al.*, 2001). On the other hand, Nout (1994) pointed out that histamine concentration should be lower than in the range of 50-100 mg/kg in sausages. Histamine concentration in sucuks that were collected from local markets in Ankara varied in the range of 6.72 to 362.22 mg/kg (Şenöz *et al.*, 2000).

Histamine formation in sucuk types A1-A3 and B1-B9 was followed during the ripening and storage periods at 20, 30 and 40°C with 50, 65 and 80 % RH and their results are given in Figures 4.78 - 4.86. It was ranged from 0.00 to 279.32 mg/kg during the ripening and from 0.00 to 292.66 mg/kg during the storage periods. Histamine concentration in sucuk retailed in Gaziantep was found to be in the range 0.00-724.24 mg/kg and 0.00-951.53 mg/kg in traditional and firm-made sucuks, respectively (Figure 4.108).

4.2.5.1.1. Histamine Formation during the Ripening Periods

Histamine concentrations increased ($P<0.05$) during the first 10 days of ripening period (Figures 4.78 and 4.86). The level of increase was depending on presence or absence of additives and lactic acid bacteria. Dierick *et al.* (1974) found that histamine concentration were increased at the beginning of ripening periods and decreased during the storage periods of dry-sucuks.

Additives affected ($P<0.05$) the histamine formation. Sucuks made with additives had lower ($P<0.05$) histamine formation than the others. The concentration of histamine was greater than 100 mg/kg in sucuk types made without additives A1, B1 and B4, but lower than 75 mg/kg in sucuk types made with high level of additives A3, B3, B6 and B9 after 15 days of ripening after 10 days. Lower levels of histamine concentration (<75 mg/kg) were obtained in sucuks type A3, B5, B7, B8 and B9; A3, B1, B2, B3, B4, B5, B6, B7, B8 and B9; and A3, B1, B7, B8 and B9 after ripening period at 20°C for 50, 65 and 80 % RH, respectively. The concentrations of histamine in sucuk types A1, A2 and B1 stored at 30°C and at 65 % RH were greater than 100 mg/kg after 15 days and lower than 60 mg/kg in other sucuk types.

Sucuks made without starter culture (A1-A3) had higher ($P<0.05$) histamine formation than sucuks made with starter culture (B1-B9).

4.2.5.1.2. Histamine Formation during the Storage Periods

Histamine concentration was greater than 100 mg/kg in sucuk types A1, A2 B1, B2, B3 and B6 stored at 20°C with 50 % RH at the end of 33 days, but lower than 50 mg/kg in sucuk types A3, B5, B7, B8 and B9. All of sucuks had about 20 mg/kg of histamine after 60 days (Figure 4.78). At 20°C with 65 % RH, the histamine concentration decreased ($P<0.05$) until 48 days to about 30 mg/kg (Figure 4.79). There were great variations in the concentration of histamine in sucuks stored at 20°C with 80 % RH during the storage periods (Figure 4.80). Histamine concentration decreased from 15 to 22 days and than increased (except sucuk type B9). The lower levels of histamine were observed in all sucuks stored at 20°C with 50 and 65 % RH after 46 days. Sucuk type B9 had contained about 30 mg/kg histamine after 28 days at 20°C with 80 % RH (Figure 4.80).

The concentrations of histamine were lower than 30 mg/kg after 53 days except sucuk type A1 and A2 at 30°C with 65 % RH. On the other hand, histamine concentrations were greater than 100 mg/kg in all sucuks stored at 30°C with 50 % and 80 % RH.

Histamine level decreased to safe level (lower than 100 mg/kg; Shalaby, 1996) in sucuk types A2, A3, B3, B6 and B9 stored at 40°C with 50 % RH (Figure 4.84); all sucuk types except B5 stored at 40°C with 65 % RH (Figure 4.85). Histamine concentrations were greater than safe level in all sucuks stored at 40°C with 80 % (Figure 4.86).

Statistical analysis pointed out that additives had reduction effects ($P<0.05$) on histamine formation in sucuks. Histamine level in sucuk A1 prepared without starter culture and additives was the highest ($P<0.05$; Figures 4.78-4.86). The concentration of histamine was higher ($P<0.05$) in sucuks (B4 and B7) made with only nitrite/nitrate than sucuks made with other additives (B2 and B3). There is a little information in the literature about commercial food additives on the biogenic amine formation. Shalaby (1996) pointed out that potassium sorbate may be used to limit the biogenic amine in foods. In our study, potassium sorbate with other commercial food additives and starter cultures limited ($P<0.05$) the histamine formation. Increasing the amount of additives decreased ($P<0.05$) the formation of histamine. In sucuks which were prepared by using high concentration of additives (nitrite, nitrate, α-tocopherol, ascorbic acid, potassium sorbate, potassium pyrophosphate and di-potassium hydrogen phosphate; in sucuk types B3, B6 and B9), had lower ($P<0.05$) histamine concentration (Figures 4.78-4.86). Using 2.5 mg/kg potassium sorbate with high levels of nitrite/nitrate (sucuk type B9) decreased the histamine formation (<100 mg/kg) during the ripening period and storage periods. This shows that the limited concentration of potassium sorbate may be around 2.5 mg/kg of sucuk for decreasing the biogenic amine formation.

The effect of starter culture on histamine formation was found to be significant ($P<0.05$). Starter culture inoculation limited ($P<0.05$) the histamine formation. Using both starter culture and additives (sucuk B9) decreased ($P<0.05$) the histamine formation to safe level. The addition of starter culture into sucuk has been suggested to limit biogenic amine formation. Roig-Sagues and Eerola (1997) found that histamine formation increased in sucuks except with starter culture inoculated sucuks. Hernandez-Jover *et al.* (1997b) found that starter cultures; *Lactobacillus plantarum-Micrococcus carnosus* mixture and *Pediococcus pentosaceus- Micrococcus carnosus* mixture decreased the biogenic amine formation during the ripening periods of sucuks. The similar results were observed in our study that is starter cultures (*Pediococcus acidilactici, Lactobacillus plantarum* and *Staphylococcus carnosus*) decreased ($P<0.05$) the biogenic amine formation.

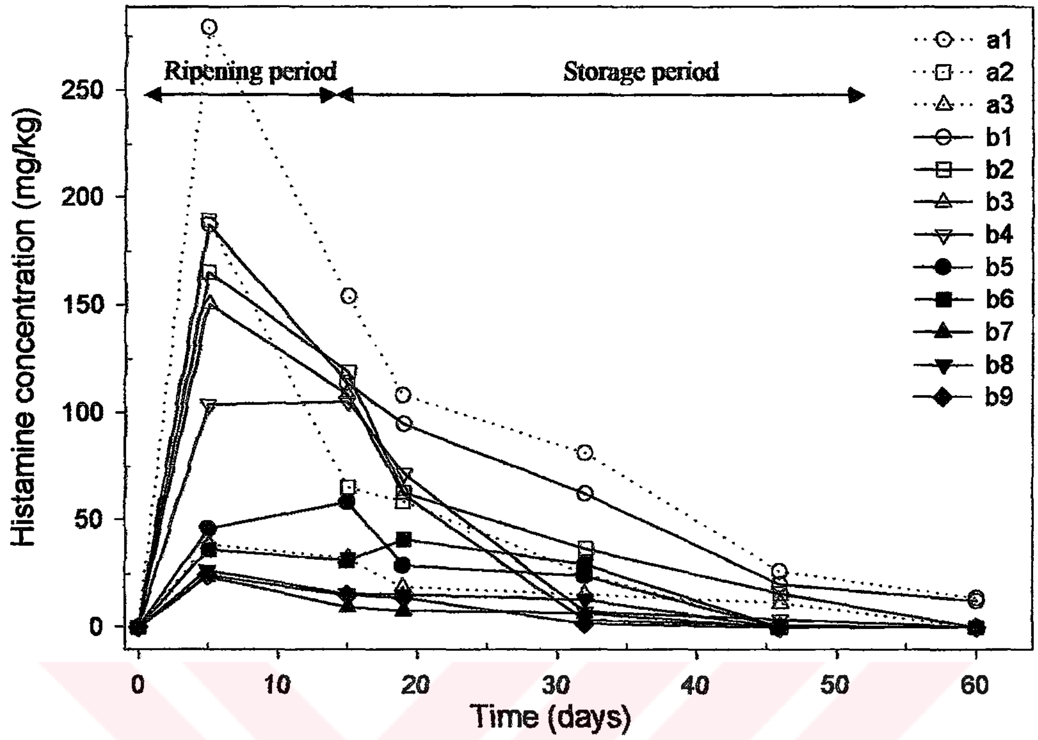


Figure 4.78 Changes of histamine concentration of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

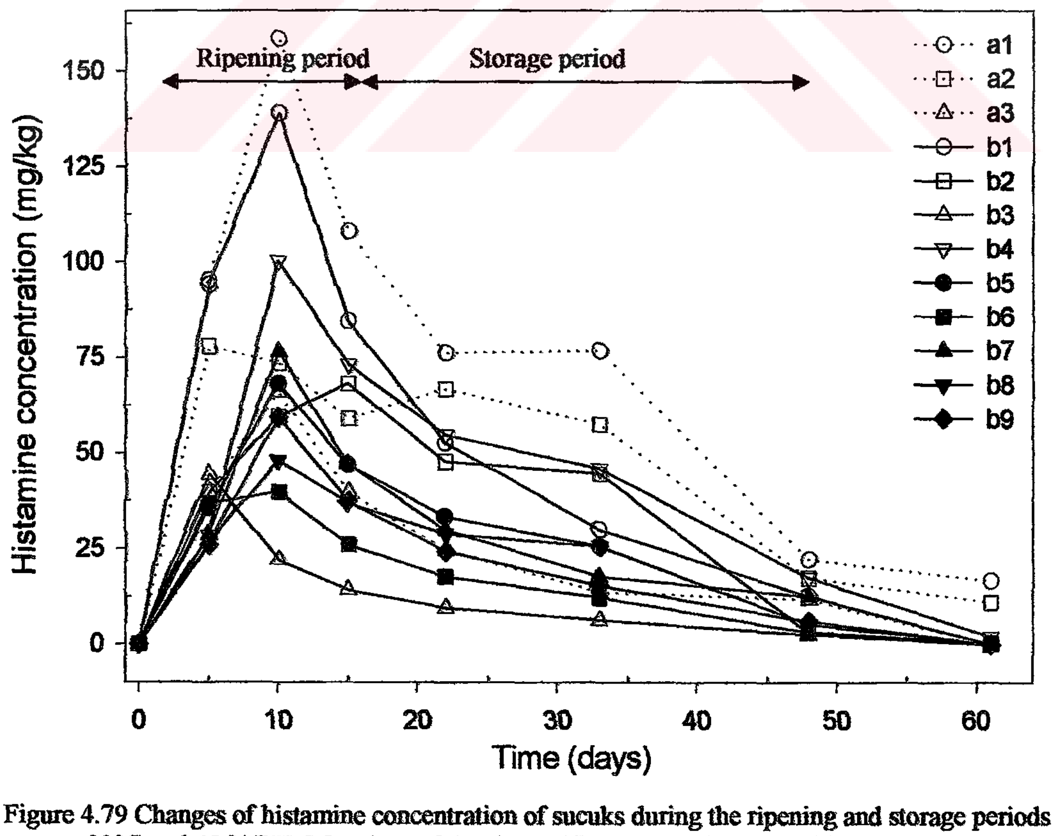


Figure 4.79 Changes of histamine concentration of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

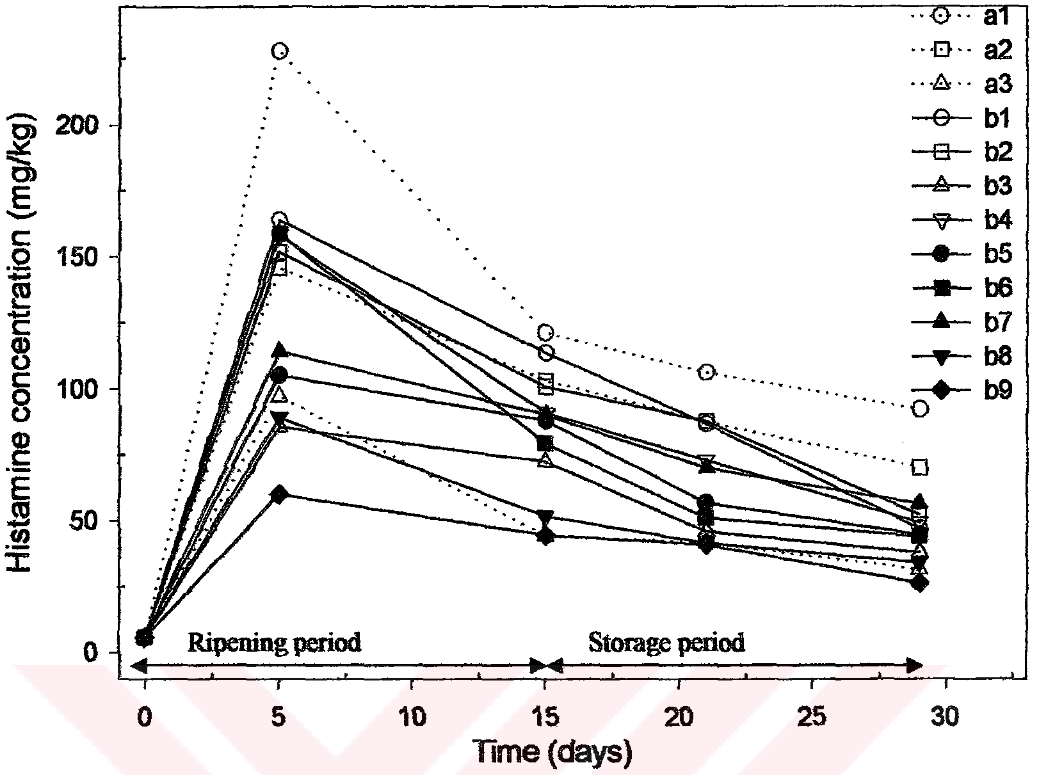


Figure 4.80 Changes of histamine concentration of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

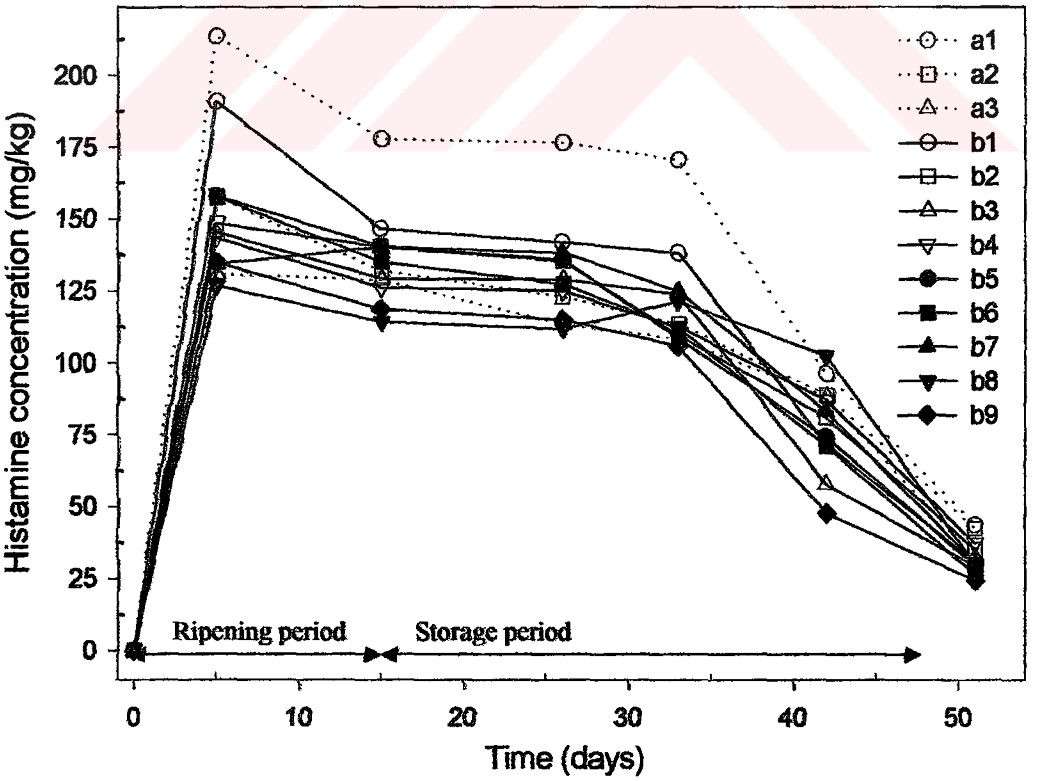


Figure 4.81 Changes of histamine concentration of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

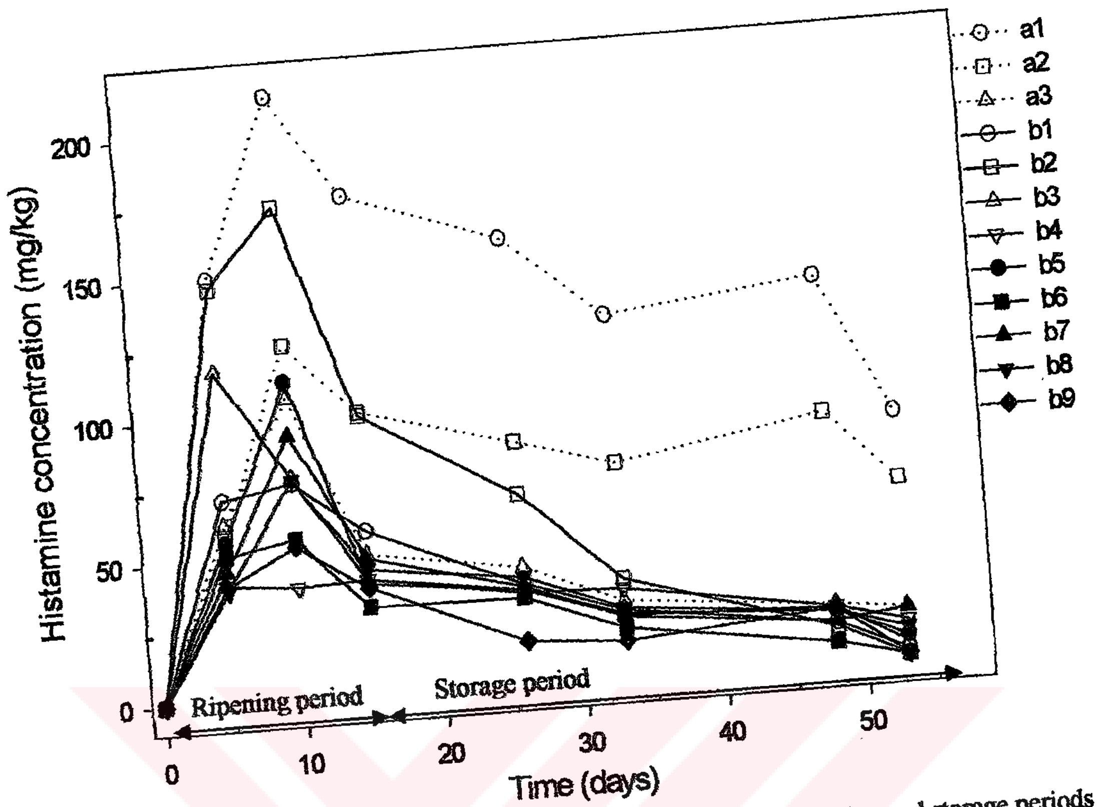


Figure 4.82 Changes of histamine concentration of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

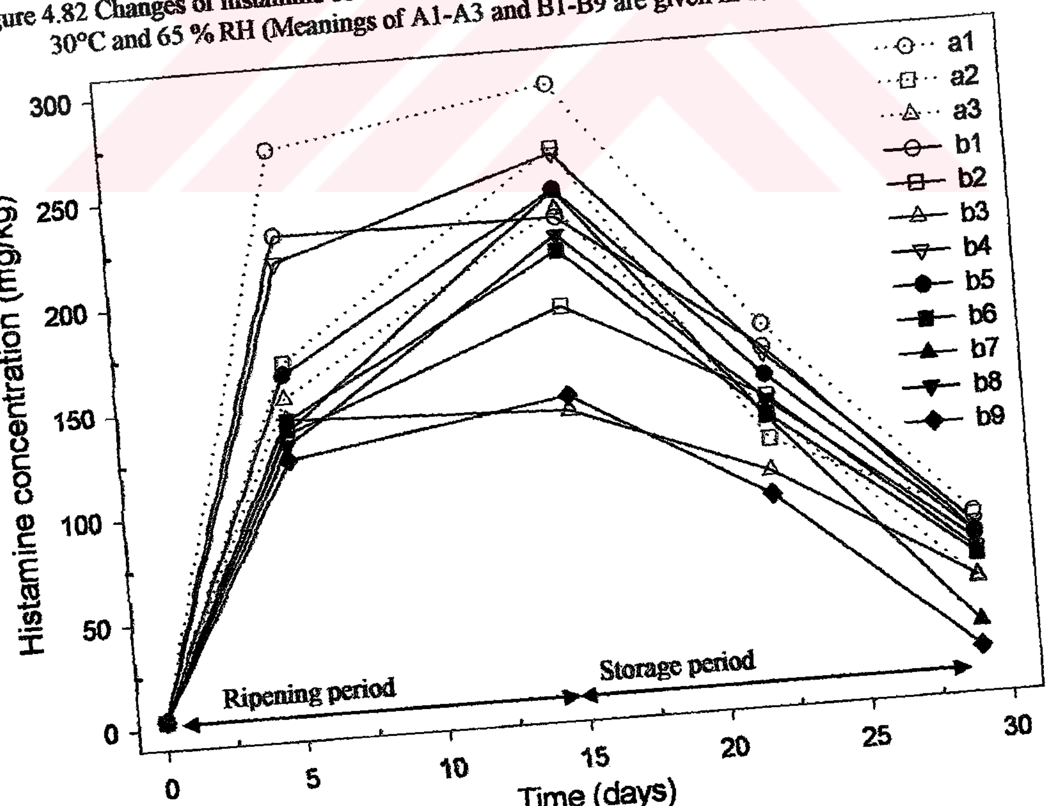


Figure 4.83 Changes of histamine concentration of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

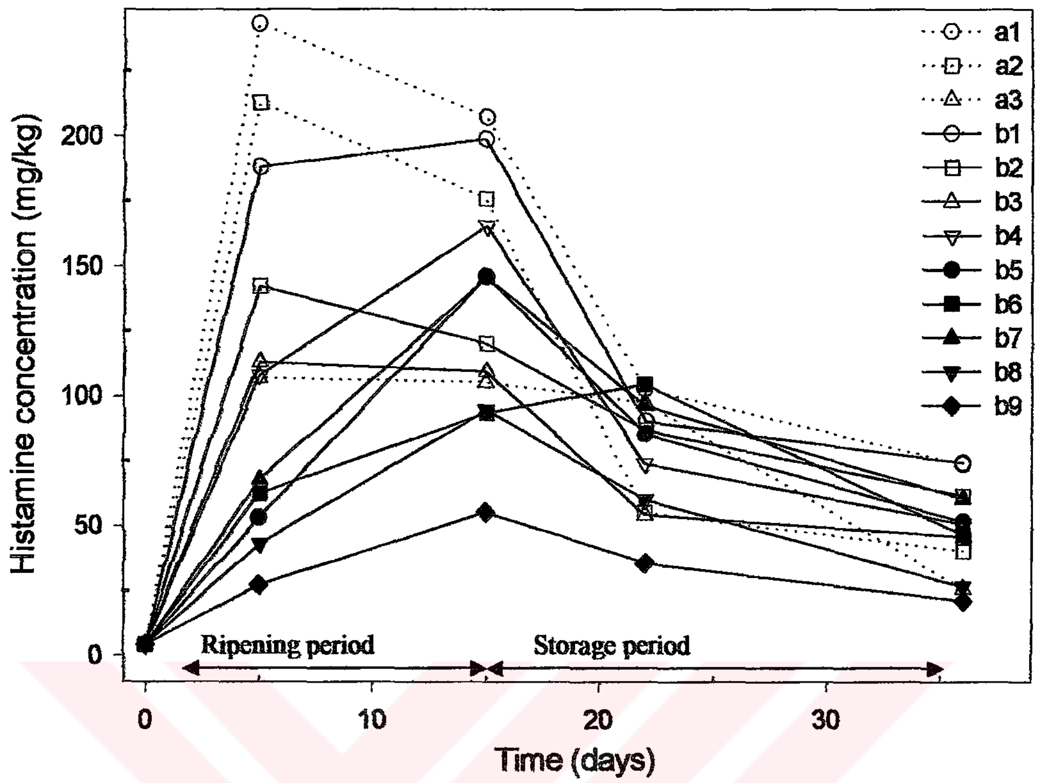


Figure 4.84 Changes of histamine concentration of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

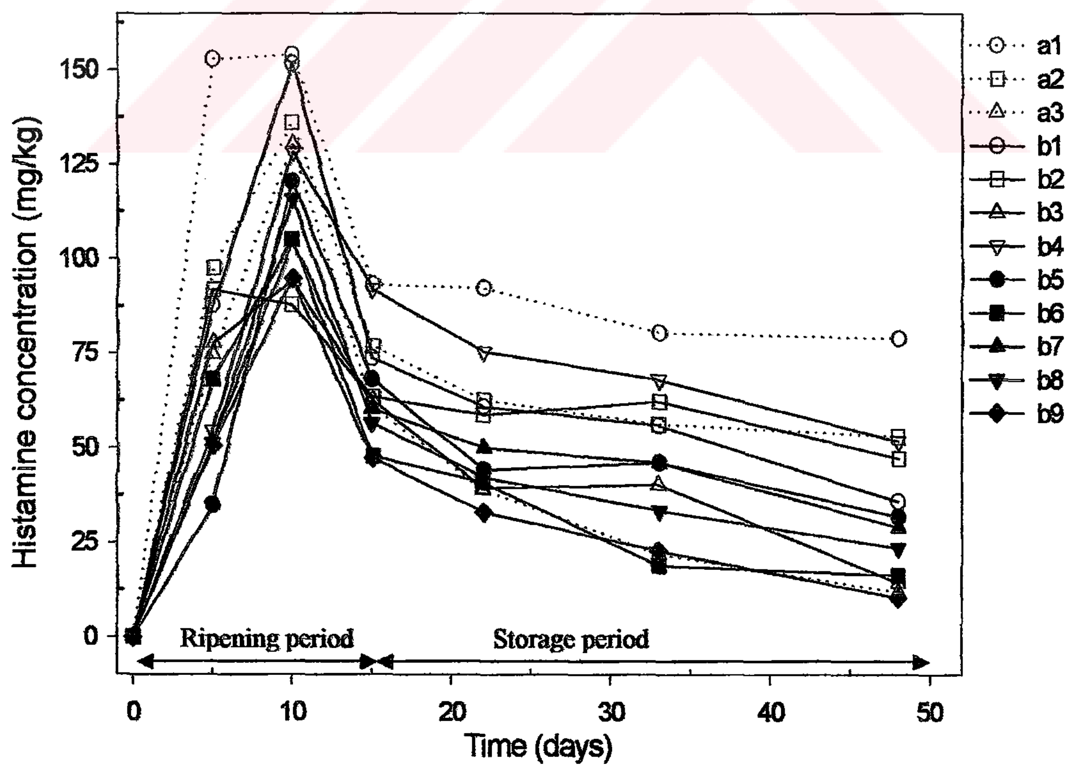


Figure 4.85 Changes of histamine concentration of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

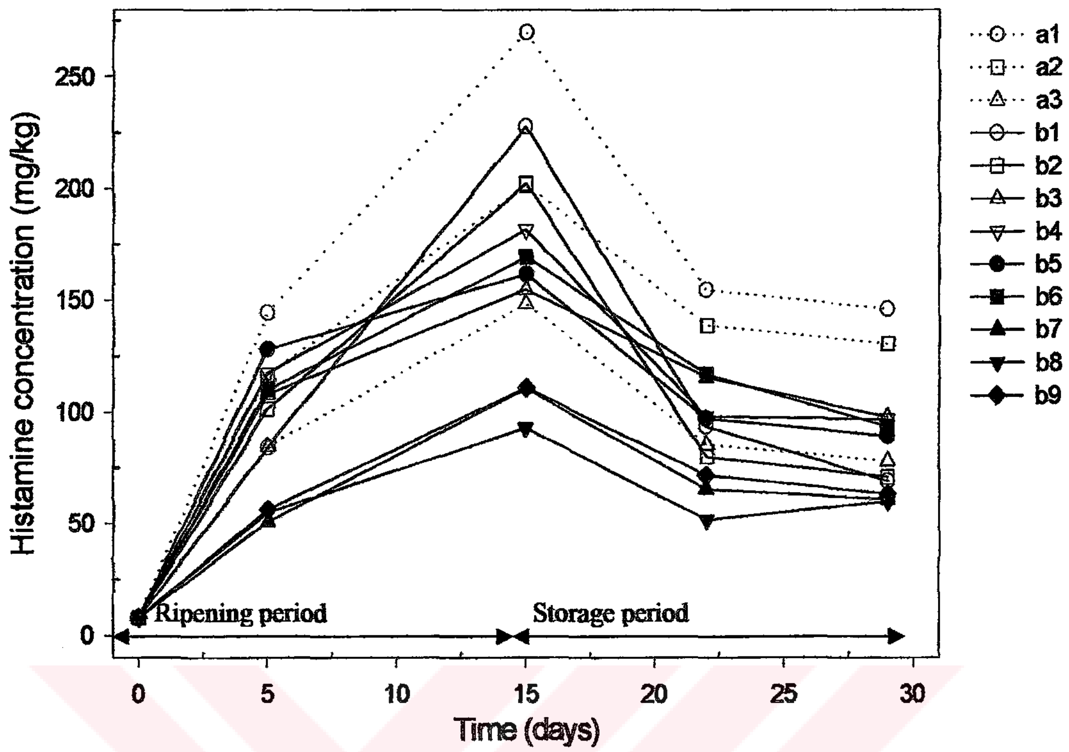


Figure 4.86 Changes of histamine concentration of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

The formation of histamine was affected significantly ($P<0.05$) from the storage conditions (temperature and % RH). Histamine formation was highest ($P<0.05$) at high RH (80 %) storage. During the storage at high RH, available water (water activity) in sucuk was high and this increased the activity of microorganisms. As a result of high microbial activity the formation of histamine increased. Histamine formation was found to be highest ($P<0.05$) at high temperature (at 40°C). Increasing the temperature from 20 to 40°C increased ($P<0.05$) the histamine formation (Figures 4.78-4.86). Similar results were observed in Shalaby's (1996) review who suggested that optimum temperature for histamine formation was 37.8°C and increasing or decreasing temperature form 37.8°C decreased the histamine formation.

Interactions among additives, temperature and % RH of the storage were studied by means of statistical analysis. This analysis indicate that two-way interactions among those factors were found to be statistically significant ($P<0.05$). It was found that addition of high levels of additives with starter culture and storage at low temperature decreased ($P<0.05$) the histamine formation largely. Addition of high levels of additives (300 ppm nitrate, 150 ppm nitrite, 2.5 mg/kg potassium pyrophosphate, 2.5 mg/kg di-potassium hydrogenphosphate, 500 ppm ascorbic acid, 200 ppm α-tocopherol and 200 ppm potassium sorbate) with starter culture at 50 % RH decreased the formation of histamine much more ($P<0.05$) than that the low additives (150 ppm nitrate, 750 ppm nitrite, 1.25 mg/kg potassium pyrophosphate, 1.25 mg/kg di-potassium hydrogenphosphate, 250 ppm ascorbic acid, 100 ppm α-tocopherol and 100 ppm potassium sorbate) and 65 and 80 % RH of the storage alone. Lowering both storage temperature and % RH lowered its formation. Three way interactions was significant ($P<0.05$) between additives, temperature and storage RH. Using high levels of additives with 50 % RH and low temperature (20°C) decreased the formation of histamine during the ripening and storage. Additives, starter culture, low % RH and low temperature can be used as hurdle factors for controlling the histamine formation in sucuk.

Pearson correlation test was performed between histamine with pH, TBA, residual nitrite levels, nitrosomyoglobin conversions, aerobic plate count, total lactic acid bacteria count and mold and yeast count of sucuks. It was observed that negative correlation ($P<0.05$) between histamine with pH and residual nitrite level of sucuks. However, a positive correlation ($P<0.05$) was observed between histamine concentration with TBA, nitrosomyoglobin conversion, overall sensory scores, APC, mold and yeast counts, putrescine and tyramine concentration of sucuks. A negative value means that lower the pH and residual nitrite level higher the histamine formation and positive values mean that higher the content of histamine higher TBA, nitrosomyoglobin conversion, overall sensory scores, APC, mold and yeast counts, putrescine and tyramine concentration of sucuks.

4.2.5.1.2.1. Prediction of Histamine Concentration during the Storage

The following empirical formula [Eqn 8] was applied to describe the histamine concentration in sucuks (B1-B9) which were collected during the storage periods. Statistical analyses was carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is $P<0.0001$. This lowest level of significance indicates that empirical formula was well fitted to the experimental data.

$$\text{Histamine Concentration (mg/kg)} = a + b\,(t) + c\,e^{A} + d\,T + f\,R + g\,R^2 + h\,T^2 \quad [8]$$

where a, b, c, d, f, g and h are the constants derived from non-linear regression, t is the storage time in day, e is the exponential term, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significance of the constants are given in Table 4.8. Significance of the constants indicates that presence of this constant necessary or not, if significance level is lower than $P<0.0001$ it is necessary to put this constant into equation. Residual data versus time (days) plot was drawn and it is given in Figure 4.87. It is observed that residual histamine concentration ranged between -79.80 to 125.98 mg/kg.

This empirical formula was compared statistically and found that it can be regarded as sufficient to describe the histamine concentration in sucuks (B1-B9) during the storage periods. It was found that histamine concentration during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 8].

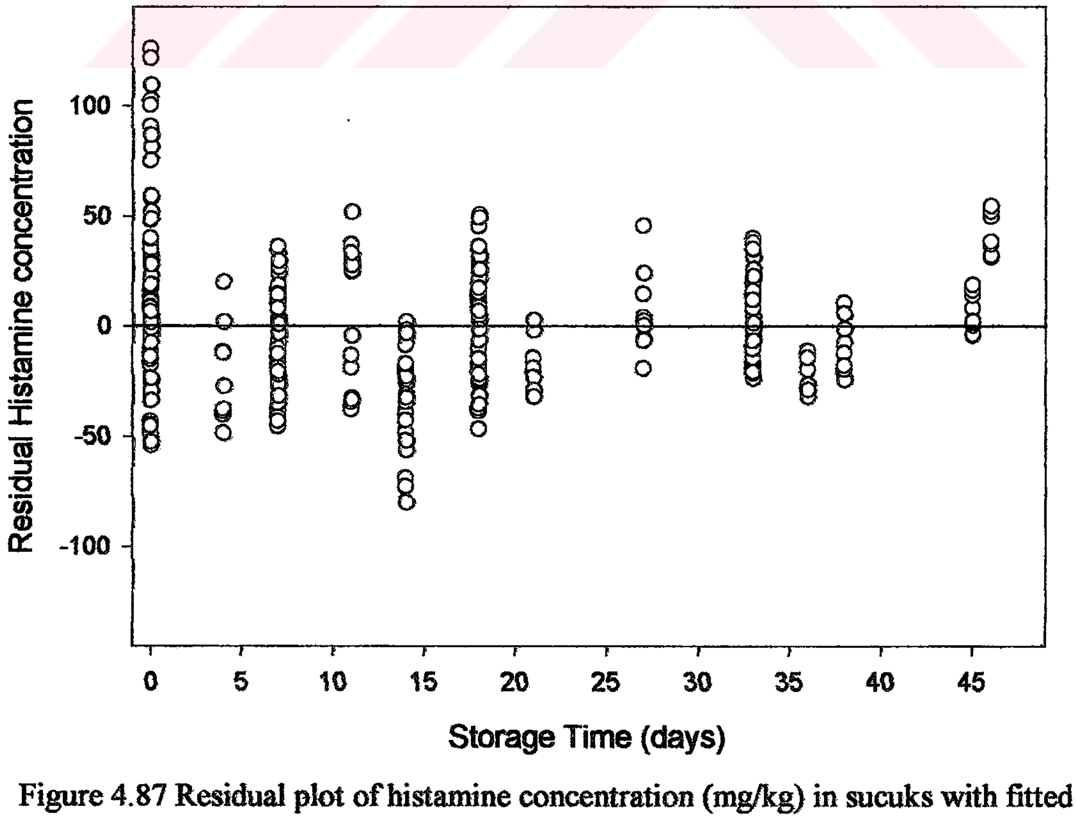


Figure 4.87 Residual plot of histamine concentration (mg/kg) in sucuks with fitted empirical formula

Table 4.8 The constants derived from the empirical formula with their standards errors and significance levels

Constant	Value	P values
a	554.1007±73.3288	<0.0001
b	-1.7175±0.1365	<0.0001
c	-1.0613±0.2124	<0.0001
d	17.6439±2.1851	<0.0001
f	-23.5499±2.1270	<0.0001
g	0.1851±0.0165	<0.0001
h	-0.2687±0.0360	<0.0001

4.2.5.2. Tyramine Formation

Tyramine is formed in meat products due to the decarboxylation of tyrosine. The importance of tyramine in foods is principally due to its toxicological implications since apart from being slightly toxic in itself; the tyramine reacts with monoamine oxidase inhibitor (MAOT) drugs giving rise to hypertensive crisis (Shalaby, 1996). Tyramine acts mainly indirectly by releasing noradrenaline from the sympathetic nervous system which causes an increase of the blood pressure by peripheral vascoconstriction and by increasing the cardiac output (Shalaby, 1996). Tyramine also causes salivation and increases respiration and the blood sugar level. The allowable maximum level of tyramine in foods is 100-800 mg/kg and 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.

The changes of tyramine concentration in sucuks A1-A3 and B1-B9 are given in Figures 4.88-4.96. Tyramine concentrations varied from 0.00 to 495.25 mg/kg in sucuks. Şenöz *et al.* (2000) detected tyramine level in sucuk collected from local market in the range of 208.66 to 1173.28 mg/kg. Hernandez-Jover *et al.* (1997a) found that about 13 % of Spanish sausage had higher level of Tyramine more than 100 mg/kg.

4.2.5.2.1. Tyramine Formation during the Ripening Periods

The tyramine concentrations after five days of ripening were different ($P<0.05$) (Figures 4.88 and 4.96). Eerola *et al.* (1997) reported that tyramine concentrations were increased up to 7 days of storage at 4°C and the concentration of tyramine decreased during the 58 days of storage. Eitenmiller *et al.* (1978) found that tyramine concentration increased in sausages during the first 3 days of ripening then decreases during 20 days of storage.

The lowest ($P<0.05$) tyramine concentration observed in sucuk type B9 made with starter culture and higher concentration of additives (Figures 4.88-4.96). Sucuk type A1 made without starter culture and additives had the highest ($P<0.05$) tyramine concentration. Starter culture containing sucuks (B1-B9) had lower ($P<0.05$) tyramine concentration than sucuks made without starter culture. This could be due to the inhibition of decarboxylase positive bacteria by additives and starter culture in sucuk type B9. Lactic acid bacteria produced organic acids and acids reduce sucuk pH value and the formation of biogenic amine depends on the pH value of sucuk (Shalaby, 1996). The decreasing of pH increased ($P<0.05$) biogenic amine formation. This result was in agreement with the results of Parente *et al.* (2001). These results indicate that, starter culture and additives should be used together for preparing the safe sausage in a proper proportion.

4.2.5.2.2. Tyramine Formation during the Storage Periods

The changes of tyramine concentration in sucuks stored at 20°C with 50, 65 and 80 % RH are given in Figures 4.88, 4.89 and 4.90, respectively. Tyramine concentration decreased ($P<0.05$) during the storage periods. Eerola *et al.* (1997) found that the concentration of tyramine decreased during the 58 days of storage. Eitenmiller *et al.* (1978) found that tyramine concentration decreased during 20 days of storage in sucuks. Tyramine concentrations in sucuks stored at 80 % RH were higher ($P>0.05$) than sucuks stored at 50 and 65 % RH. Available water in high % RH is higher than in low % RH, this could enhance the activity of microorganisms (Figures 4.1-4.30). Therefore, tyramine formation in high % RH was higher than sucuks stored at lower % RH. The highest ($P<0.05$) tyramine concentration was observed in sucuk type A1.

Tyramine concentration in sucuks during the storage periods at 30°C with 50, 65 and 80 % RH are given in Figures 4.91, 4.92 and 4.93, respectively. It was observed that tyramine concentration in sucuks decreased ($P<0.05$) during the storage periods. The lowest tyramine formation was observed in sucuk type B9 and the highest tyramine in sucuk type A1. Sucuks made with starter culture and additives had lower (P<0.05) tyramine concentration.

The changes of tyramine concentration in sucuks during the storage periods at 40°C with 50, 65 and 80 % RH are given in Figures 4.94, 4.95 and 4.96, respectively. Tyramine concentration in sucuks decreased (P<0.05) during the storage periods at all % RH of storage environment.

Additives decreased (P<0.05) the tyramine formation. Sucuks made with highest levels of additives (A3, B3, B6 and B9; Table 3.2) had lower (P<0.05) tyramine concentration during the storage period. This would be due to the antimicrobial effects of additives (nitrite, nitrate and potassium sorbate) and they could inhibit the growth of amine positive bacteria. Therefore, tyramine formation was lower (P<0.05) in sucuks made with high level of nitrite/nitrate and potassium sorbate.

Low temperature could reduce the growth of tyrosine decoxylase positive (tyramine producing) microorganisms. In this study, tyramine formation decreased (P<0.05) with increasing or decreasing the temperature from optimum temperature for tyrosine decarboxylase positive bacteria. The optimum temperature for tyramine production was found to be 30°C (Shalaby, 1996). Vidal-Carou *et al.* (1990) found that tyramine formation in meat product increased with increasing the temperature and its level occurred increased much more quickly at room temperature.

Percent RH of the storage conditions had significant effect (P<0.05) on the tyramine formation. The lowest tyramine formation was observed in sucuks stored at 50 % RH. This could be due to the activity of microorganism at low % RH. Tyramine production was lower (P<0.05) at low % RH than at high % RH.

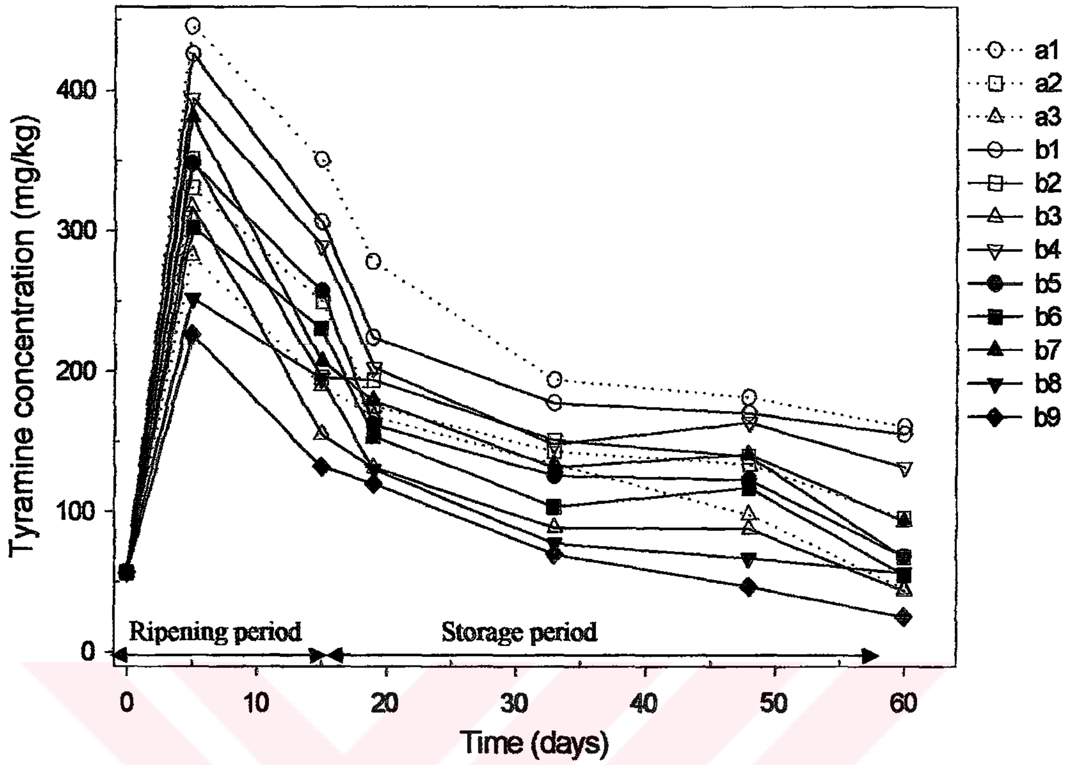


Figure 4.88 Changes of tyramine concentration of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

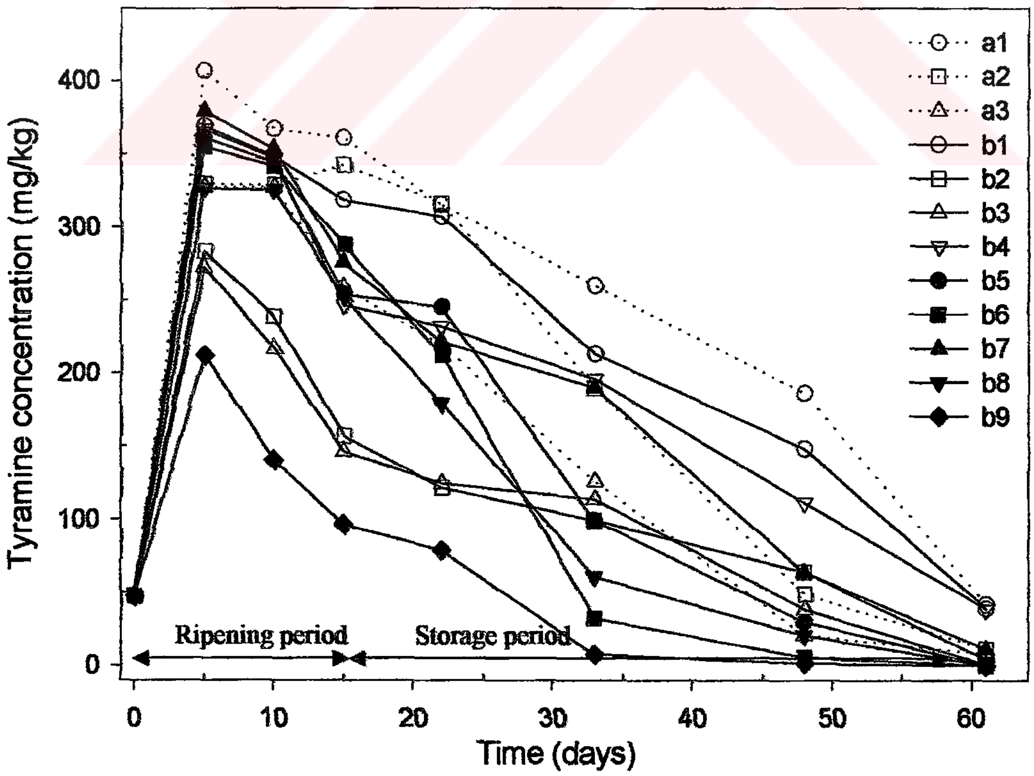


Figure 4.89 Changes of tyramine concentration of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

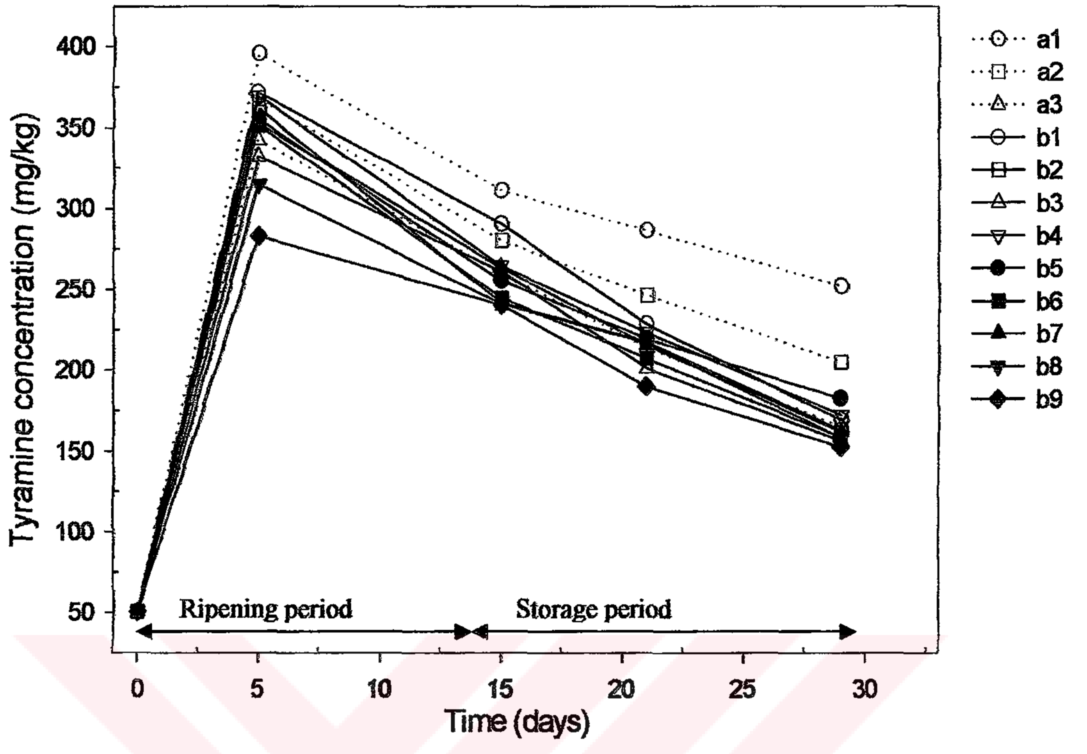


Figure 4.90 Changes of tyramine concentration of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

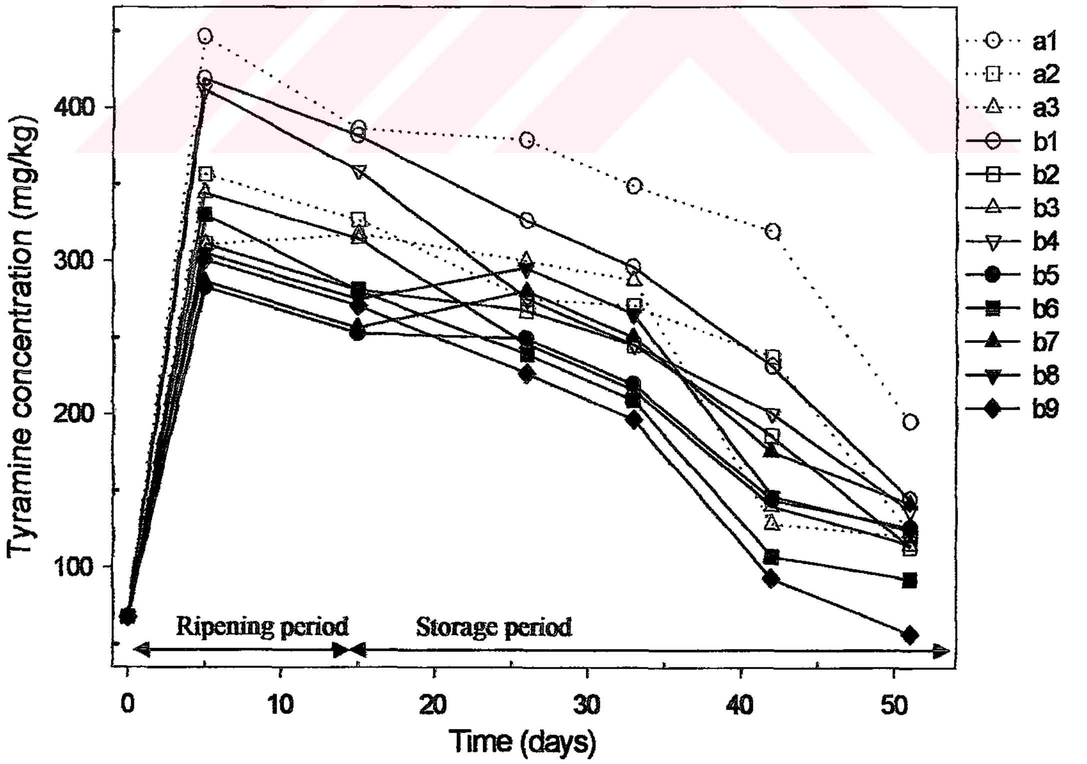


Figure 4.91 Changes of tyramine concentration of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

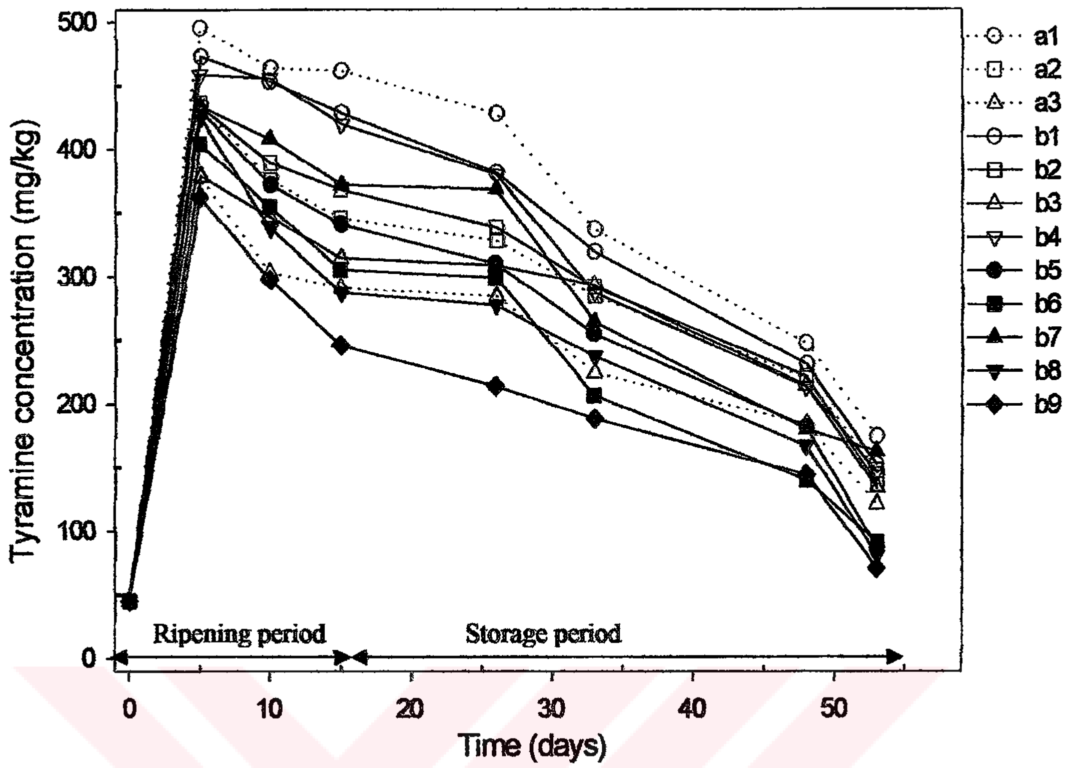


Figure 4.92 Changes of tyramine concentration of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

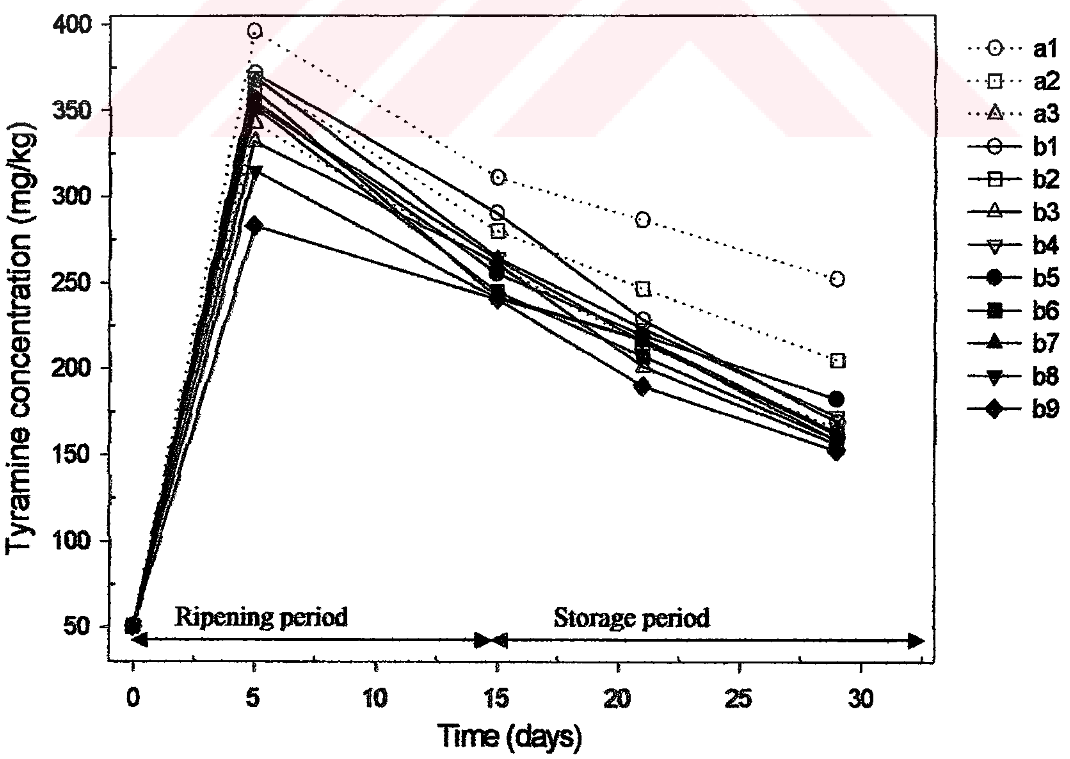


Figure 4.93 Changes of tyramine concentration of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

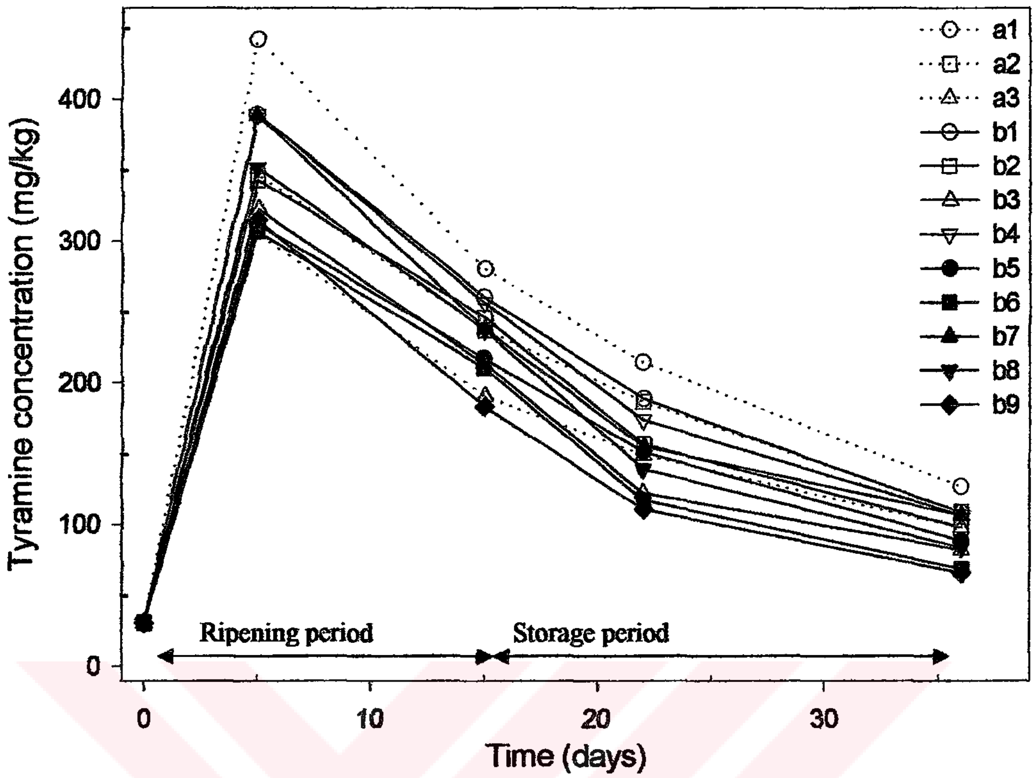


Figure 4.94 Changes of tyramine concentration of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

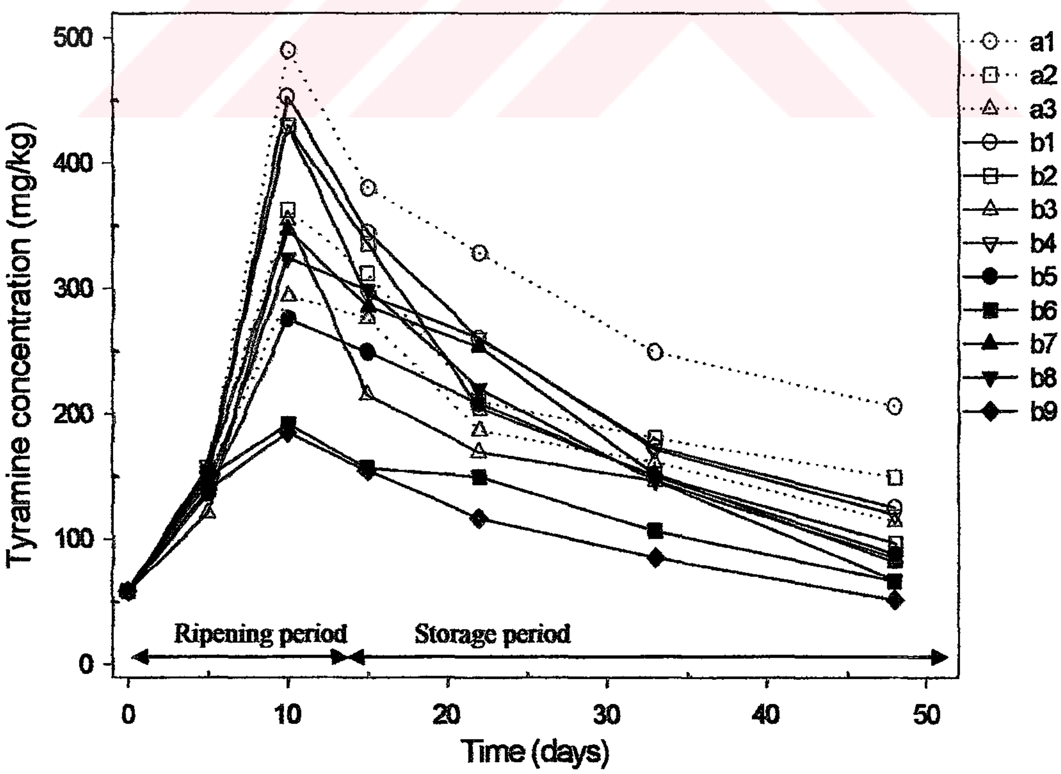


Figure 4.95 Changes of tyramine concentration of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

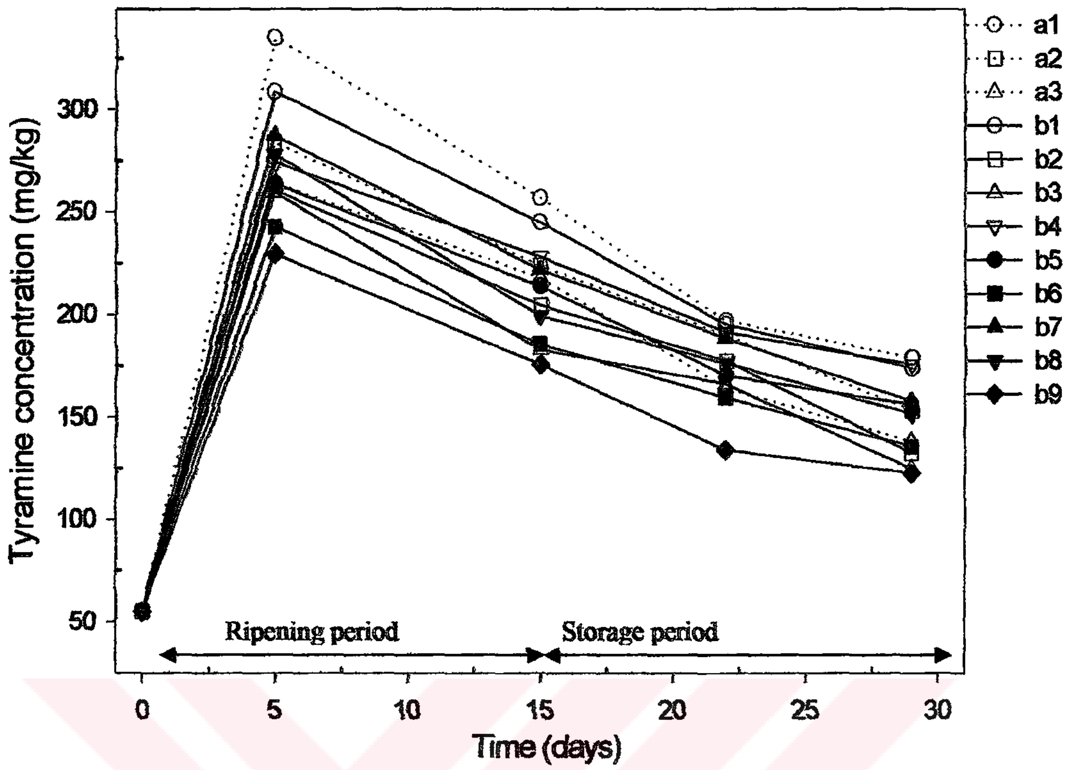


Figure 4.96 Changes of tyramine concentration of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

Statistical analyses indicated that there were additives-% RH and temperature-% RH interactions ($P<0.05$). This means that decreasing the concentration of additives and increasing the % RH increased ($P<0.05$) the tyramine formation in sucuks. Especially addition of high concentration of nitrite/nitrate with low temperatures decreased tyramine formation much more than the other cases. Also, increasing the temperature and % RH of storage increased the tyramine formation more than that increasing temperature or % RH alone. From that it could be concluded that to get low concentration of tyramine, additives should be added in high concentration and stored at low storage temperature (20°C) and % RH (50 % RH).

Positive significant correlation ($P<0.05$) was found among tyramine concentration and nitrosomyoglobin conversion, TBA values, putrescine and histamine concentration of sucuks. It means that the higher tyramine concentration in sucuks the higher the nitrosomyoglobin conversion, TBA values, putrescine and histamine concentration of sucuks. Negative correlations were observed between tyramine concentration and APC, LAB count, mold and yeast counts, pH, residual nitrite level, overall sensory quality of sucuks. Negative correlations mean lower tyramine concentration of sucuks higher APC, LAB count, mold and yeast counts, pH, residual nitrite level and overall sensory quality of sucuks.

4.2.5.2.2.1. Prediction of Tyramine Concentration during the Storage

The following empirical formula was applied to describe the tyramine concentration in sucuks (B1-B9) which were collected during the storage periods. Statistical analyses were carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is $P<0.0001$. This lowest level of significance indicates that empirical formula was well fitted to the experimental data.

$$\text{Tyramine Concentration (mg/kg)} = a + b\,(t) + c\,e^{A} + d\,T + f\,R + g\,R^2 + h\,T^2 \quad [9]$$

where a, b, c, d, f, g and h are the constants derived from non-linear regression, t is the storage time in day, e is the exponential term, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significance of the constants are given in Table 4.9. Significance of the constants indicates that presence of this constant necessary or not, if significance level is lower than $P<0.0001$ it is necessary to put this constant into equation. Residual data versus time (days) plot was drawn and it is given in Figure 4.97. It is observed that residual tyramine concentration ranged between -131.26 to 121.42 mg/kg.

This empirical formula was compared statistically and found that it can be regarded as sufficient to describe the tyramine concentration in sucuks (B1-B9) during the storage periods. It was found that tyramine concentration (mg/kg) during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 9].

Table 4.9 The constants derived from the empirical formula with their standards error and significance level

Constant	Value	P values
a	-793.9321±111.3414	<0.0001
b	-4.5738±0.2073	<0.0001
c	-1.8844±0.3224	<0.0001
d	45.6532±3.3178	<0.0001
e	14.4162±3.2296	<0.0001
F	-0.1150±0.0251	<0.0001
g	-0.7682±0.0555	<0.0001

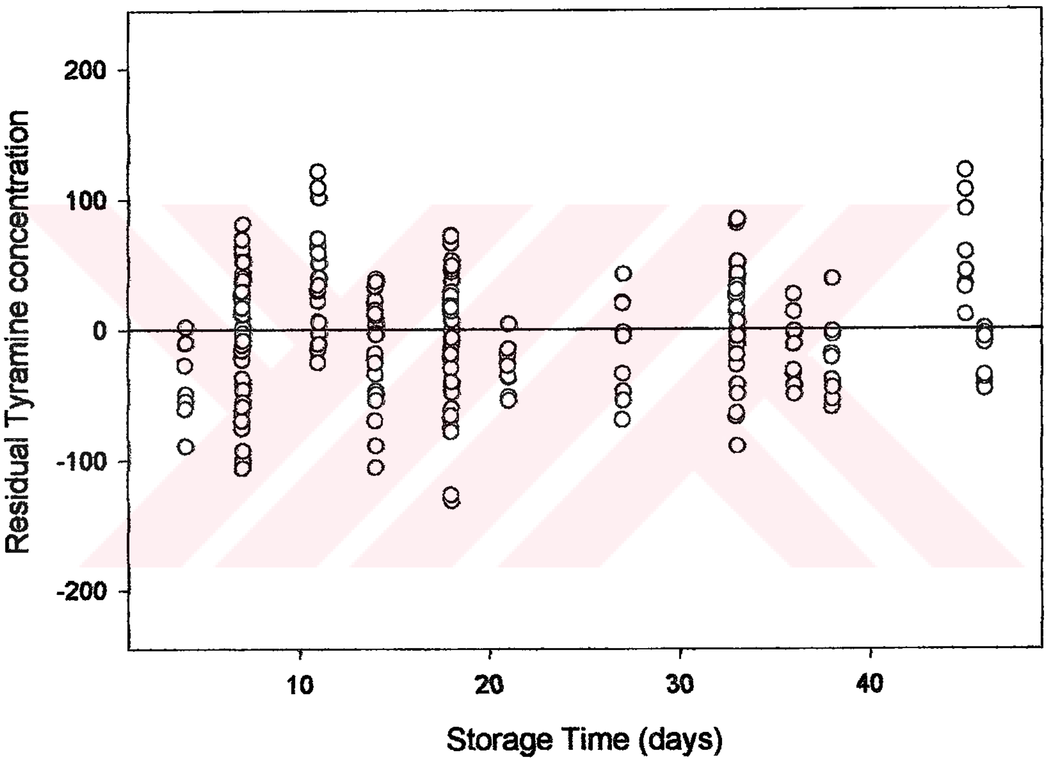


Figure 4.97 Residual plot of tyramine concentration (mg/kg) in sucuks with fitted empirical formula

4.2.5.3. Putrescine Formation

Putrescine has pharmacological effects in humans. It potentiates the toxicity of the other amines (Shalaby, 1996). Also, putrescine is important in the formation of pyrrolidine. Pyrrolidine is one of the carcinogenic nitrosamine, is formed when sausages are heated. 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.

Putrescine concentration varied between 1.08 to 396.23 mg/kg. Ayhan *et al.* (1999) detected putrescine formation increased about 412 mg/kg in sucuks stored at +4°C with 90 % RH.

4.2.5.3.1. Putrescine Formation during the Ripening Periods

Putrescine formations were followed during the ripening and storage periods in sucuk types A1-A3 and B1-B9 and the changes of putrescine in sucuks are given in Figures 4.98-4.106. Putrescine formation increased ($P<0.05$) during the first days of the ripening and after that their concentration decreased during the further ripening period as inversely related with the pH values of sucuks (Figures 4.34-4.42). The highest putrescine formation was observed in sucuk type A1 and the lowest in sucuks made with high level of additives (B9).

Sucuks (B1-B9) made with starter culture had lower ($P<0.05$) putrescine formation than sucuks (A1-A3) made without starter culture. The starter culture might produce some chemicals which inhibits or decreases the activity of putrescine forming bacteria.

During the ripening periods, it was observed that sucuks made with additives had lower ($P<0.05$) putrescine concentration. Increasing the concentration of additives decreased ($P<0.05$) the putrescine formation.

4.2.5.3.2. Putrescine Formation during the Storage Periods

The formation of putrescine in sucuks stored at 20°C with 50, 65 and 80 % RH are given in Figures 4.98, 4.99 and 4.100, respectively. Putrescine concentration decreased ($P<0.05$) during the storage periods in all sucuks as parallel to the microbial growth (Figures 4.1-4.27). The lowest ($P<0.05$) putrescine formation was observed in sucuk type B9 made with starter culture and high levels of additives. It was observed that sucuk type A1 had the highest ($P<0.05$) putrescine concentration. Its concentration in sucuks decreased ($P<0.05$) gradually during the storage periods.

The changes of putrescine in sucuks stored at 30°C with 50, 65 and 80 % RH are shown in Figures 4.101, 4.102 and 4.103, respectively. Sucuk type A1 had the highest ($P<0.05$) putrescine concentration besides and sucuk type B9 had the lowest

(P<0.05). Sucuks stored at 50 % RH had lower putrescine concentration than the others.

Figures 4.104, 4.105 and 4.106 show the changes of putrescine concentration in sucuks stored at 40°C with 50, 65 and 80 % RH, respectively. It was found that sucuks made with additives and starter culture decreased (P<0.05) the formation of putrescine in all RH. After 29 days, putrescine concentration was varied between 118.26-220.94, 41.42-173.59 and 84.15-179.46 mg/kg in sucuks stored at 50, 65 and 80 % RH, respectively.

Formations of putrescine in sucuks were affected (P<0.05) from temperature and % RH of the storage. Increasing the temperature of the storage increased the formation of putrescine significantly (P<0.05). Putrescine formation in sucuks increased (P<0.05) with increasing the % RH of the storage.

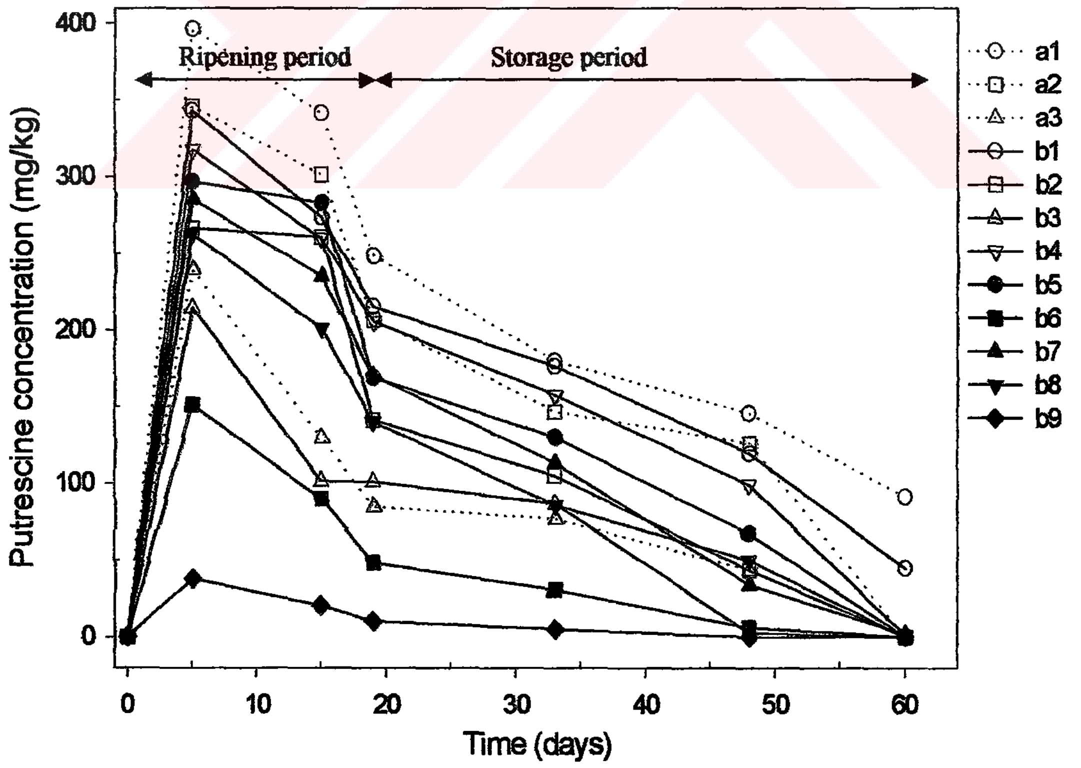


Figure 4.98 Changes of putrescine concentration of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

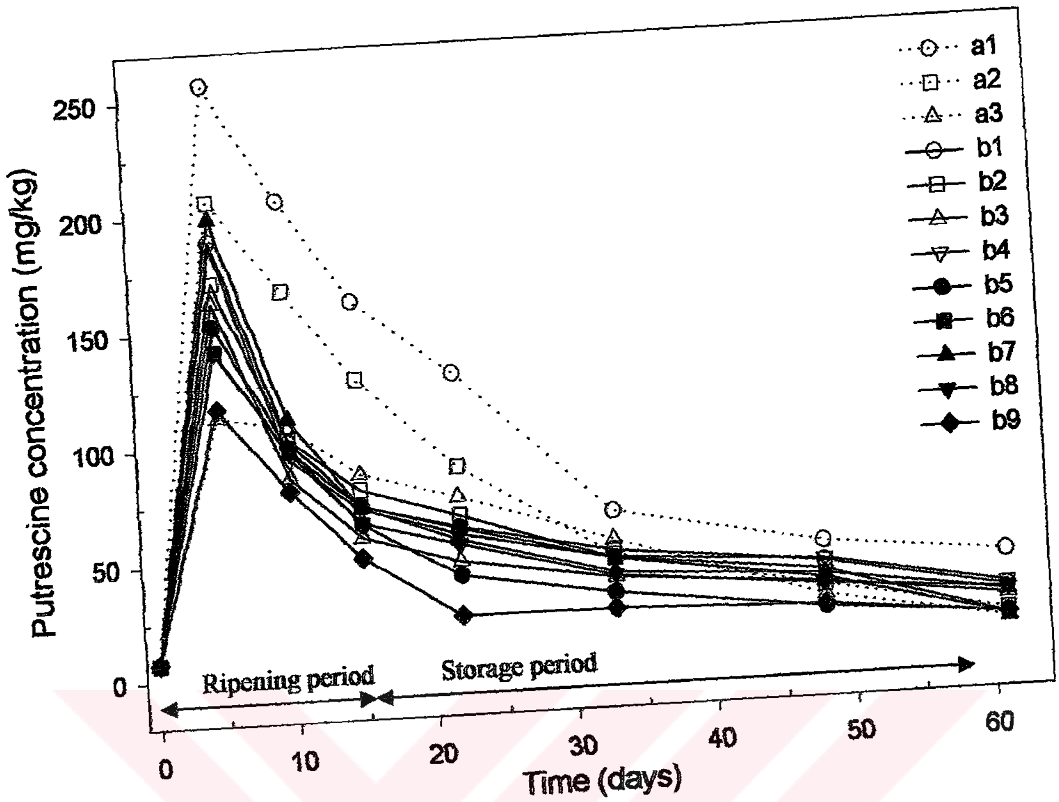


Figure 4.99 Changes of putrescine concentration of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

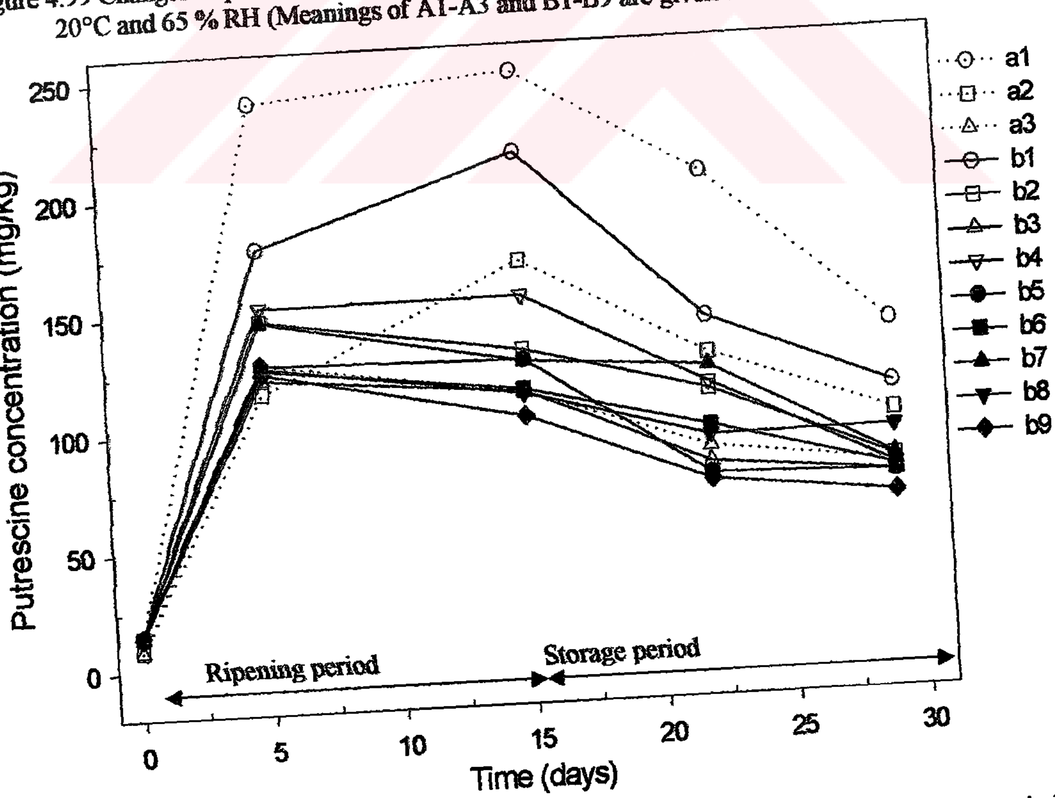


Figure 4.100 Changes of putrescine concentration of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

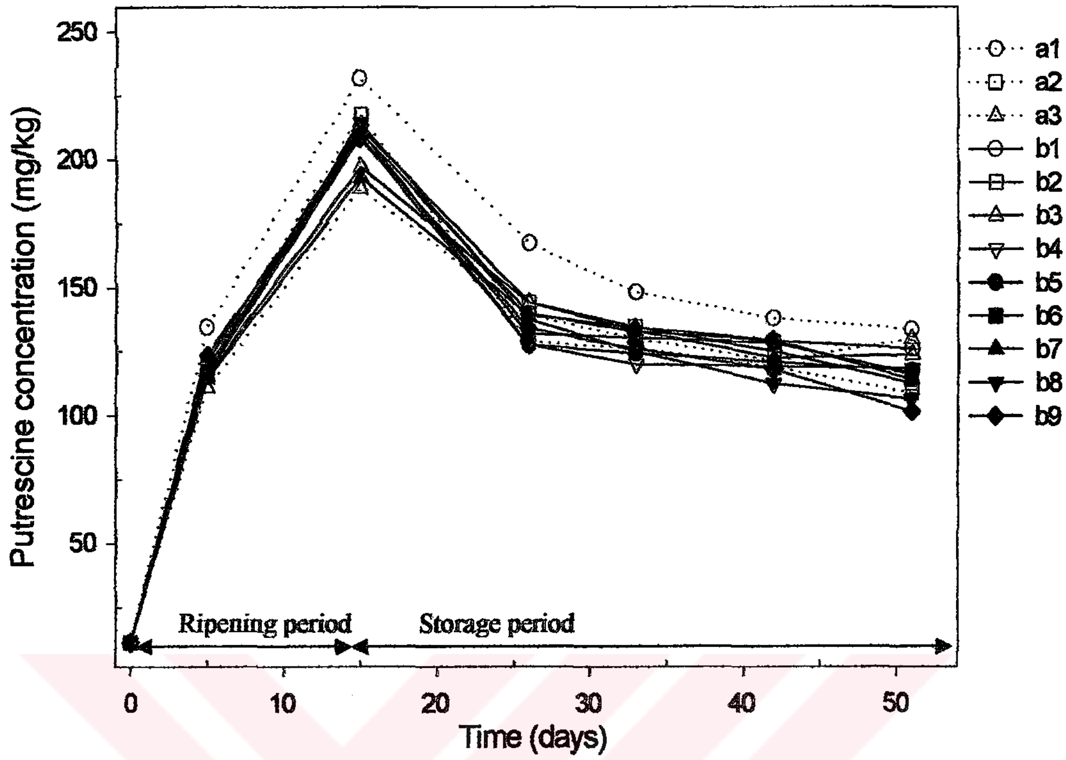


Figure 4.101 Changes of putrescine concentration of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

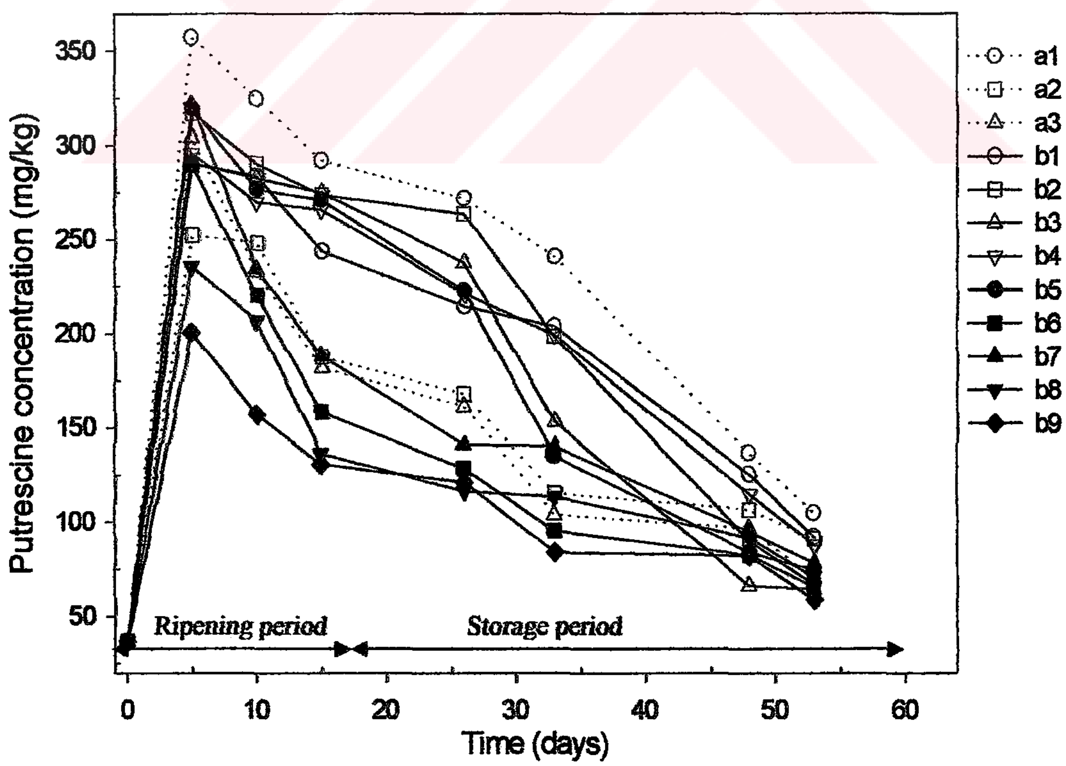


Figure 4.102 Changes of putrescine concentration of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

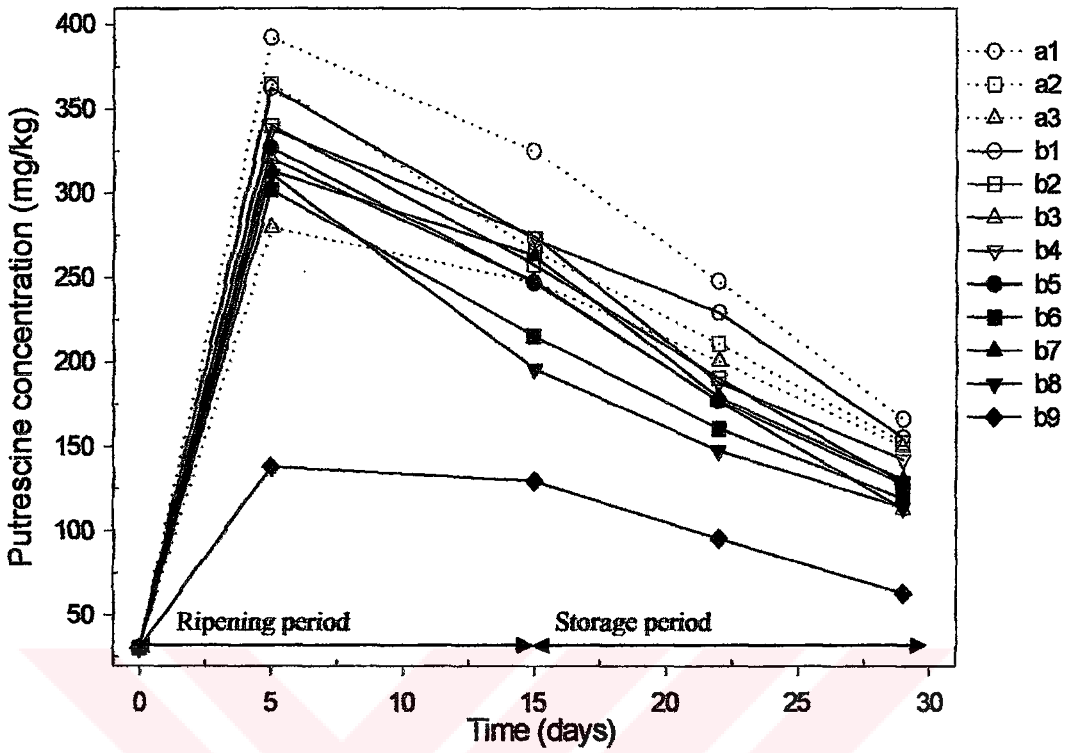


Figure 4.103 Changes of putrescine concentration of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

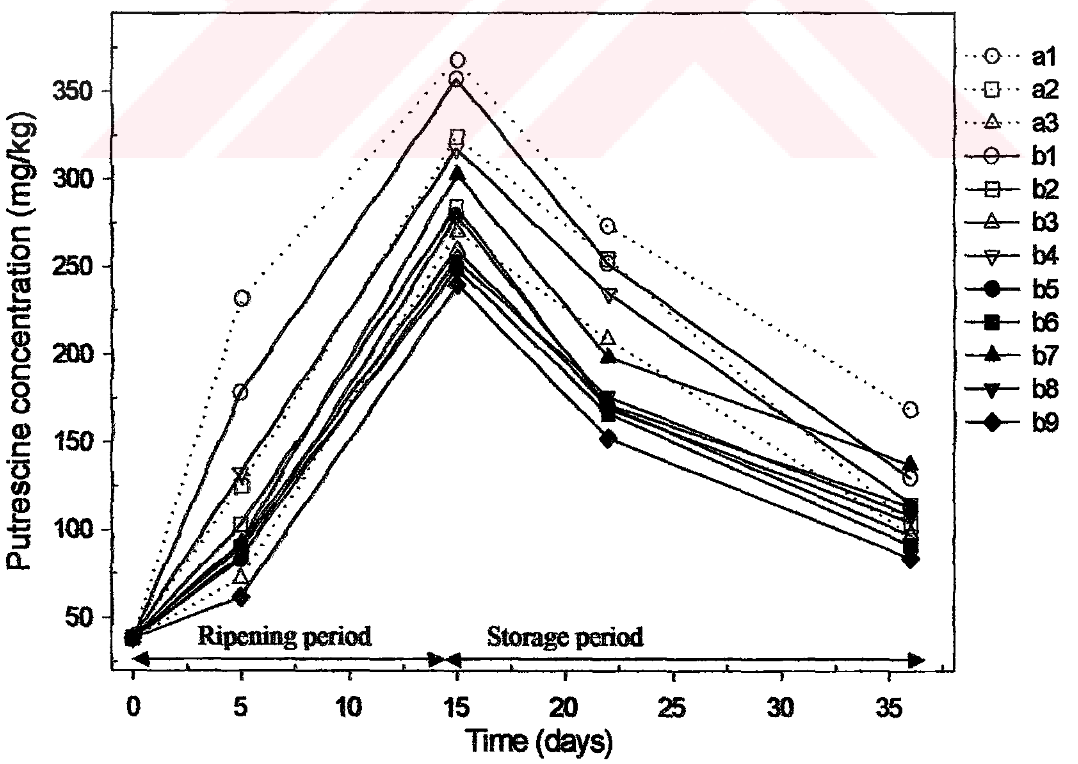


Figure 4.104 Changes of putrescine concentration of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

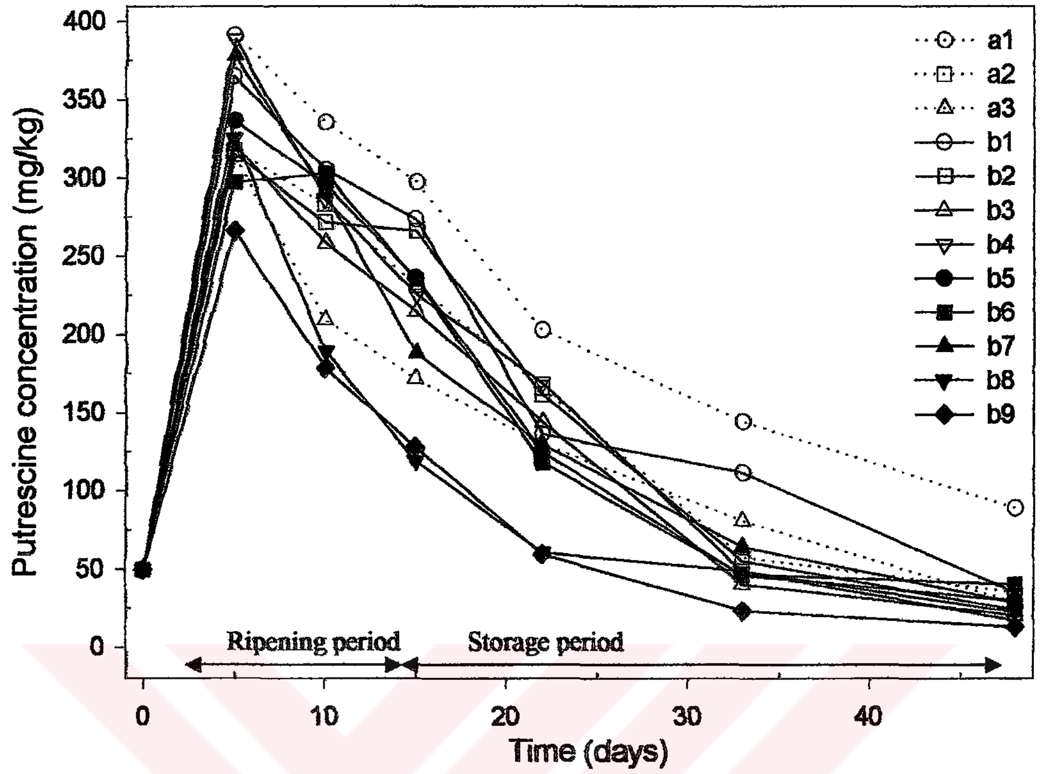


Figure 4.105 Changes of putrescine concentration of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

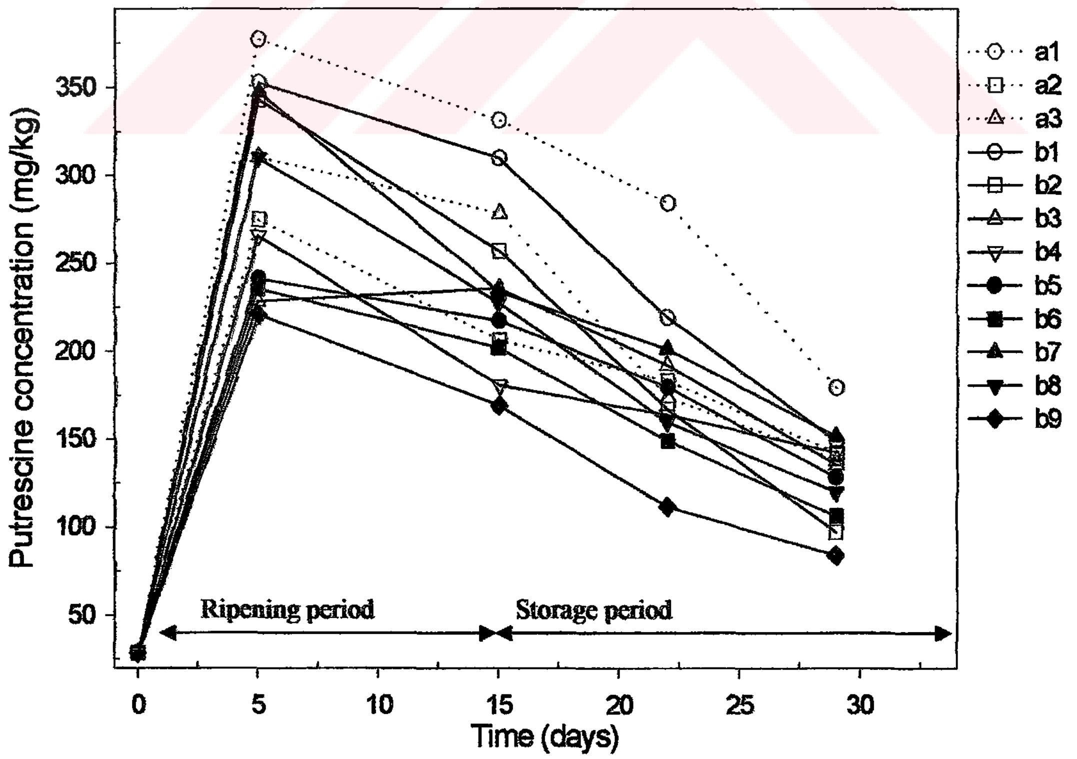


Figure 4.106 Changes of putrescine concentration of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

Putrescine levels in sucuk were also affected ($P<0.05$) from the additives and starter culture. Its formation decreased ($P<0.05$) with addition of additives. The highest putrescine concentration was found in sucuk A1. However, sucuks made with additives (nitrite, nitrate and potassium sorbate) had lower ($P<0.05$) putrescine concentration than sucuks made without additives. In the literature there was no enough information about the effect of additives on the putrescine formation, only it was proposed that potassium sorbate decreased the formation of biogenic amines (Shalaby, 1996).

Statistical analyses indicate that there was a temperature and RH interaction ($P<0.05$). Increasing both temperature and % RH of the storage increased ($P<0.05$) putrescine formation more than the increasing alone. It was concluded that decreasing of temperature and % RH of the storage was one of the important controlling (hurdle) factors for the formation of putrescine.

Positive significant correlation ($P<0.05$) was found among putrescine concentration and APC, LAB count, nitrosomyoglobin conversion, TBA values, histamine and tyramine concentration of sucuks. That is the higher putrescine concentration in sucuks the higher APC, LAB count, nitrosomyoglobin conversion, TBA values, histamine and tyramine concentration of sucuks. Negative correlations were observed between putrescine concentration and pH and residual nitrite level of sucuks. Negative correlations means the lower putrescine concentration of sucuks the higher pH and residual nitrite level of sucuks.

4.2.5.3.2.1. Prediction of Putrescine Concentration during the Storage

The following empirical formula [Eqn. 10] was applied to describe the putrescine concentration in sucuks (B1-B9) which were collected during the storage periods. Statistical analyses were carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is $P<0.0001$. This lowest level of significance indicates that empirical formula was well fitted to the experimental data.

$$\text{Putrescine Concentration (mg/kg)} = a + b\,(t) + c\,e^{A} + d\,T + f\,R + g\,R^2 + h\,T^2 \quad [10]$$

where a, b, c, d, f, g and h are the constants derived from non-linear regression, t is the storage time in day, e is the exponential term, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significance of the constants are given in Table 4.10. Significance of the constants indicate that presence of this constant necessary or not, if significance level is lower than $P<0.0001$ it is necessary to put this constant into equation. Residual data versus time (days) plot was drawn and it is given in Figure 4.107. It is observed that residual putrescine concentration ranged between -107.98 to 134.23 mg/kg.

This empirical formula was compared statistically and found that it can be regarded as sufficient to describe the putrescine concentration in sucuks (B1-B9) during the storage periods. It was found that putrescine concentration (mg/kg) during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 10].

Table 4.10 The constants derived from the empirical formula with their standards errors and significance levels

Constant	Value	P values
a	265.0604±102.4980	0.0102
b	-3.3480±0.1909	<0.0001
c	-1.8114±0.2968	<0.0001
d	28.7847±3.0543	<0.0001
f	-15.6486±2.9731	<0.0001
g	0.1152±0.0231	<0.0001
h	-0.4340±0.0511	<0.0001

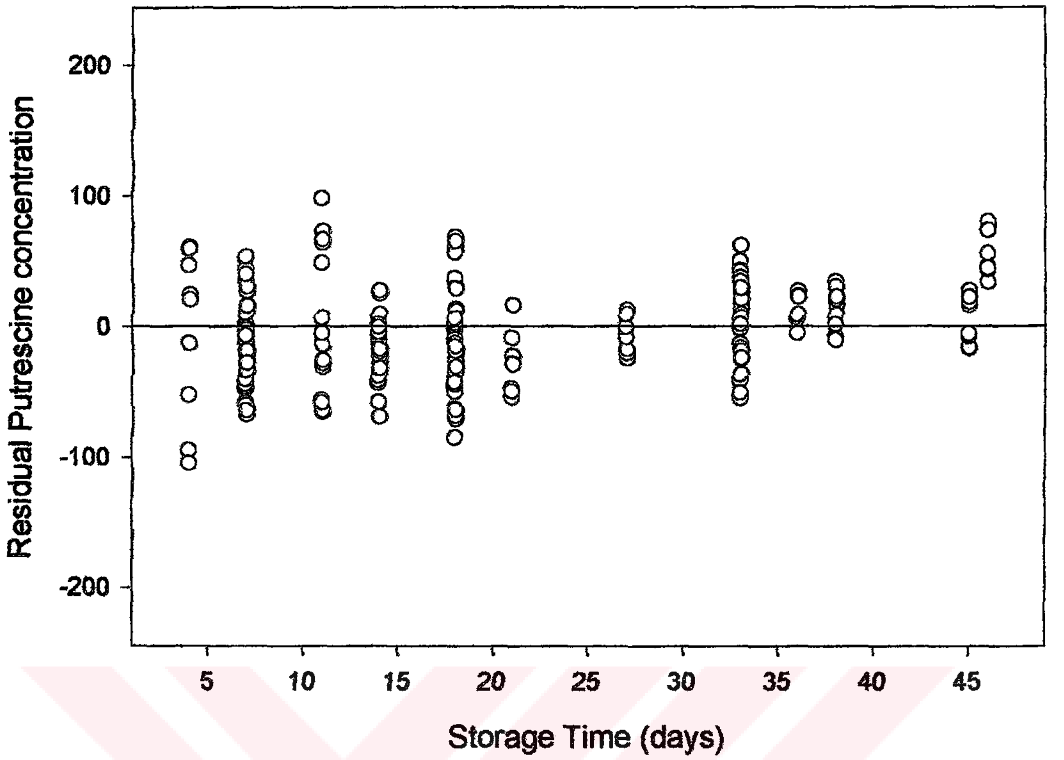


Figure 4.107 Residual plot of putrescine concentration (mg/kg) in sucuks with fitted empirical formula

4.2.5.4. Tryptamine Formation

Tryptamine has a toxic effect on the human beings as increasing the blood pressure therefore causes hypertension. There was no regulation for maximum amount for tryptamine in sucuks.

Tryptamine formation in sucuks was followed during ripening and storage periods and the results are given in Table 4.11. It was observed that tryptamine formation in sucuks fluctuated during the ripening and storage periods. Tryptamine formation ranged from 0.00 to 78.08 mg/kg during the ripening and storage periods of sucuks. It was about 0.00-40.46 mg/kg and 0.00-126.91 mg/kg in traditional and firm-made sucuks collected from Gaziantep, respectively. Şenöz *et al.* (2000) detected tryptamine in 15 of 20 sucuks retailed in local market varied between 25 to 619 mg/kg.

It was found that additives had no effect (P>0.05) on the tryptamine formation. The maximum temperature for tryptamine formation was found to be

30°C. Increasing or decreasing temperature from 30°C decreased ($P<0.05$) the tryptamine formation.

Increasing % RH of the storage from 50 % RH to either 65 or 80 % RH increased ($P<0.05$) the formation of tryptamine. Their mean concentration values were found to be about 11.52, 23.11 and 19.39 mg/kg at 50, 65 and 80 % RH, respectively.

Statistical analyses indicated that temperature-% RH interaction exist ($P<0.050$) on the formation of tryptamine. It means that increasing or decreasing temperature from 30°C and RH from 65 % decreased ($P<0.05$) the formation of tryptamine. This shows that optimum temperature and % RH of tryptamine formation could be 30°C and 65 % RH, respectively. Therefore, to control the formation of tryptamine, temperature and % RH should be adjusted to rather than 30°C and 65 % RH.

As a result, adjusting temperature and % RH of the storage could control tryptamine formation to have safe food products.

4.2.5.5. β-Phenyl Ethylamine Formation

Some pharmacological effects of β-phenyl ethylamine were reported as; to release noradrenaline from sympathetic nervous system, to increase the blood pressure and to cause migraine (Shalaby, 1996). Nout (1994) proposed that β-phenyl ethylamine concentration should be lower than 30 mg/kg for "Good Manufacturing" of sausages.

β-Phenyl ethylamine formation in sucuks was followed and its changes are given in Table 4.12. Generally, β-phenyl ethylamine was not found in sucuks except sucuks stored at 40°C with 50 and 80 % RH (Table 4.12). Its concentration varied between 0.00 to 69.37 mg/kg in sucuks.

Table 4.11 Changes of tryptamine concentration (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
20	50	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	16.51	0.00	0.00	0.00	8.76	0.00	0.00	0.00	0.00	0.00	0.00
		15	14.72	6.60	0.00	11.02	0.00	3.50	0.00	0.00	0.00	4.39	0.97	0.00
		19	36.79	0.00	0.00	27.54	0.00	0.00	0.00	0.00	0.00	10.97	2.43	0.00
		33	38.63	25.71	18.22	25.28	23.05	10.18	0.00	14.99	9.46	16.06	8.18	8.06
		48	0.00	0.00	0.00	19.73	0.00	0.00	0.00	0.00	0.00	12.32	0.00	0.00
		60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.98
	65	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	10.56	11.19	6.41	8.19	9.64	4.20	8.02	8.40	8.40	12.91	10.34	8.63
		10	21.46	19.08	19.80	19.85	22.64	11.11	19.68	22.90	15.53	17.64	21.92	19.17
		15	39.93	38.74	39.10	39.12	12.12	10.76	31.04	12.65	8.96	23.62	12.16	10.78
		22	12.38	18.17	12.20	12.16	13.83	7.91	20.33	19.53	13.25	15.74	11.45	10.44
		33	8.27	43.13	11.48	11.20	12.54	11.79	52.48	40.39	27.45	34.63	2.42	4.27
		48	1.22	1.29	0.99	1.65	1.21	0.98	0.45	1.25	1.24	2.09	0.00	0.00
		61	0.00	5.69	28.54	0.00	4.93	3.00	18.62	18.34	10.18	63.68	35.93	0.00
	80	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	18.73	26.18	19.86	29.34	28.89	24.30	22.50	26.65	25.16	24.30	24.91	21.37
		15	19.98	26.29	29.36	42.34	43.67	23.18	42.34	38.84	45.50	46.14	34.94	33.48
		22	0.64	6.15	25.06	6.83	19.74	7.74	6.89	24.67	17.84	17.39	27.96	4.55
		29	14.11	23.81	10.02	22.05	23.10	23.13	25.88	14.39	15.51	8.91	25.40	19.87
30	50	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00	0.00
		15	21.39	0.00	11.28	10.67	13.28	6.82	14.22	8.17	9.24	6.47	13.20	10.37
		26	54.16	57.50	50.67	23.16	35.63	29.83	46.48	31.80	25.25	54.77	43.12	21.87
		33	52.10	56.25	52.36	28.04	44.30	36.68	48.92	40.95	33.96	60.37	45.55	29.86
		42	50.04	55.00	54.05	32.91	52.98	43.52	51.36	50.10	42.66	65.97	47.98	37.86
		51	36.44	20.86	35.43	35.77	46.72	46.03	57.56	35.63	38.75	53.29	50.07	25.29
	65	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	25.13	26.65	15.25	19.49	22.95	10.01	17.80	20.28	19.97	30.75	24.61	20.55
		10	51.09	45.44	47.15	47.26	53.90	26.45	46.85	54.53	36.97	42.00	52.20	45.64
		15	55.77	53.75	47.44	36.26	41.55	30.76	23.52	46.55	41.02	42.03	44.04	39.45
		26	60.46	62.05	47.73	25.27	29.20	35.07	0.19	38.57	45.08	42.06	35.88	33.27
		33	52.39	56.56	62.72	26.37	25.85	30.33	16.99	31.46	29.69	44.73	24.56	40.83
		48	44.32	51.07	77.70	27.47	22.50	25.59	33.79	24.35	14.30	47.40	13.24	48.38
		53	25.15	46.83	22.10	29.12	30.38	25.22	30.89	41.33	43.16	78.08	42.33	47.66
	80	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	37.47	52.35	39.72	47.57	42.71	48.61	36.50	42.05	41.93	35.74	38.08	35.15
		15	0.00	23.12	18.25	44.97	48.76	6.38	49.80	49.12	54.59	46.90	44.27	42.78
		22	0.00	11.56	17.53	22.48	24.38	6.68	27.15	27.57	34.94	25.01	27.37	33.89
		29	0.00	0.00	16.81	0.00	0.00	6.98	4.50	6.01	15.28	3.13	10.47	25.00

Table 4.11 Continued

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
			Sucuks											
		0	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55	3.55
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	50	15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	0.00	0.00	0.00	0.00	0.00	0.00	4.29	0.00	0.00	0.00	0.00	0.00
		36	0.00	0.00	0.00	0.00	0.00	0.00	8.58	0.00	0.00	0.00	0.00	0.00
		0	25.76	27.31	15.63	19.98	23.53	10.26	17.42	11.15	19.98	31.51	25.22	21.07
		5	52.37	46.58	48.33	0.00	0.00	27.11	48.02	55.90	37.89	43.05	53.51	46.78
		10	29.18	29.29	27.16	0.00	0.00	26.55	29.01	30.95	29.95	29.52	29.75	29.39
40	65	15	43.37	49.58	38.97	33.34	38.06	25.27	37.18	47.69	37.08	40.70	36.33	32.77
		22	34.36	52.59	29.60	18.24	20.87	23.43	26.33	39.48	36.26	38.34	19.15	18.77
		33	22.77	26.18	39.34	14.56	11.86	13.28	17.12	12.80	7.77	24.74	6.62	24.19
		48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	11.11	15.08	0.00	8.49	11.26	8.39	12.87	11.74	7.58
	80	15	39.96	29.47	40.47	39.72	38.58	39.98	34.88	28.55	36.42	45.39	25.62	24.17
		22	1.28	0.00	40.47	0.00	17.35	9.05	9.28	43.33	0.00	31.66	27.93	4.33
		29	28.22	47.61	13.23	44.09	46.21	39.27	51.75	28.78	15.73	17.82	40.33	37.97

β-Phenyl ethylamine formations in sucuks were affected ($P<0.05$) from temperature and % RH of the storage. Increasing the temperature of storage increased ($P<0.05$) the formation of β-phenyl ethylamine in sucuks. The reasons of this might be due to the lower decarboxylation activity of microorganisms at low temperatures. When temperature increased from 20°C to 40°C, its formation increased ($P<0.05$). Shalaby (1996) concluded that increasing the storage temperatures increased biogenic amine formation.

Percent relative humidity affected ($P<0.05$) β-phenyl ethylamine formations in sucuks. β-Phenyl ethylamine formations in sucuks stored at 80 % RH was higher ($P<0.05$) than in sucuks stored at 50 and 65 % RH.

Statistical analyses show that there was temperature and % RH interaction ($P<0.05$). Increasing both temperature and % RH of the storage increased ($P<0.05$) β-phenyl ethylamine formations in sucuks. Therefore, these results indicate that temperature and % RH could be used as hurdle factors of β-phenyl ethylamine formations in sucuks. Low temperature and % RH of storage should be used to limit the formations of β-phenyl ethylamine.

4.2.5.6. 1,7-Diamino Heptane Formation

1,7-diamino heptane formation was monitored in sucuks during the ripening and storage periods at 20, 30 and 40°C with 50, 65 and 80 % RH. Their concentrations are given in Table 4.13. It was observed that 1,7-diamino heptane concentration was changed between 0.00 to 43.54 mg/kg in sucuks during the ripening and storage periods.

1,7-diamino heptane formation was affected from changing of temperature and % RH of the storage, but not from additives. Increasing the temperature decreased ($P<0.05$) the 1,7-diamino heptane formation in sucuks. The highest ($P<0.05$) 1,7-diamino heptane was observed in sucuks stored at 20°C. Besides that, increasing the % RH increased ($P<0.05$) the 1,7-diamino heptane formation in sucuks. The lowest ($P<0.05$) 1,7-diamino heptane formation was observed in sucuks stored at 80 % RH.

Statistical analyses showed that temperature - % RH interaction existed ($P<0.05$). The highest ($P<0.05$) 1,7-diamino heptane formation was observed in sucuks stored at 20°C with 80 % RH. The lowest ($P<0.05$) 1,7-diamino heptane was observed in sucuks stored at 40°C with 80 % RH. Therefore, to limit the formation of 1,7-diamino heptane sucuks should be stored at 40°C with 80 % RH.

4.2.5.7. Serotonin Formation

The formation of serotonin was followed during the ripening and storage periods at 20, 30 and 40°C with 50, 65 and 80 % RH and its concentration are given in Table 4.14. It was observed that there is no linear relationship between time and serotonin formation in sucuks. It means that serotonin concentration fluctuated during the ripening and storage periods. Its concentration varied between 0.00 to 57.26 mg/kg.

It was observed in statistical analysis that additives, temperature and % RH of the storage did not affect ($P<0.05$) serotonin formation in sucuk.

Table 4.12 Changes of β-phenyl ethylamine concentration (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
20	50	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.06
		15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.53
		19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	48.27
		33	0.00	0.00	0.00	0.00	0.00	0.00	5.55	0.00	0.00	0.00	0.00	0.00
		48	0.00	0.00	0.00	0.00	0.00	7.99	0.00	27.74	0.00	0.00	0.00	0.00
		60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	65	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	1.72	0.00	0.00	0.00	0.00	0.00	0.48	0.61	0.00	0.00	0.00	1.55
		33	3.44	0.00	0.00	0.00	0.00	0.00	0.97	1.22	0.00	0.00	0.00	3.10
		48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		61	0.00	3.87	2.02	6.73	18.04	12.76	2.31	0.00	11.99	0.00	0.00	0.00
	80	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.50
		29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.38
30	50	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	65	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		10	0.00	0.00	4.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		15	0.00	0.00	2.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		33	7.73	0.00	0.00	3.30	16.76	19.29	21.97	8.82	15.70	28.47	18.17	0.00
		48	5.45	0.00	0.00	4.60	13.51	8.57	23.94	17.65	3.41	6.94	6.34	0.00
		53	4.92	8.47	5.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	80	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4.12 Continued

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
			Sucuks											
		0	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
		5	0.00	0.00	0.00	0.00	0.00	0.00	4.12	0.85	0.00	2.14	1.30	0.00
	50	15	4.53	4.54	2.52	6.65	2.86	4.03	9.87	6.73	4.06	6.18	3.06	3.94
		22	12.92	4.36	2.31	14.57	3.04	4.45	7.35	5.53	2.78	11.10	8.22	3.79
		36	21.30	4.17	2.09	22.49	3.23	4.86	4.83	4.33	1.50	16.03	13.38	3.65
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40	65	15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		48	6.65	0.00	0.00	4.32	4.21	4.29	4.73	1.98	1.69	7.13	4.01	0.00
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	2.32	0.00	0.00	1.19	1.52	1.02	0.00	0.46	0.63	0.00	0.00	0.00
	80	15	46.52	28.58	36.56	15.60	21.28	23.52	15.58	9.79	19.23	16.21	15.38	17.77
		22	37.33	34.72	54.67	13.48	19.08	16.28	0.00	0.00	7.21	28.15	22.63	12.27
		29	24.54	20.76	33.32	4.83	9.37	7.46	4.29	27.37	24.10	3.66	3.67	3.98

Temperature - % RH interaction affect ($P<0.05$) the formation of serotonin in sucuks. The lowest ($P<0.05$) serotonin was observed in sucuks stored at 20°C with 65 % RH. Therefore, to limit the formation of serotonin sucuks should be stored at 20°C with 65 % RH.

4.2.5.8. Polyamines Formation

Polyamines (spermidine and spermine) occur in sucuk due to two reasons; from fresh meat quality and drying of sucuk during the manufacturing process (Martuscelli *et al.*, 2000). Polyamines have physiological functions related to cell stabilization and DNA, RNA and protein synthesis; however, they have some pathological cases (tumors) (Shalaby, 1996). Hernandez-Jover *et al.* (1997a) pointed out that those polyamines may be naturally occurring amines in meat and their formation is not indicating the loss of quality.

Polyamines; spermidine and spermine, were followed in sucuks during the ripening and storage periods at 20, 30 and 40°C with 50, 65 and 80 % RH and their results are given in Table 4.15 and 4.16, respectively. Spermidine and spermine in sucuks during the ripening and storage periods were ranging between 0.00-30.60 mg/kg and 0.00-31.29 mg/kg, respectively.

Table 4.13 Changes of 1-7- diamino heptane concentration (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks											
			A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
20	50	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	1.43	1.10	1.64	24.83	0.00	1.44	1.06	0.58	0.93	0.00	0.00
		15	5.40	2.01	1.91	2.23	11.98	2.18	2.53	3.68	2.46	2.11	0.00	3.15
		19	13.49	3.59	3.68	3.93	5.12	5.44	4.88	8.14	5.57	4.35	0.00	7.87
		33	4.06	4.94	6.68	4.61	2.90	6.10	20.37	6.87	2.27	4.36	4.64	31.29
		48	0.00	0.00	1.54	1.79	1.54	0.00	0.00	1.11	0.00	2.61	0.00	0.00
		60	0.00	0.00	0.00	0.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	65	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	7.21	7.63	4.63	34.71	5.25	4.42	19.95	14.23	33.11	24.89	20.21	18.05
		10	21.77	24.98	10.45	0.00	25.31	8.98	27.77	36.79	0.00	10.32	24.13	23.62
		15	31.89	31.49	28.22	0.00	31.66	29.49	28.88	29.39	0.00	28.16	25.06	24.81
		22	43.33	38.47	5.22	2.75	13.66	26.10	13.88	18.39	0.66	5.87	12.75	11.81
		33	4.88	1.96	0.00	5.51	0.00	3.22	0.00	0.00	1.32	1.42	1.38	0.00
		48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		61	18.83	0.00	7.04	5.01	0.00	0.00	0.00	0.00	0.00	1.91	2.40	1.48
	80	0	1.41	1.41	1.41	1.41	1.41	1.41	1.41	1.41	1.41	1.41	1.41	1.41
		5	29.86	29.67	18.41	33.03	30.21	40.49	24.24	30.73	28.64	20.92	28.92	25.27
		15	39.84	25.10	23.65	37.52	43.54	3.89	34.65	40.81	38.52	34.10	41.57	42.95
		22	19.00	7.25	11.56	7.61	6.62	2.75	21.95	21.74	16.20	19.09	16.14	0.00
		29	5.48	1.70	1.13	0.78	0.00	0.00	1.04	1.73	0.31	2.18	1.24	0.52
30	50	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	4.65	0.00	1.50	2.17	2.58	3.34	0.00	3.12	4.00	3.05	3.76	4.25
		15	3.35	8.55	1.66	3.92	4.34	3.86	4.35	6.44	5.08	1.45	0.00	4.87
		26	0.00	0.00	0.00	0.00	0.00	0.78	0.00	0.00	1.49	1.07	2.45	0.00
		33	0.00	0.00	14.92	0.00	0.00	2.67	0.00	0.52	0.75	0.54	1.22	0.00
		42	0.00	0.00	29.84	0.00	0.00	4.55	0.00	1.03	0.00	0.00	0.00	0.00
		51	0.00	0.00	0.00	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	65	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	6.01	5.36	4.41	3.06	5.72	42.30	19.00	13.55	3.53	23.70	9.25	17.19
		10	7.88	7.41	9.95	0.00	7.73	6.65	6.44	15.04	0.00	9.55	12.98	22.50
		15	8.94	9.76	7.71	1.71	13.97	0.00	15.99	17.52	1.88	8.60	11.49	14.31
		26	0.00	4.12	5.48	3.42	4.20	0.00	5.54	0.00	3.77	1.65	0.00	6.12
		33	1.72	4.10	4.69	3.55	3.72	0.00	3.91	1.45	3.74	3.26	0.00	4.46
		48	3.44	4.09	3.90	3.68	3.25	3.71	2.29	2.89	3.72	4.88	0.00	2.79
		53	3.46	0.00	2.24	2.47	3.19	3.11	0.00	0.00	0.00	3.88	3.61	3.59
	80	0	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11	3.11
		5	9.43	8.70	7.64	12.14	12.85	11.97	9.95	11.77	11.56	2.17	13.61	11.08
		15	15.00	9.07	9.73	14.04	13.07	14.61	13.59	11.38	12.15	13.11	15.02	16.68
		22	8.50	5.93	8.61	7.07	8.54	7.30	7.48	8.16	7.70	7.06	8.32	17.50
		29	16.00	6.79	4.50	3.11	0.00	0.00	2.37	6.93	1.25	0.00	3.62	5.32

Table 4.13 Continued

Temp (°C)	RH (%)	Time (days)	Sucuks											
			A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
		5	2.21	2.31	1.99	2.18	1.55	0.29	2.61	3.88	3.87	3.44	2.70	4.11
	50	15	2.77	2.09	1.87	1.52	1.08	1.60	1.20	1.96	1.61	1.42	1.71	1.08
		22	1.39	1.56	1.84	0.76	0.54	0.80	0.60	0.98	0.80	0.71	0.86	0.54
		36	0.00	1.04	1.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	6.81	5.43	5.29	9.67	6.86	5.77	22.80	16.26	37.84	28.44	23.10	20.63
		10	9.45	8.69	11.94	0.00	8.07	8.98	31.73	42.05	0.00	11.66	27.57	26.99
40	65	15	13.73	15.84	12.97	0.00	16.04	3.99	28.87	24.02	0.00	33.33	28.79	28.50
		22	6.73	5.31	9.25	2.05	5.56	7.99	19.19	21.02	2.26	8.32	13.79	17.17
		33	0.00	4.94	6.57	4.11	5.05	0.00	6.64	0.00	4.52	1.98	0.00	7.35
		48	4.12	4.91	4.68	4.42	3.89	4.46	2.74	3.47	4.47	5.86	0.00	3.35
		0	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.17	0.00	1.49	2.08	0.00
	80	15	0.37	1.33	1.86	5.06	1.07	0.94	0.00	1.85	1.94	2.28	1.27	2.10
		22	0.00	3.34	0.00	3.65	0.00	4.01	3.43	0.00	0.00	0.00	0.00	0.00
		29	5.92	0.00	0.00	0.00	0.00	0.00	1.80	0.00	0.00	1.60	1.34	1.00

Spermine and spermidine concentrations were changed (P<005) with time. However, Parente *et al.* (2001) found that spermidine and spermine concentration did not increase significantly during the ripening of sucuk. Bover-Cid *et al.* (2001) found that polyamines (spermidine and spermine) decreased significantly during the storage of ripened sucuks.

Additives did not affect (P<0.05) the formation of polyamines (spermidine and spermine). It means that addition of nitrite, nitrate, ascorbic acid, α-tocopherol, potassium pyrophosphate and di-potassium hydrogenphosphate had no affect on the formation of spermidine and spermine.

Spermidine formation was affected (P<0.05) by temperature and % RH of the storage. Decreasing the temperature and % RH of the storage increased (P<0.05) the formation of spermidine. So, to limit spermidine in food product during the manufacturing of sucuk, factors (such as temperature and % RH of the storage) should be controlled. However, spermine did not affect from temperature and % RH of the storage.

Table 4.14 Changes of serotonin concentration (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	9.55	20.54	16.60	0.00	9.12	6.07	8.31	5.07	6.65	5.35	4.76
		15	0.00	7.72	13.28	8.90	2.06	19.19	16.31	10.55	10.10	4.58	7.53	6.00
	50	19	0.00	9.74	12.66	5.64	5.15	8.86	4.70	8.07	10.18	0.80	5.48	2.25
		33	16.58	17.36	12.95	15.55	15.85	20.01	0.00	17.66	1.33	12.08	13.17	2.07
		48	4.27	0.00	1.22	1.85	1.22	1.96	1.59	1.61	1.62	2.65	4.04	0.00
		60	5.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.07	0.00	0.00	1.76
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.08	0.00
20		10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	65	15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38	1.44	6.40	0.00
		33	2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.77	2.88	12.79	0.00
		48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		61	5.94	0.00	5.13	1.37	0.00	0.00	0.00	0.00	0.00	1.03	1.87	3.22
		0	3.53	3.53	3.53	3.53	3.53	3.53	3.53	3.53	3.53	3.53	3.53	3.53
		5	2.70	1.69	1.67	2.07	2.35	1.35	2.89	1.89	2.57	2.18	2.57	1.96
	80	15	4.05	1.71	1.40	1.21	1.95	1.09	11.93	10.47	3.16	12.74	1.68	1.16
		22	5.80	1.38	0.97	0.00	2.74	1.49	7.26	0.74	9.78	0.94	1.41	2.08
		29	7.55	1.87	1.63	1.48	1.36	0.00	1.14	0.98	2.22	1.15	1.75	2.49
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	10.00	9.29	7.27	8.62	10.33	9.28	10.10	9.50	9.36	8.56	9.00	11.21
		15	14.39	14.24	5.51	12.54	12.12	10.31	13.14	13.69	13.04	10.35	12.98	10.74
	50	26	17.83	12.19	16.84	10.76	12.65	12.92	13.78	12.88	14.48	14.90	15.13	14.90
		33	15.53	12.40	18.12	11.96	13.95	12.83	13.64	12.69	15.17	13.21	12.55	13.05
		42	13.24	12.62	19.41	13.17	15.26	12.74	13.49	12.50	15.86	11.52	9.97	11.19
		51	20.58	13.29	9.91	11.56	10.85	13.70	13.67	13.49	11.52	11.93	7.95	12.64
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.98	0.00
30		10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	65	15	15.51	15.09	17.59	17.44	22.00	18.68	18.50	17.94	21.34	19.20	17.94	17.92
		26	31.02	30.18	35.19	34.88	44.00	37.36	37.00	35.87	42.68	38.40	35.88	35.84
		33	33.07	27.88	32.56	34.99	36.76	32.19	33.10	41.80	33.43	37.76	33.70	32.66
		48	35.12	25.58	29.93	35.10	29.51	27.02	29.21	47.72	24.18	37.12	31.53	29.48
		53	35.97	25.96	31.96	40.71	26.49	14.72	26.39	30.16	33.29	38.45	41.40	31.80
		0	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
		5	3.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	80	15	10.41	1.87	0.00	0.00	0.00	0.00	3.69	0.00	8.22	0.00	0.00	0.00
		22	19.22	18.19	16.17	15.78	13.55	19.39	22.86	18.07	21.96	22.07	16.64	0.00
		29	28.03	34.50	32.35	31.57	27.10	38.77	42.04	36.15	35.70	44.15	33.27	0.00

Table 4.14 Continued

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
			Sucuks											
		0	29.47	29.47	29.47	29.47	29.47	29.47	29.47	29.47	29.47	29.47	29.47	29.47
		5	13.42	14.77	11.64	14.62	13.55	13.16	15.80	14.26	21.83	11.78	19.37	17.69
	50	15	19.09	2.02	2.71	1.95	1.27	2.25	2.92	2.28	3.43	2.98	17.45	3.59
		22	11.08	2.59	1.36	1.82	1.68	1.13	2.32	2.54	3.20	1.49	8.72	2.43
		36	3.08	3.17	0.00	1.68	2.09	0.00	1.73	2.79	2.97	0.00	0.00	1.27
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.38	0.00
		10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40	65	15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	18.61	18.11	21.11	20.93	26.40	22.41	22.20	21.52	25.61	23.04	21.53	21.50
		33	37.22	36.22	42.23	41.86	52.80	44.83	44.40	43.05	51.21	46.08	43.06	43.01
		48	42.15	30.70	35.91	42.12	35.42	32.42	35.05	57.26	29.02	44.55	37.83	35.37
		0	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10	11.10
		5	7.01	6.74	6.67	8.27	9.39	5.38	7.87	7.55	10.28	8.70	10.28	7.86
	80	15	5.77	4.97	5.60	4.85	7.78	4.35	5.68	5.72	4.43	6.82	6.73	4.66
		22	4.18	3.52	3.88	0.00	3.86	2.17	2.68	2.95	3.43	3.75	3.97	3.96
		29	2.16	3.97	4.17	4.35	5.44	0.00	4.56	3.91	6.21	4.60	3.73	4.75

4.2.5.9. Biogenic Amine Content of Retailed Sucuks

Statistical analyses were carried out to determine any significant differences of the biogenic amine contents between the traditional and firm-made sucuks. ANOVA test indicates that traditional and firm-made sucuks had similar ($P>0.05$) amount of biogenic amines.

One of the most studied biogenic amine is the histamine. Histamine ranged between 0.00– 951.53 mg/kg and 0.00 – 724.24 in firm-made and traditional sucuks, respectively (Figure 4.108). Şenöz *et al.* (2000) observed that histamine level in sucuks obtained from local market was varied between 3.72 to 362 mg/kg. However, statistical differences were not found among histamine level in traditional and firm-made sucuks, firm-made sucuks had higher amount of histamine level. Mean values for histamine amount in traditional sucuks and firm-made sucuks were found about 78.04 mg/kg and 119.04 mg/kg, respectively. Nout (1994) pointed out that histamine contents should be in the range of 50-100 mg/kg in sausages processed according to "Good Manufacturing Practice". Histamine level in 16 of 65 firm-made sucuks higher level than this proposed level, besides that 4 of 25 traditional sucuks had higher than this level. Generally, histamine formation occurs during the fermentation period and could be continued during the ripening and storage periods due to the activity of bacteria.

Optimum temperature for histamine formation is around 37.8 °C (Nout, 1994) and during the manufacturing of firm-made sucuks conditions for histamine formation is more suitable than that in the manufacturing of traditional sucuks.

Table 4.15 Changes of spermidine concentration (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks											
			A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	4.58	5.40	24.82	23.61	19.51	29.98	6.10	1.41	30.60	4.20	5.34
		15	9.05	11.18	5.01	12.40	16.97	8.90	13.24	9.92	3.49	14.28	11.25	11.50
	50	19	17.62	4.36	7.12	6.18	13.32	2.75	3.13	22.21	7.32	23.09	2.93	3.40
		33	7.37	13.91	15.78	12.57	13.94	13.58	9.70	11.13	11.69	12.93	11.04	15.43
		48	4.99	3.36	2.15	8.61	2.15	12.98	2.06	3.11	4.32	1.01	7.14	1.40
		60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.37
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		10	3.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	65	15	24.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	7.94	6.16	0.00	0.00	0.00	5.82	3.45	3.81	4.70	6.67	5.53	0.00
		33	11.88	12.32	0.00	0.00	0.00	11.63	6.90	7.63	9.39	13.34	11.06	0.00
		48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		61	2.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.38	3.36
		0	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52	2.52
		5	1.34	0.83	0.00	1.08	0.00	1.53	1.25	0.90	0.79	0.83	1.23	1.15
	80	15	0.44	0.51	0.00	0.60	0.00	0.00	1.41	0.47	0.86	0.00	0.45	0.45
		22	0.06	0.00	0.00	0.43	0.04	0.30	0.23	0.00	0.00	0.00	0.00	0.57
		29	0.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	50	26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.42
		33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.71
		42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		10	3.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	65	15	1.90	0.28	0.93	0.39	0.98	0.00	0.48	0.65	4.20	0.00	0.21	0.23
		26	0.00	0.56	1.87	0.78	1.97	0.00	0.96	1.31	8.41	0.00	0.42	0.46
		33	0.00	0.28	0.93	0.39	0.98	0.00	0.48	0.65	4.20	0.00	0.21	0.23
		48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	5.04	5.04	5.04	5.04	5.04	5.04	5.04	5.04	5.04	5.04	5.04	5.04
		5	5.34	3.33	0.00	4.34	0.00	6.11	4.99	3.61	3.15	3.34	4.93	4.60
	80	15	0.00	0.00	0.00	2.38	0.00	0.00	2.98	1.87	3.44	0.00	1.81	1.79
		22	0.98	0.00	0.00	1.19	0.00	0.00	1.49	0.93	1.72	0.00	0.91	1.04
		29	1.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30

Table 4.15 Continued

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	9.89	9.89	9.89	9.89	9.89	9.89	9.89	9.89	9.89	9.89	9.89	9.89
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.89	9.98	0.00	0.00
	50	15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	0.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		36	1.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		10	4.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40	65	15	25.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		22	2.28	0.34	1.12	0.47	1.18	0.00	0.57	0.78	5.05	0.00	0.25	0.28
		33	0.00	0.68	2.24	0.93	2.36	0.00	1.15	1.57	10.09	0.00	0.50	0.56
		48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	5.06	5.06	5.06	5.06	5.06	5.06	5.06	5.06	5.06	5.06	5.06	5.06
		5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	80	15	1.76	2.03	0.00	0.00	0.00	0.00	2.67	0.00	0.00	0.00	0.00	0.00
		22	0.00	0.00	0.00	0.70	0.17	1.18	0.91	0.00	0.00	0.00	0.00	1.08
		29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Tyramine level ranged between 0.00-547.51 mg/kg and 0.00-244.41 mg/kg in firm-made and traditional sucuks, respectively (Figure 4.109). There was no statistical difference (P>0.05) between the tyramine level in traditional and firm-made sucuks. The allowable maximum level of tyramine in foods is 100-800 mg/kg and 1080 mg/kg of tyramine is toxic (Shalaby, 1996). According to these levels, tyramine levels of traditional and firm-made sucuks stated in the acceptable range. However, Şenöz *et al.* (2000) detected tyramine level in sucuk collected from local market in the range of 208.66 to 1173.28 mg/kg, some sucuks are therefore considered to be toxic. Hernandez-Jover *et al.* (1997a) found that about 13 % of Spanish sausage had higher level of tyramine than 100 mg/kg. Tyramine level in 18 of 65 firm-made and 3 of 25 traditional sucuks were higher than the level of 100 mg/kg.

Putrescine level varied in traditional and firm-made sucuks about 0.00 to 551.62 mg/kg and 0.00 to 1135.01 mg/kg, respectively (Figure 4.110). Statistical analyses indicate that putrescine level were not different in traditional and firm-made sucuks. Ayhan *et al.* (1999) detected putrescine level increased to about 412 mg/kg during the storage of sucuk at + 4 °C at 90 % RH.). Changes in putrescine concentrations during the storage of vacuum-packed meat are proposed as indicators of spoilage and concentrations of putrescine is correlate with microbial, physical and organoleptic quality attributes in pork and beef (Eerola *et al.*, 1997; Sayem-El-Daher *et al.*, 1984).

Table 4.16 Changes of spermine concentration (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks											
			A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	9.05	9.74	5.86	4.25	9.23	8.64	8.09	6.50	7.07	4.87	3.70
		15	3.58	4.30	9.10	3.16	3.26	6.08	6.77	5.28	5.41	4.03	3.12	2.34
	50	19	8.95	1.69	13.02	2.05	3.89	5.98	8.29	5.12	7.03	3.00	2.92	2.15
		33	13.83	21.27	27.66	12.89	5.61	17.96	2.67	8.34	6.51	8.38	7.55	4.34
		48	1.64	3.94	0.80	6.72	0.80	1.45	4.21	1.33	3.10	3.33	4.53	0.00
		60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	6.67	0.00	0.00	0.00	25.48	2.02	0.00	0.00	0.00	2.03	0.00
20		10	1.42	2.76	0.00	13.46	3.13	6.21	0.00	2.18	2.47	1.07	1.69	1.09
	65	15	23.71	24.38	0.00	27.73	24.57	26.11	0.00	17.09	14.24	23.54	13.85	13.55
		22	3.66	2.34	0.00	6.73	1.57	3.11	0.00	1.09	1.81	0.54	1.83	0.55
		33	5.91	1.92	0.00	0.00	0.00	0.00	0.00	0.00	1.15	0.00	1.98	0.00
		48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.35	15.50
		0	19.62	19.62	19.62	19.62	19.62	19.62	19.62	19.62	19.62	19.62	19.62	19.62
		5	2.05	2.40	2.97	2.32	1.49	3.25	2.16	2.06	1.76	1.89	1.71	1.84
	80	15	8.09	2.04	0.74	2.01	0.24	0.00	1.59	1.64	2.37	2.59	1.68	1.94
		22	4.04	2.57	0.70	1.62	0.63	1.99	1.33	1.75	2.10	2.46	1.95	1.03
		29	1.94	2.70	2.21	2.13	1.96	0.00	0.58	1.73	0.76	1.15	1.80	1.28
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	7.83	4.82	5.39	5.58	7.51	5.59	4.49	4.70	4.76	5.73	5.56	5.87
		15	4.06	4.66	0.00	2.62	5.31	2.23	3.60	5.22	5.85	2.85	4.36	6.18
	50	26	3.67	3.52	4.16	2.37	2.61	2.61	4.71	3.01	2.14	3.35	3.70	0.00
		33	1.84	3.27	2.08	2.29	3.05	3.29	3.39	3.27	3.43	2.76	3.21	1.99
		42	0.00	3.01	0.00	2.20	3.49	3.97	2.06	3.54	4.71	2.17	2.71	3.97
		51	7.51	2.90	3.05	2.55	2.55	2.70	2.89	3.25	2.15	2.36	2.81	2.38
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	6.35	0.00	0.00	0.00	24.26	1.93	0.00	0.00	0.00	1.93	0.00
30		10	1.35	2.63	0.00	12.81	2.98	5.92	0.00	2.07	2.36	1.02	1.61	1.04
	65	15	2.17	4.96	3.88	9.85	5.95	7.11	4.12	4.83	3.61	3.24	5.36	4.04
		26	2.99	7.29	7.76	6.90	8.92	8.31	8.25	7.59	4.86	5.46	9.11	7.04
		33	4.14	6.39	6.71	6.19	7.53	6.33	6.58	6.22	5.23	4.74	6.32	5.17
		48	5.28	5.50	5.67	5.48	6.13	4.35	4.91	4.86	5.60	4.02	3.52	3.31
		53	2.86	3.52	4.55	3.31	2.31	4.00	3.41	3.41	2.41	3.62	1.70	5.48
		0	4.61	4.61	4.61	4.61	4.61	4.61	4.61	4.61	4.61	4.61	4.61	4.61
		5	5.01	6.91	0.00	6.42	2.99	7.61	5.11	5.47	3.60	3.05	3.14	3.25
	80	15	31.29	6.94	7.90	5.76	0.96	0.00	6.35	4.26	6.78	8.08	4.35	5.12
		22	19.52	7.12	6.79	5.05	3.51	3.19	5.23	4.29	5.56	6.33	3.98	4.72
		29	7.75	7.31	5.67	4.34	6.06	6.39	4.10	4.32	4.33	4.58	3.61	4.33

Figure 4.16 Continued

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72	1.72
		5	5.72	6.18	8.55	11.15	9.04	7.46	5.92	12.46	2.08	2.18	6.75	7.96
	50	15	7.80	7.42	6.60	8.78	7.53	8.74	7.28	10.19	9.06	8.98	8.65	5.81
		22	6.05	4.39	5.24	5.81	4.89	4.37	4.43	6.50	5.30	5.16	4.32	3.68
		36	4.31	1.36	3.87	2.83	2.26	0.00	1.59	2.81	1.54	1.34	0.00	1.56
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	0.00	7.62	0.00	0.00	0.00	29.12	2.31	0.00	0.00	0.00	2.32	0.00
		10	1.62	3.15	0.00	15.38	3.58	7.10	0.00	2.49	2.83	1.23	1.93	1.25
40	65	15	23.81	24.58	0.00	20.69	14.79	16.55	0.00	14.24	14.41	13.61	13.97	13.62
		22	2.61	5.95	4.65	11.83	7.14	8.53	4.95	5.80	4.33	3.89	6.44	4.85
		33	3.59	8.75	9.31	8.27	10.71	9.97	9.90	9.10	5.83	6.55	10.94	8.45
		48	6.34	6.59	6.81	6.57	7.35	5.21	5.90	5.83	6.72	4.82	4.23	3.97
		0	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86
		5	3.21	2.68	3.97	2.84	2.96	5.40	3.53	2.78	3.46	4.50	3.69	4.13
	80	15	1.06	1.22	2.97	2.28	0.00	0.00	0.00	2.31	2.71	2.27	2.36	2.65
		22	3.02	3.26	2.80	1.48	2.53	1.58	1.24	2.69	4.07	3.06	3.70	1.97
		29	0.00	3.49	3.18	4.19	1.78	0.00	2.32	2.61	3.04	0.00	3.57	2.43

Generally, biogenic amine concentrations in sucuk types (A1-B9) were found to be higher ($P<0.05$) than the traditional and firm-made sucuks. Figure 4.108 represents means of histamine concentration in sucuks types (A1-B9) and retailed sucuks (traditional and firm-made). The lowest ($P<0.05$) biogenic amine concentrations were observed in traditional sucuks. This can be due to low fermentation period during the manufacturing of traditional sucuks, that were only dried under ambient conditions (temperature around 10°C and RH around 60 %). Sucuk types A1-B9 fermented at high RH about 80-90 % during the first 4 days of ripening period (Table 3.3). During this period, bacteria would be very active, so contaminated amine-positive bacteria could readily produce biogenic amines. Firm-made sucuks had lower ($P<0.05$) biogenic amine concentration than sucuk types. This can be due to after the short-ripening, sucuks could be pasteurized at 60°C for 1 hr. The application of heat could be convert the produced biogenic amine into nitrosamines which are canceriogenic substances (Özdemir *et al.*, 1984). Thus biogenic amines in the firm-made sucuks could be found lower than those in sucuk types.

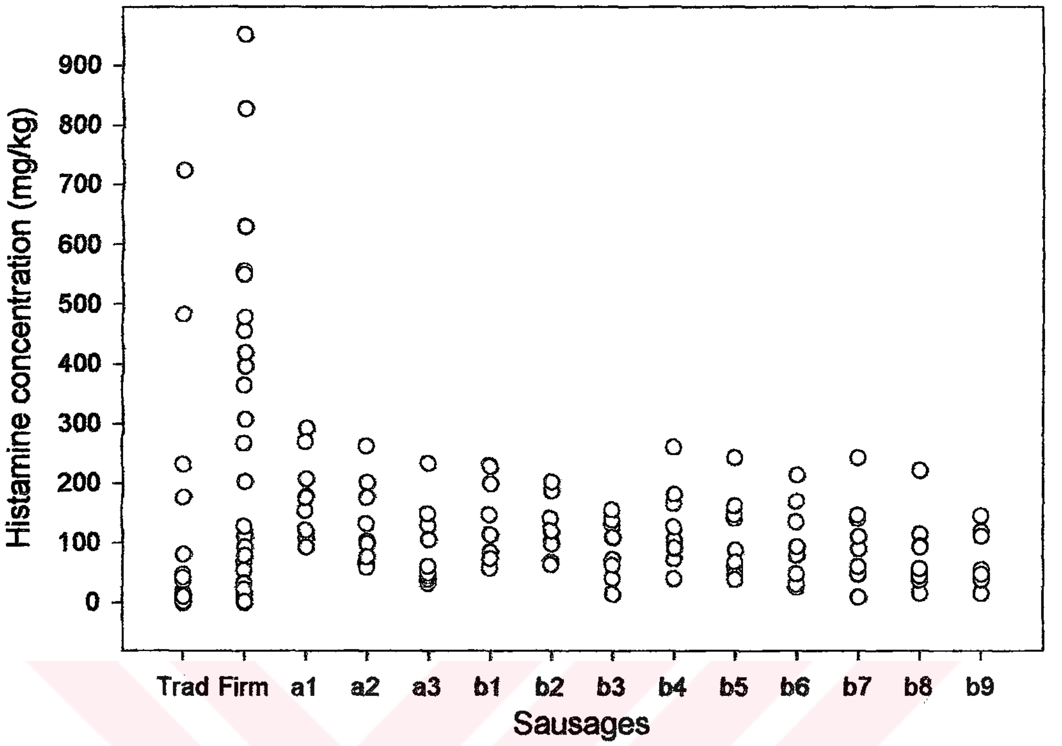


Figure 4.108 Histamine concentration (mg/kg) of sucuk types and retailed sucuks (Trad: traditional; Firm: Firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

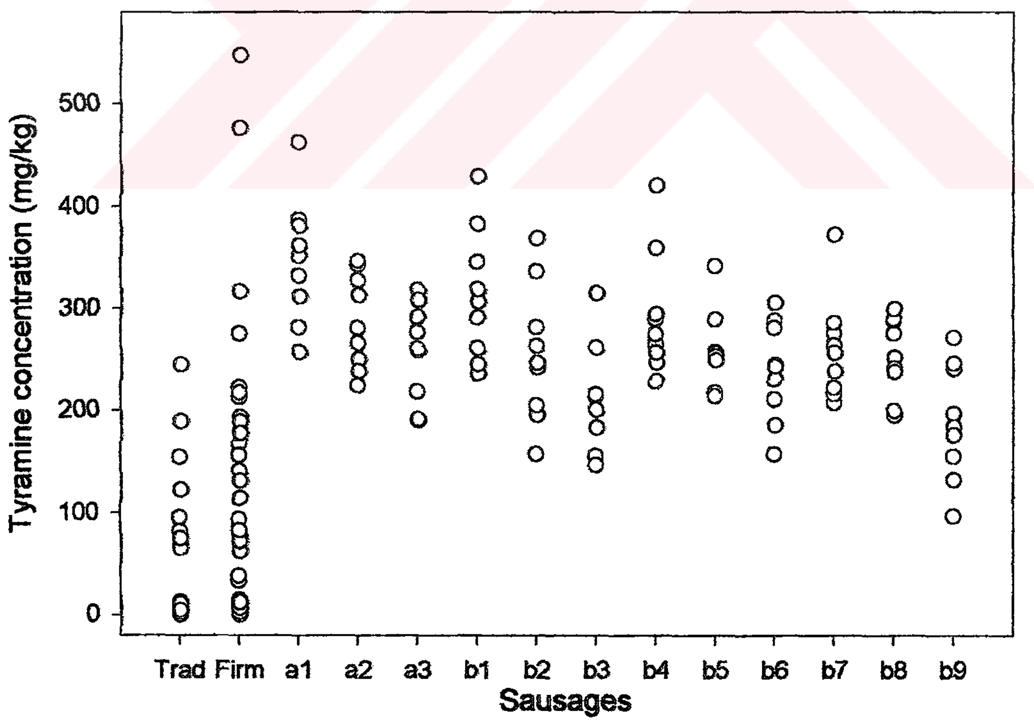


Figure 4.109 Tyramine concentration (mg/kg) of sucuk types and retailed sucuks (Trad: traditional; Firm: Firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

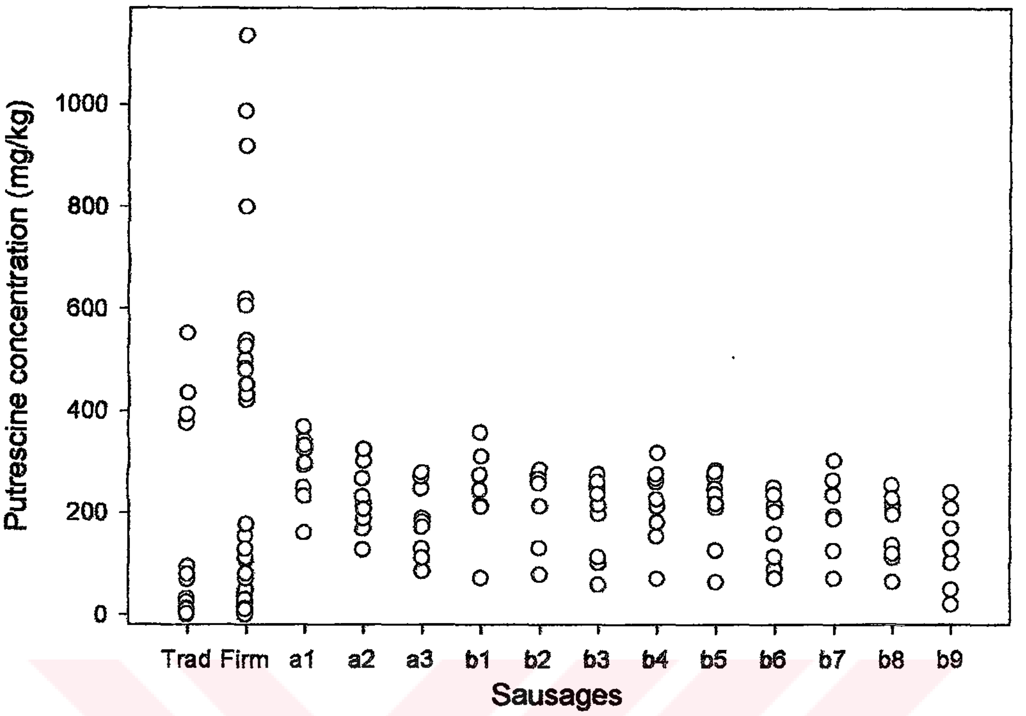


Figure 4.110 Putrescine concentration (mg/kg) of sucuk types and retailed sucuks (Trad: traditional; Firm: Firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

4.2.6. Organoleptic Properties

Organoleptic properties of sucuks were monitored by overall sensory quality (flavor, color and cutting scores) of sucuk types A1-A3 and B1-B9 during the ripening and storage periods. Overall sensory quality of sucuks was evaluated from the similar expression described by Bruna *et al.* (2000), where overall sensory quality equals to the sums of 50 % of flavor scores, 25 % color scores and 25 % cutting scores. Overall sensory qualities of traditional and firm-made sucuks were determined by flavor, color and cutting scores.

4.2.6.1. Overall Sensory Quality of Sucuks during the Ripening Periods

Overall sensory quality of sucuks depends primarily on formation of desired aroma. Formation of nitrosomyoglobin (color scores) and resistance to cut of sucuks (cutting scores) are also responsible for overall sensory quality. 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.

The changes of overall sensory quality of sucuk types A1-A3 and B1-B9 during the ripening periods are given in Figures 4.111-4.119. During the first days of ripening, as parallel to the nitrosomyoglobin conversion, color scores increased and this increased overall sensory quality. The lowest overall sensory quality was observed in sucuk type A1 in all batches. The reason of this could be due to the absence of additives. Generally, sucuk made with starter culture and additives had higher ($P<0.05$) overall sensory qualities than that of the others. Inoculation of starter culture enhanced the formation of flavor and desired color (nitrosomyoglobin conversion in sucuks made with starter culture was higher) and cutting scores (formation of structure). Vural (1998) found that Turkish semi-fermented sucuks had better overall sensory scores when they were produced with a starter culture.

During the ripening period, rancidity (lipid oxidation) started because of the high % RH and temperatures (90 % RH and 25°C; Table 3.3). Aldehydes (e.g. malonaldehyde) and ketones formed, these compounds increased flavor scores, so the overall sensory quality of sucuks.

4.2.6.2. Overall Sensory Quality of Sucuks during the Storage Periods

Overall sensory quality of sucuk types during the storage periods at 20°C with 50, 65 and 80 % RH are given in Figures 4.111, 4.112 and 4.113, respectively. Overall sensory qualities of sucuks decreased ($P<0.05$) during the storage periods, as a result of break down of flavoring compounds and nitrosomyoglobin. However, cutting scores increased due to the moisture loss, but ratio of cutting scores on the overall sensory quality (1/4) is not as high as flavor plus color scores (3/4). Therefore, overall sensory quality of sucuks decreased ($P<0.05$). The lowest overall sensory quality was observed in sucuk type A1and the highest was in sucuk type B9 at the end of the storage periods. Sucuk type B7 had good color scores but its cutting scores were lower than in sucuk B8 and B9. Nitrite, nitrate, salt and phosphates have buffering capacity. In addition to this, increasing the levels of additives increased ($P<0.05$) flavor scores of sucuks. Sucuks stored at low % RH (50 % RH), had lower ($P<0.05$) overall sensory quality than high % RH (65 and 80 % RH). The reason could be due to the higher drying of sucuks at low % RH, so undesired flavoring compounds (from browning reactions).

The changes of overall sensory qualities of sucuks at 30°C with 50, 65 and 80 % RH are shown in Figures 4.114, 4.115 and 4.116, respectively. During the storage periods, overall sensory quality of sucuks decreased ($P<0.05$), because of some chemical reactions (browning, lipid oxidation, etc), microbial and enzymatic activities (proteolysis, lipolysis; Ordonez *et al.*, 1999). Overall sensory qualities of sucuks were higher at high % RH than that at low % RH. At low % RH sucuks dried rapidly and color changes rapidly to dark brown color. Undesirable color and flavor may have been formed due to lipid oxidation. The lowest overall sensory score was obtained for sucuk type A1, the highest one for B9.

Figures 4.117, 4.118 and 4.119 show the changes of overall sensory qualities of sucuks during the storage periods at 40°C with 50, 65 and 80 % RH, respectively. Sucuks made without starter culture had lower ($P<0.05$) overall sensory qualities than sucuks made with starter culture. Overall sensory quality decreased ($P<0.05$) rapidly in sucuks stored at 50 % RH. This could be due to acceleration of chemical reaction and decreasing microbial and enzymatic activity at high temperature and low % RH. Overall sensory qualities of sucuks at high % RH was significantly higher ($P<0.05$) than low % RH. The lowest ($P<0.05$) overall sensory quality was observed in sucuk type A1.

Sucuk made with starter culture (B1) had higher ($P<0.05$) overall sensory quality than in sucuk made without starter culture (A1), in a similar way sucuk types B5 and B9 had higher overall sensory qualities than that in sucuks made with the same additives A2 and A3, respectively. Starter cultures improved aroma and textural attributes of sucuk. During storage of sucuks, some chemical reactions took place, especially, browning and lipid oxidation. Some aromatic compounds might be formed from these reactions, so these reactions enhanced the flavor formation.

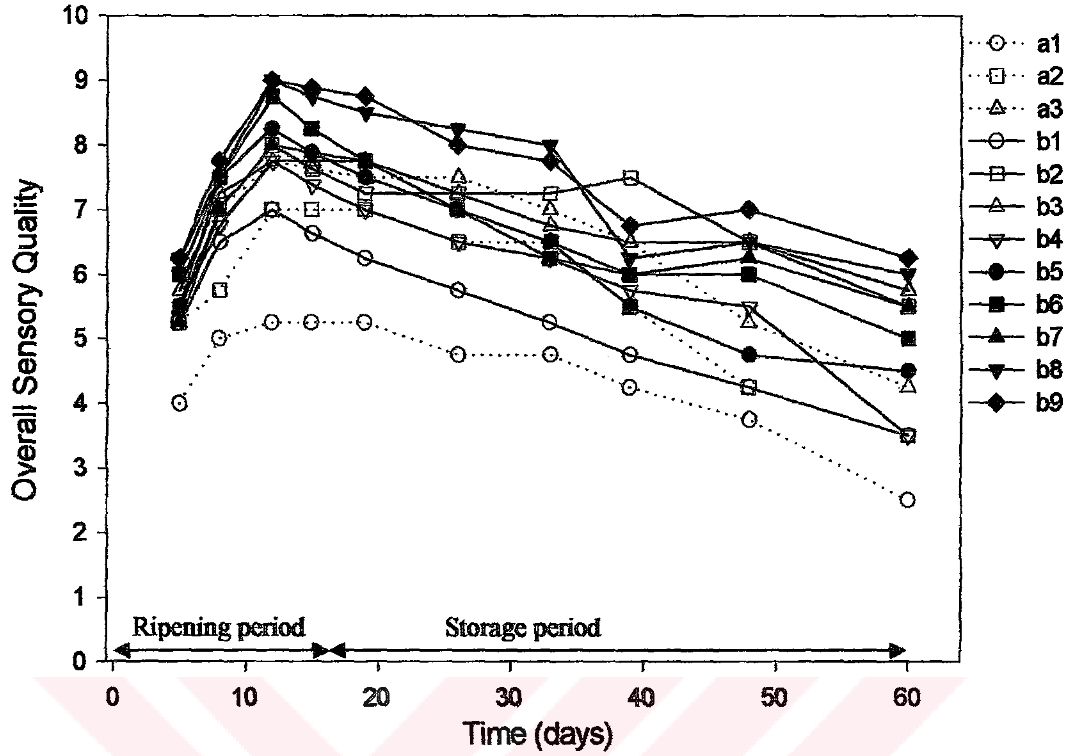


Figure 4.111 Changes of overall sensory quality of sucuks during the ripening and storage periods at 20°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

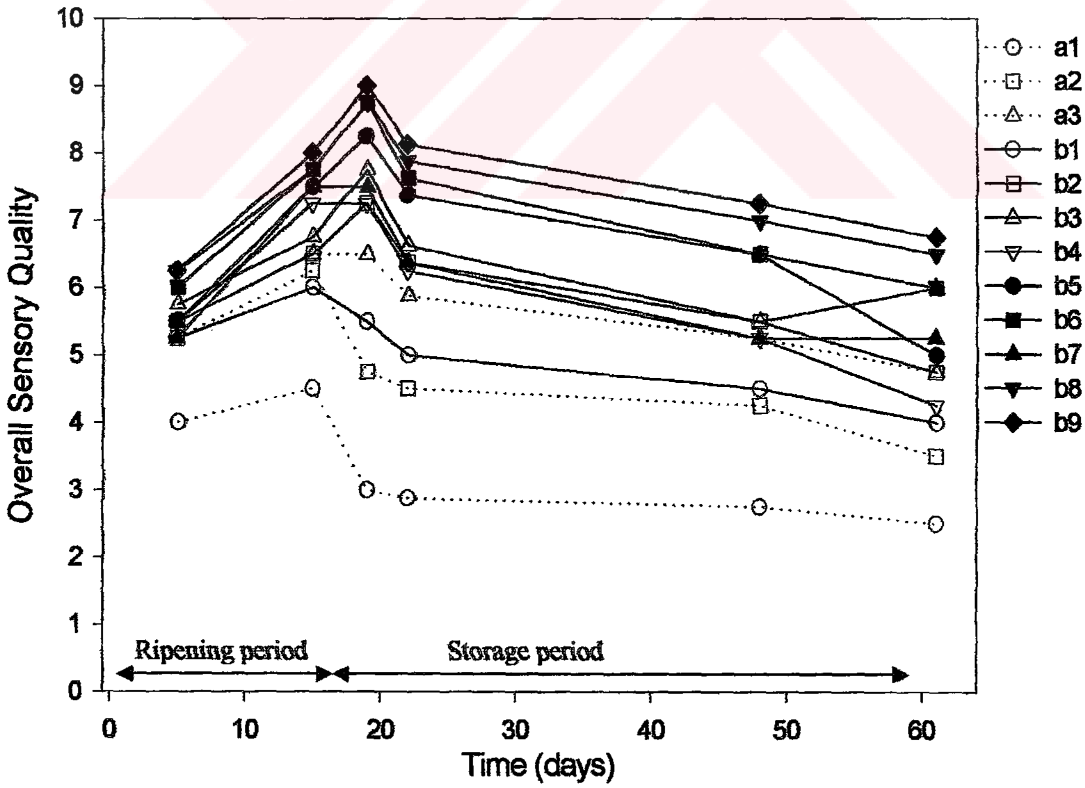


Figure 4.112 Changes of overall sensory quality of sucuks during the ripening and storage periods at 20°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

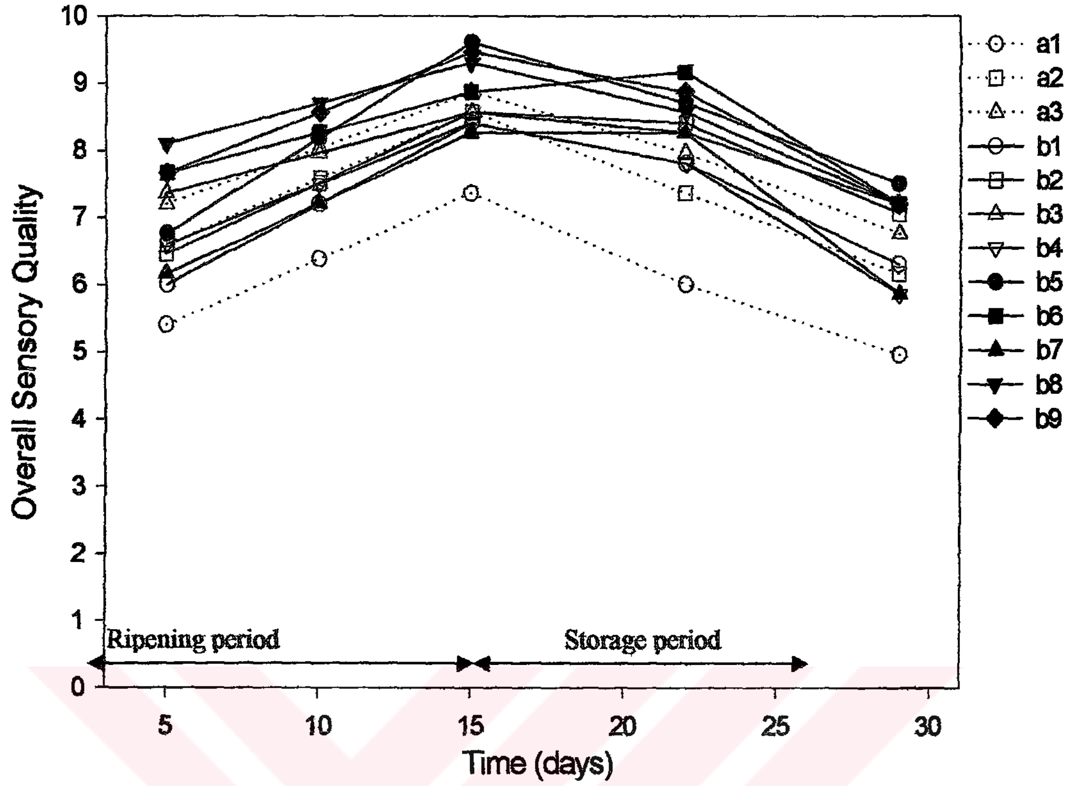


Figure 4.113 Changes of overall sensory quality of sucuks during the ripening and storage periods at 20°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

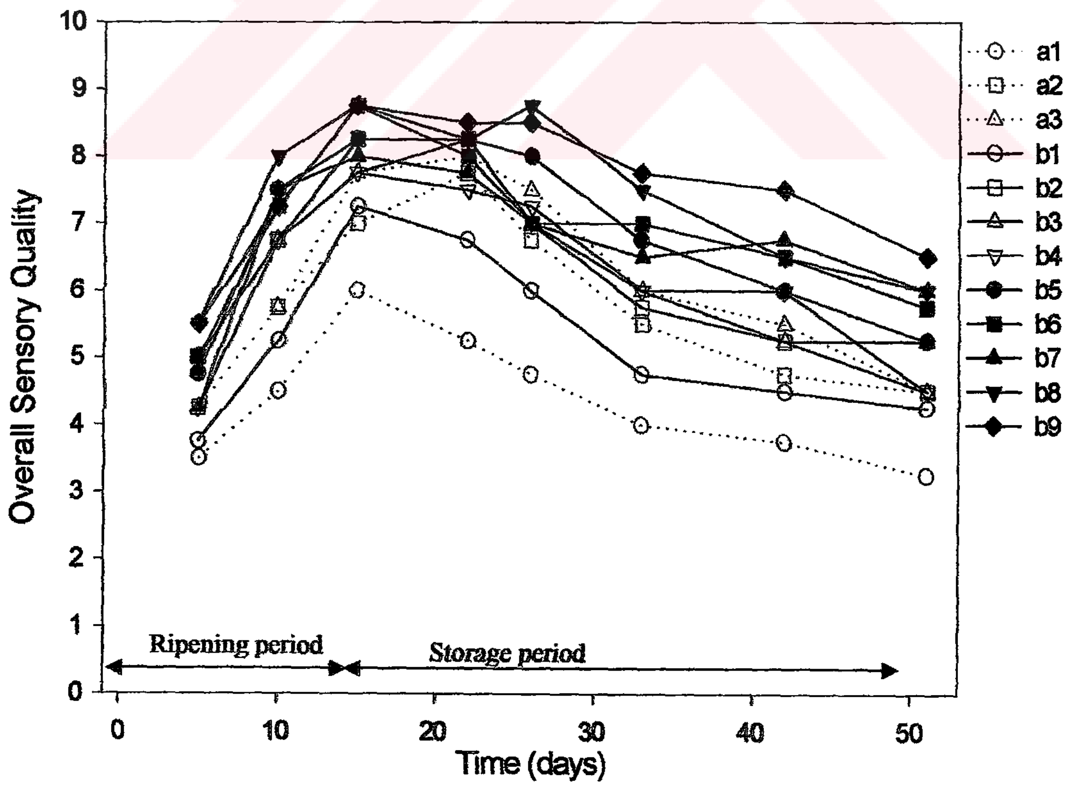


Figure 4.114 Changes of overall sensory quality of sucuks during the ripening and storage periods at 30°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

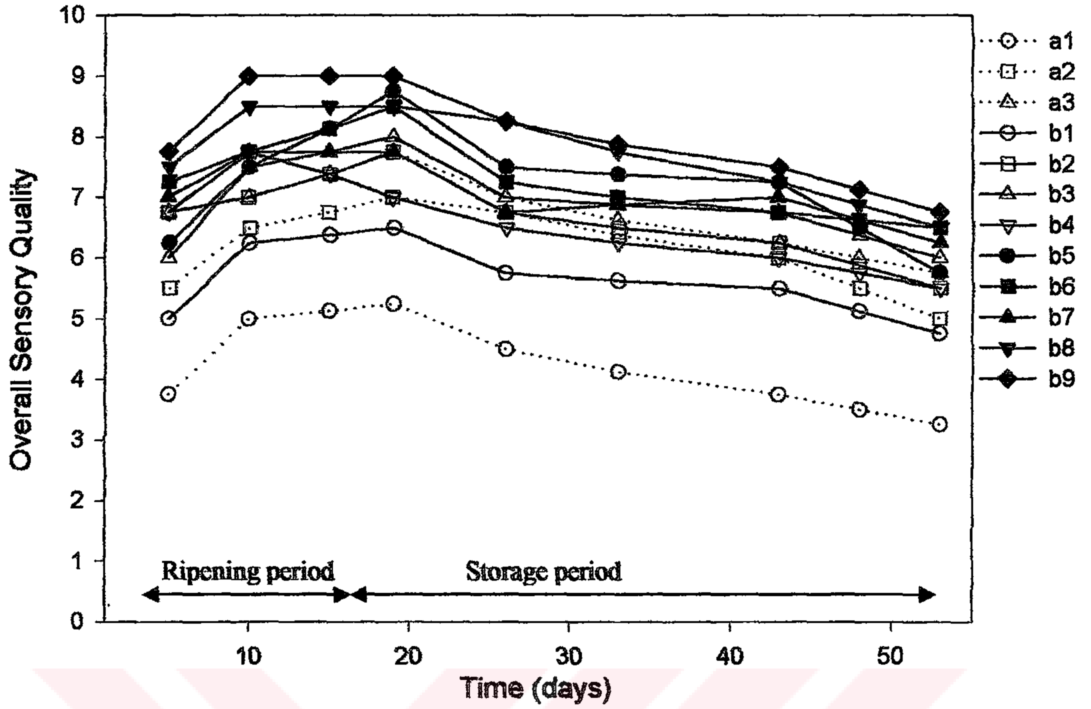


Figure 4.115 Changes of overall sensory quality of sucuks during the ripening and storage periods at 30°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

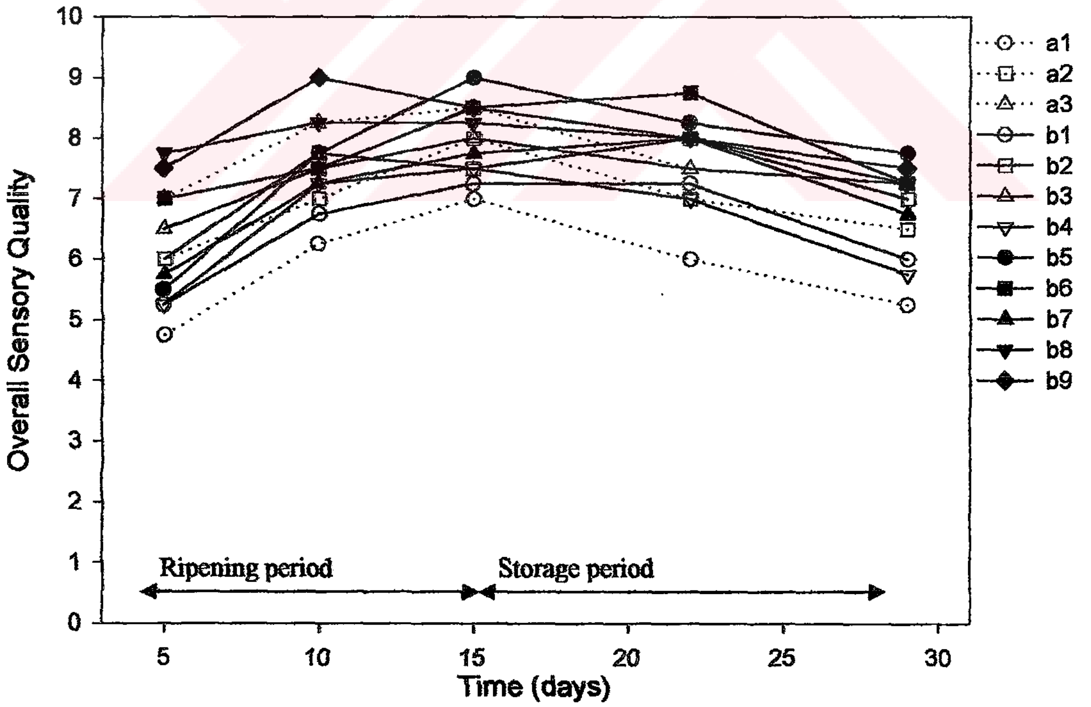


Figure 4.116 Changes of overall sensory quality of sucuks during the ripening and storage periods at 30°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

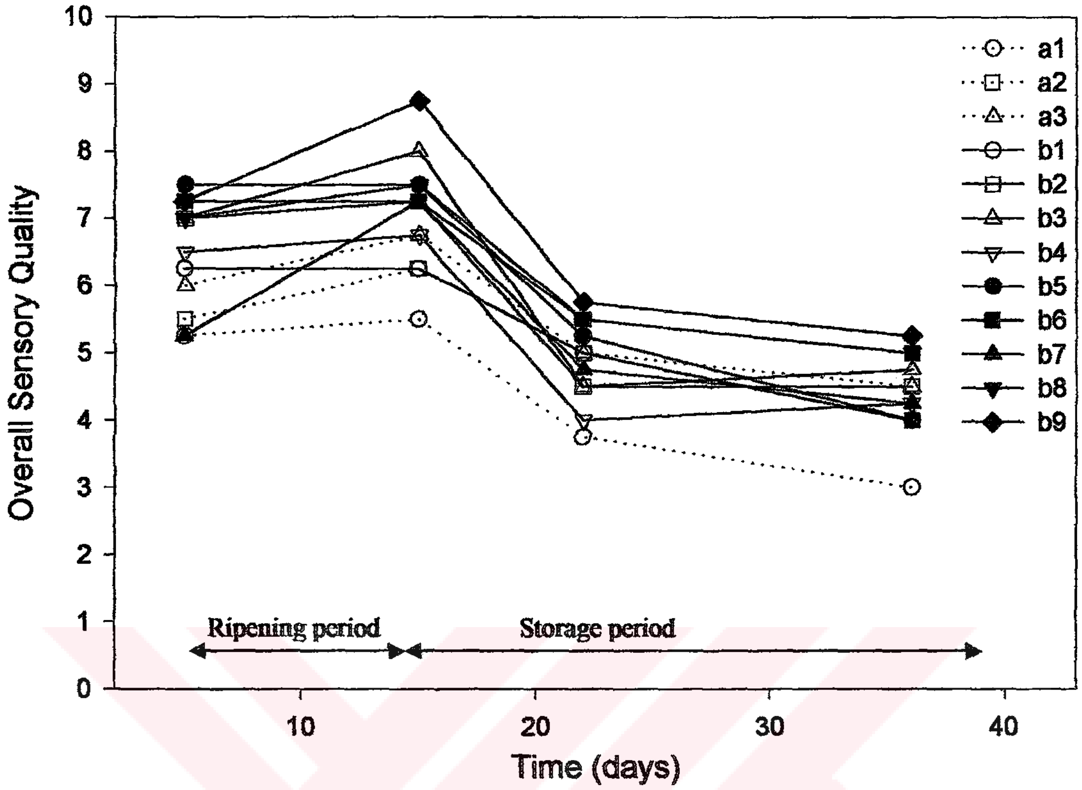


Figure 4.117 Changes of overall sensory quality of sucuks during the ripening and storage periods at 40°C and 50 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

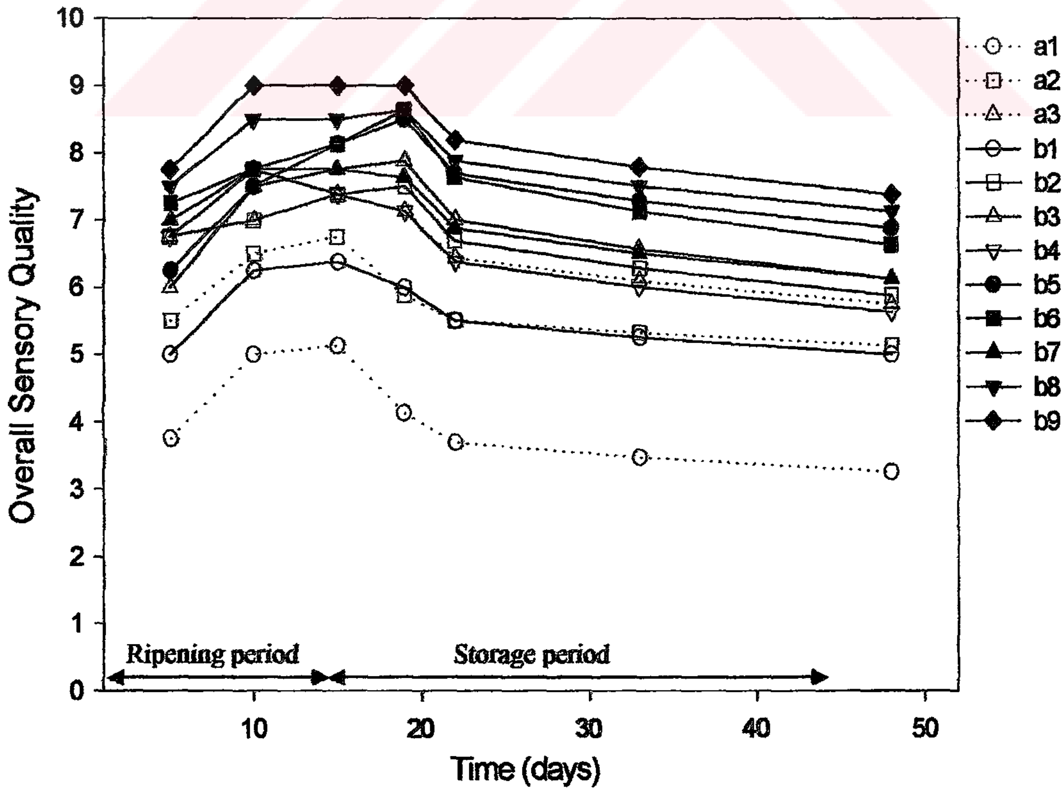


Figure 4.118 Changes of overall sensory quality of sucuks during the ripening and storage periods at 40°C and 65 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

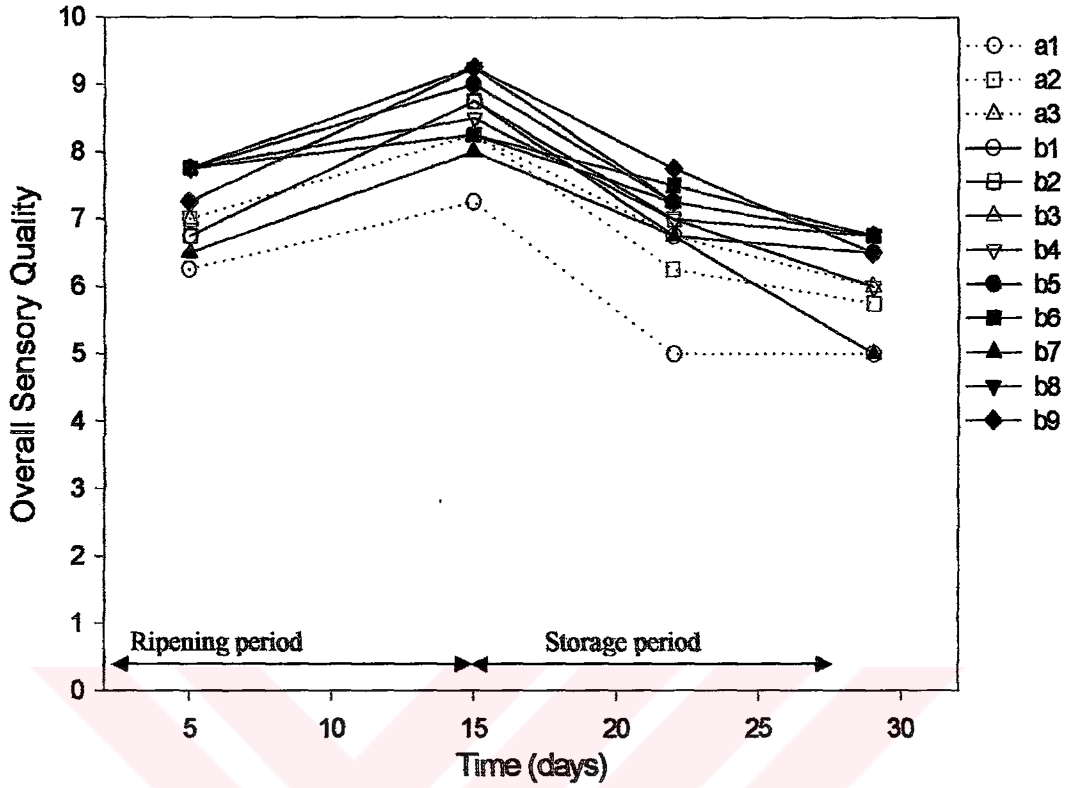


Figure 4.119 Changes of overall sensory quality of sucuks during the ripening and storage periods at 40°C and 80 % RH (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

Additives improved ($P<0.05$) the overall acceptability of sucuks. Nitrite/nitrate played important role in the formation of desired color (nitrosomyoglobin); ascorbic acid and α-tocopherol reduced the lipid oxidation; and phosphates improved textural properties of sucuks. So, sucuk made with all of these additives (B9) had the highest ($P<0.05$) overall sensory quality. It was observed that sucuks made with only nitrite/nitrate addition had lower overall sensory qualities than the other additives containing sucuks. Using of additives other than nitrite/nitrate increased ($P<0.05$) overall sensory quality (B9 > B8 > B7).

Statistical analyses indicated that increasing the storage temperature increased ($P<0.05$) overall sensory quality. Overall sensory quality increased ($P<0.05$) with increasing the % RH of the storage. The highest overall sensory quality was observed in sucuks stored at 80 % RH.

It was found that there were additives- % RH and temperature-% RH interactions. This means that effect of additives on the overall sensory qualities of sucuks increased with increasing the temperature, therefore using of additives at high temperature were more effective to improve the overall sensory qualities of sucuks than at low temperatures. In a similar way, effect of % RH on the overall sensory qualities of sucuks increased with increasing the temperature.

Positive significant correlation ($P<0.05$) was found among overall sensory quality and APC, LAB count, nitrosomyoglobin conversion and histamine concentration of sucuks. It means that the higher the overall sensory quality the higher the APC, LAB count, nitrosomyoglobin conversion and histamine concentration of sucuks. Negative correlations were observed between overall sensory quality and pH, TBA and mold and yeast counts of sucuks. Negative correlations means the lower overall sensory quality of sucuks the higher pH, TBA and mold and yeast counts of sucuks.

4.2.6.2.1. Prediction of Overall sensory quality Concentration during the Storage

The following empirical formula [Eqn. 11] was applied to describe the overall sensory quality concentration in sucuks (B1-B9) which were collected during the storage periods. Statistical analyses were carried out in the residual data (difference between experimental and predicted data) by means of one-way ANOVA and significance level (P value) of regression is $P<0.0001$. This lowest level of significance indicates that empirical formula was well fitted to the experimental data.

$$\text{Overall sensory quality} = a + b\,(t) + c\,A + d\,T + e\,R \qquad [11]$$

where a, b, c, d, e, f and g are the constants derived from non-linear regression, t is the storage time in day, A additives given in Table 3.2, T is temperature in °C and R is the percentage relative humidity of the storage.

The constants with their standard errors and significance of the constants are given in Table 4.17. Significance of the constants indicates that presence of this constant necessary or not, if significance level is lower than $P<0.0001$ it is necessary to put this constant into equation. Residual data versus time (days) plot was drawn and it is given in Figure 4.120. It is observed that residual overall sensory quality concentration ranged between -2.76 to 1.70.

This empirical formula was compared statistically and found that it can be regarded as sufficient to describe the overall sensory quality concentration in sucuks (B1-B9) during the storage periods. It was found that overall sensory quality concentration (mg/kg) during the storage periods (20-40°C and 50-80 % RH) could be predicted by using the empirical formula [Eqn. 11].

Table 4.17 The constants derived from the empirical formula with their standards error and significance level

Constant	Value	P values
A	5.9988±0.3035	<0.0001
B	-0.0580±0.0031	<0.0001
C	0.5116±0.0496	<0.0001
d	-0.0255±0.0053	<0.0001
e	0.0232±0.0037	<0.0001

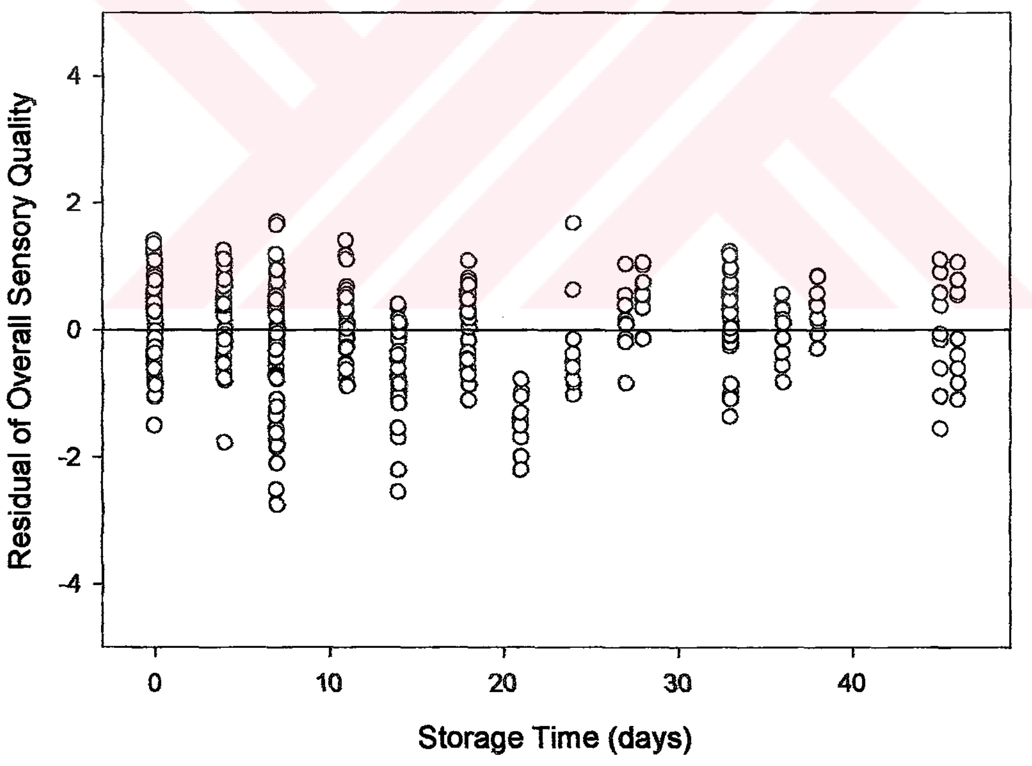


Figure 4.120 Residual plot of overall sensory quality concentration (mg/kg) in sucuks with fitted empirical formula

4.2.6.3. Organoleptic Properties of Retailed Sucuks

Overall sensory quality were followed by flavor, color and cutting scores and calculated from flavor (50%), color (25 %) and cut scores (25 %) of sucuks (Bruna *et al.*, 2000).

Traditional sucuks had spicier and a sharp which dominant aroma come from the added spices. Generally, firm-made sucuks had not a desired aroma. Firm-made sucuks had good color scores than the traditional sucuks. Generally, darken color was observed in traditional sucuks than firm-made sucuks due to unsuitable ripening and storage conditions. Cut scores were found to be higher in the traditional sucuks than that in firm-made sucuks.

Overall sensory quality of firm-made and traditional sucuks was ranged between 2.75-8.93 and 3.03-8.00, respectively. Statistical analysis indicates that there were no differences ($P>0.05$) between sensory quality of firm-made and traditional sucuks. However, traditional sucuks had higher overall sensory quality than firm-made sucuks.

Statistical analyses were carried out to determine any significant differences exist between sucuk types (A1-B9) and retailed sucuks with respect to their overall sensory quality.

It was found that sucuk types (a1-B9) had different ($P<0.05$) overall sensory quality than the retailed sucuks. Sucuk type produced with starter culture (B1-B9) had higher ($P<0.05$) overall sensory scores than traditional and firm-made sucuks (Figure 4.121). Sucuk type B9 was found to be the most acceptable had about 8.20 overall sensory scores. These results show that desired aroma, color and resistance to cut (cut scores) were obtained during the ripening period in sucuk types.

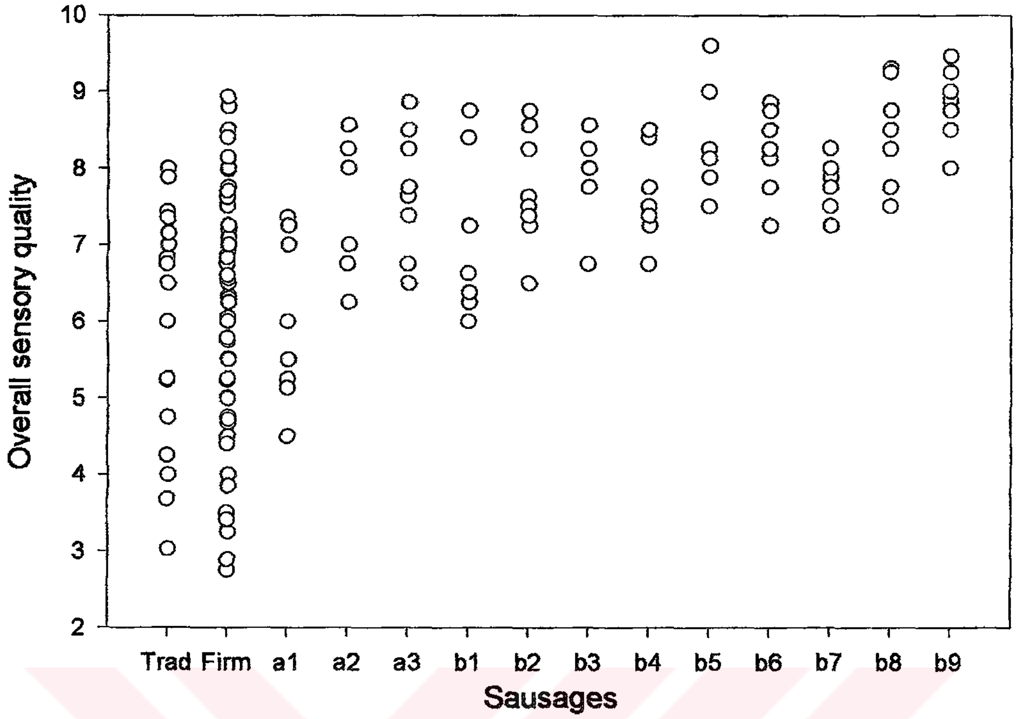


Figure 4.121 Overall sensory quality of sucuk types (A1-B9) and retailed sucuks (Trad: traditional; Firm: firm-made sucuks) (Meanings of A1-A3 and B1-B9 are given in Table 3.2; page 40)

CHAPTER IV
CONCLUSIONS

In this work, aerobic plate counts (APC), mold and yeast counts and lactic acid bacteria (LAB) counts; pH values, 2-thiobarbituric acid (TBA) values, nitrosomyoglobin conversion, residual nitrite level and biogenic amine formations; and overall sensory quality were monitored for microbial, chemical and organoleptic changes in sucuks during the ripening and storage periods. These microbial, chemical and organoleptic properties were also monitored for sucuks collected from local markets (firm-made) and butchers (Traditional).

The following results were obtained from the microbial, chemical and overall sensory quality changes of sucuks during the ripening and storage periods.

- APC and LAB increased ($P<0.05$) during the first 10 days of ripening and decreased ($P<0.05$) during the further ripening and storage periods. Starter culture had no effect ($P>0.05$) on the reduction of APC. Additives had significant reduction on the APC, LAB and mold and yeasts counts. The highest mold and yeasts counts were observed in sucuk type A1 and the lowest in sucuk type B9. Increasing the storage temperature and % RH increased ($P<0.05$) APC and LAB count. Traditional sucuks had higher APC and mold and yeasts counts than firm-made sucuks. There were no statistical differences exist between LAB count of traditional and firm-made sucuks.
- pH values of sucuks were sharply decreased ($P<0.05$) during the ripening. Addition of additives increased ($P<0.05$) pH values. Increasing or decreasing temperature from optimum temperature of lactic acid bacteria (about 30°C) decreased or increased ($P<0.05$) pH values. Sucuks stored at 80 % RH had the lower ($P<0.05$) pH values than that stored at 50, and 65 % RH. pH values of firm-made sucuks were higher ($P<0.05$) than that of traditional sucuks.
- TBA values of sucuk samples increased ($P<0.05$) up to about 2.0 mg/kg during first five days of the ripening period. During the storage periods, TBA values of sucuks continued to increase ($P<0.05$). Additives and starter culture decreased ($P<0.05$) TBA values. Increasing % RH of sucuk increased the

TBA values significantly ($P<0.05$). There were no statistical differences ($P>0.05$) exist between the TBA values of traditional and firm-made sucuks.

- Nitrosomyoglobin conversions in sucuks increased ($P<0.05$) to about 80 % during the ripening period parallel to the reduction of pH. Starter culture and additives also increased ($P<0.05$) the nitrosomyoglobin conversion. It was found that increasing the storage temperature and % RH increased ($P<0.05$) the nitrosomyoglobin conversion. Nitrosomyoglobin conversion in traditional sucuks was significantly different ($P<0.05$) than the firm-made sucuks.
- The residual nitrite level of sucuk sharply decreased ($P<0.05$) in sucuks made with nitrate/nitrite during the first five days of ripening periods. Residual nitrite was found to be low ($P<0.05$) in sucuks stored at 30°C. Firm-made sucuks had higher ($P<0.05$) residual nitrite level than that in traditional sucuks.
- Histamine concentration ranged from 0.00 to 279.32 mg/kg during the ripening and from 0.00 to 292.66 mg/kg during the storage periods. Histamine concentration in sucuk collected in Gaziantep was found to be in the range of 0.00-724.24 mg/kg and 0.00-951.53 mg/kg in traditional and firm-made sucuks, respectively. Starter culture and additives limited ($P<0.05$) the histamine formation. Histamine formation was highest ($P<0.05$) at high RH (80 %) stored sucuks.
- The lowest ($P<0.05$) tyramine concentration observed in sucuk type B9. Starter culture and additives decreased ($P<0.05$) tyramine concentration. There was no statistical difference ($P>0.05$) exist between the tyramine level in traditional and firm-made sucuks.
- Putrescine formation increased ($P<0.05$) during the first days of the ripening and after that their concentration decreased during the further ripening period as inversely related with the pH values of sucuks. Its formation decreased ($P<0.05$) with addition of additives and starter culture into sucuks. Statistical analyses indicate that putrescine level was not different in traditional and firm-made sucuks.
- It was found that additives had no effect ($P>0.05$) on the tryptamine formation. Increasing % RH of the storage from 50 % RH to either 65 or 80 % RH increased ($P<0.05$) the formation of tryptamine.

- Increasing the temperature of storage increased ($P<0.05$) the formation of β-phenyl ethylamine in sucuks.
- It was observed that 1,7-diamino heptane was changed between 0.00 to 43.54 mg/kg in sucuks during the ripening and storage periods.
- Serotonin concentration fluctuated during the ripening and storage periods. Its concentration varied between 0.00 to 57.26 mg/kg.
- Spermidine and spermine in sucuks during the ripening and storage periods were ranging between 0.00-30.60 mg/kg and 0.00-31.29 mg/kg, respectively. Additives did not affect ($P<0.05$) the formation of polyamines (spermidine and spermine).
- Overall sensory quality increased during the first days of ripening. The lowest overall sensory quality was observed in sucuk type A1 in all batches. Starter culture and additives increased ($P<0.05$) overall sensory quality. Nitrite/nitrate played important role in the formation of desired color (nitrosomyoglobin); ascorbic acid, and α-tocopherol reduced the lipid oxidation; and phosphates improved textural properties of sucuks. Traditional sucuks had spicier and the dominant aroma was come from spices. Generally, firm-made sucuks had not desired aroma.
- Generally, temperature - % RH and additives - % RH interactions were found to be significant for microbial, chemical and organoleptic changes in sucuks. These show that temperature - % RH and additives - % RH could be used as a hurdle factors in the production and storage of sucuks to get safer sucuks.
- Empirical equations were well fitted to the microbial, chemical and overall sensory quality during the storage periods of sucuks. Therefore they could be used to describe these changes in sucuks during the storage periods.

Recommendations

- Majority of microbial, chemical and organoleptic changes occurred during the ripening periods. Therefore ripening periods should be strictly controlled to produce safe and high quality sucuks.
- Starter culture should be used to decrease biogenic amine formation, residual nitrite level and to increase nitrosomyoglobin conversion, overall sensory quality.

- Nitrite/nitrate should be used to reduce TBA value, increase nitrosomyoglobin conversion, microbial activity and overall sensory quality; potassium sorbate should be used to decrease microbial activity and biogenic amine formation.
- Sucuks should be stored at low temperature (below 20°C).
- Sucuks should be stored at 65 % RH because 1) at high % RH microbial activity, TBA value, biogenic amines formation were high, 2) nitrosomyoglobin conversion and overall sensory quality were low.
- Biogenic amine concentration increased with decreasing the pH.
- Biogenic amine concentration could be used as an indicator of overall sensory quality of sucuks.
- Empirical equations could be used to predict the microbial, chemical and organoleptic changes during the storage periods.
- Temperature - % RH and additives - % RH could be used as hurdle factors.

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APPENDICES

Table A1 Changes of APC (log CFU/g) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp	RH	Time	Sucuks											
(°C)	(%)	(days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
20	50	0	5.28	5.28	5.28	5.28	5.28	5.28	5.28	5.28	5.28	5.28	5.28	5.28
		5	5.78	5.75	5.78	5.90	6.03	5.94	5.86	5.75	5.87	5.75	5.71	5.89
		8	6.02	5.90	5.82	6.48	6.58	6.10	5.86	5.98	5.97	5.87	5.99	6.16
		12	5.71	5.73	5.71	5.81	6.06	6.03	5.88	5.91	5.94	5.73	5.92	5.85
		15	5.58	5.56	5.61	5.72	6.00	5.88	5.87	5.85	5.92	5.69	5.68	5.76
		26	5.45	5.39	5.52	5.63	5.94	5.74	5.87	5.79	5.91	5.66	5.45	5.68
		33	5.42	5.32	5.45	5.28	5.32	5.63	5.32	5.39	5.45	5.45	5.37	5.45
		39	5.07	4.75	5.11	5.50	5.18	5.60	5.29	5.03	5.15	5.24	5.33	5.33
		48	4.90	4.69	4.69	5.38	5.15	5.57	5.18	4.90	4.69	5.10	5.18	4.97
		60	4.58	4.41	4.41	4.75	4.48	5.01	4.58	3.99	4.20	4.33	4.90	4.41
	65	0	5.75	5.75	5.75	6.32	6.32	6.32	6.32	6.32	6.32	6.32	6.32	6.32
		5	7.42	7.09	6.78	7.52	7.48	7.36	6.75	6.78	6.80	6.38	6.39	6.38
		10	7.18	6.52	6.32	7.12	6.98	7.02	6.65	6.70	6.40	6.35	6.34	6.29
		15	7.05	6.49	6.22	7.03	6.95	6.98	6.63	6.65	6.48	6.31	6.32	6.25
		19	6.91	6.45	6.11	6.93	6.91	6.94	6.60	6.59	6.56	6.24	6.30	6.20
		22	6.86	6.23	5.90	6.90	6.90	6.88	6.57	6.56	6.53	6.07	5.92	5.85
		26	6.81	6.01	5.69	6.87	6.89	6.81	6.54	6.52	6.50	5.89	5.54	5.50
		33	6.38	5.85	5.19	6.48	6.32	6.40	6.47	6.46	6.32	5.79	5.42	5.33
		48	6.03	5.66	5.06	6.09	6.10	6.06	6.01	5.99	6.09	5.51	5.30	5.22
		54	5.71	5.28	4.43	5.75	5.88	5.65	5.45	5.60	5.28	5.30	4.98	4.90
		61	5.40	4.98	4.22	5.47	5.53	5.46	4.98	4.95	4.94	4.83	4.60	4.71
	80	0	5.70	5.66	5.76	5.69	5.72	5.73	5.71	5.76	5.69	5.71	5.78	5.74
		5	6.23	6.15	5.96	6.49	6.43	6.30	6.31	6.32	6.34	6.41	6.36	6.36
		10	5.92	5.87	5.42	5.73	5.73	5.90	5.84	5.74	5.76	5.83	5.78	5.79
		15	5.67	5.46	5.49	5.76	5.69	5.72	5.90	5.31	5.80	5.90	5.95	5.94
		22	5.11	5.15	4.98	5.34	5.48	5.66	5.54	5.06	5.07	5.30	5.08	5.20
		29	5.06	4.56	4.61	4.69	4.75	4.90	4.79	4.78	4.55	4.41	4.79	4.69
30	50	0	5.18	5.18	5.18	5.19	5.19	5.19	5.19	5.19	5.19	5.19	5.19	5.19
		5	5.19	5.56	5.30	5.28	5.32	5.65	5.54	5.34	5.38	5.52	5.41	5.43
		10	6.22	5.99	5.98	6.24	5.96	5.90	6.07	5.90	6.17	6.09	5.96	6.08
		15	5.98	5.76	5.66	5.85	5.80	5.70	5.91	5.70	5.61	5.76	5.72	5.71
		22	4.60	4.66	4.82	5.19	5.13	5.01	5.13	5.38	4.82	4.88	4.92	4.99
		26	4.12	3.92	4.10	4.49	4.29	4.59	4.14	4.42	4.42	3.96	4.31	4.02
		33	4.05	3.59	3.98	3.99	3.72	4.10	3.88	4.20	4.14	3.92	3.68	3.87
		42	3.78	3.08	3.64	3.77	3.70	3.89	3.81	3.43	3.70	3.61	3.65	3.65
	65	0	5.54	5.54	5.54	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75
		2	6.80	5.70	6.68	6.88	6.78	7.10	6.86	6.73	5.83	7.01	6.19	6.63
		5	7.19	6.18	6.00	7.20	6.66	7.29	6.31	7.07	6.45	7.31	6.47	7.05
		10	7.54	6.00	5.60	7.13	6.96	6.75	7.14	7.07	7.03	7.20	6.75	6.53
		15	7.17	5.74	5.57	6.71	6.58	6.68	7.04	6.67	6.37	6.81	6.68	6.62
		19	7.21	5.48	5.54	6.45	6.41	6.51	7.05	6.26	5.75	6.83	6.48	6.42
		26	6.64	5.33	5.00	6.44	6.08	6.48	6.08	6.11	5.60	6.01	6.23	5.81
		33	6.10	5.28	4.95	6.02	5.99	5.84	5.84	5.96	5.30	5.46	5.92	5.36
		43	5.85	5.18	4.85	5.50	5.55	5.15	5.55	4.85	5.10	5.55	4.85	4.85
		48	5.45	4.89	4.75	5.10	5.00	4.65	5.00	4.57	4.95	5.15	4.54	4.50
		53	5.10	4.50	4.70	4.74	4.50	4.20	4.50	4.33	4.20	4.80	4.28	4.20

Table A1. Continued

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
			Sucuks											
30	80	0	6.15	6.15	6.15	6.26	6.26	6.26	6.26	6.26	6.26	6.26	6.26	6.26
		5	6.88	6.94	6.90	7.07	6.68	6.60	6.91	6.63	6.54	6.73	6.75	6.41
		10	7.52	6.92	6.90	7.47	7.03	7.38	7.41	7.23	6.90	7.45	7.37	6.78
		15	7.41	6.84	6.82	7.21	7.03	6.97	7.16	6.95	6.90	7.19	7.19	6.84
		22	7.18	6.83	6.81	7.16	6.83	6.89	7.14	6.81	6.80	7.04	7.04	6.71
		29	7.04	6.81	6.57	7.00	6.61	6.49	6.98	6.65	6.55	6.86	6.75	6.53
40	5	0	6.20	6.20	6.20	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30	6.30
		5	7.26	6.86	6.80	7.39	7.22	7.05	7.29	7.17	7.02	7.31	7.24	7.16
		15	7.16	7.04	6.91	7.12	7.19	7.07	6.97	7.03	7.00	7.11	7.03	6.81
		22	6.51	6.26	5.86	6.16	6.21	6.40	6.36	5.98	5.90	6.20	6.05	5.91
		36	5.45	5.11	4.18	4.90	4.83	5.08	5.15	4.80	4.48	5.02	4.85	4.10
	65	0	5.05	5.05	5.05	5.29	5.29	5.29	5.29	5.29	5.29	5.29	5.29	5.29
		5	6.83	6.28	6.07	6.87	6.63	6.84	6.19	6.51	6.27	6.45	6.11	6.34
		10	6.55	5.97	5.72	6.68	6.55	6.81	6.44	6.51	6.36	6.10	6.39	6.18
		15	6.42	5.85	5.67	6.66	6.43	6.43	6.44	6.17	6.22	6.15	6.22	6.11
		19	6.30	5.72	5.61	6.63	6.31	6.06	6.43	5.83	6.09	6.19	6.05	6.03
		22	6.21	5.57	5.42	6.50	6.31	6.17	6.22	5.76	6.05	6.00	5.81	5.75
		26	6.12	5.42	5.22	6.37	6.30	6.28	6.01	5.68	6.00	5.80	5.57	5.47
		33	5.88	5.35	5.03	5.95	5.93	5.83	5.76	5.45	5.82	5.56	5.25	5.19
		48	5.64	5.28	4.83	5.52	5.55	5.37	5.51	5.22	5.63	5.31	4.93	4.90
		54	5.06	4.64	4.45	4.93	4.88	4.66	4.71	4.70	4.51	4.77	4.42	4.36
	80	0	5.75	5.63	5.93	5.71	5.79	5.82	5.75	5.90	5.69	5.75	5.96	5.85
		5	7.77	7.53	7.34	7.73	7.62	7.51	7.68	7.51	7.43	7.58	7.42	7.31
		15	7.58	7.37	7.19	7.58	7.47	7.28	7.29	7.23	7.17	7.28	7.07	7.01
		22	7.00	6.89	6.40	6.88	6.80	6.70	6.90	6.55	6.68	6.98	6.76	6.21
		29	6.10	5.81	5.21	5.98	5.30	5.88	5.30	5.60	5.60	5.70	5.74	5.18

Table A2 Changes of LAB count (log CFU/g) in sucuks (A1, and B1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks A1	B1	B4	B7	B8	B9
20	50	0	4.92	4.92	4.92	4.92	4.92	4.92
		5	5.14	5.17	5.42	5.40	5.35	5.40
		8	5.42	5.58	5.48	5.46	5.46	5.43
		12	5.28	5.35	5.34	5.36	5.42	5.33
		15	5.07	5.26	5.20	5.27	5.39	5.24
		26	4.46	4.77	4.67	4.78	4.97	4.76
		33	4.32	4.61	4.47	4.63	4.44	4.14
		39	3.99	4.39	4.40	4.25	4.09	4.06
		48	3.69	4.28	4.32	3.89	3.90	3.92
	65	0	5.21	5.72	5.60	5.60	5.60	5.60
		5	5.61	6.69	6.47	6.39	5.67	5.72
		10	5.53	6.52	6.41	6.30	5.64	5.45
		15	5.38	6.46	6.38	6.01	5.60	5.32
		19	5.22	6.40	6.35	5.71	5.28	5.18
		22	5.02	6.31	6.15	5.61	5.09	4.99
		26	4.81	6.22	5.95	5.50	4.90	4.79
		33	4.55	5.84	5.46	5.15	4.59	4.50
		48	4.18	5.56	4.98	4.84	4.24	4.23
		54	3.92	5.30	4.48	4.34	4.01	4.04
		61	3.76	4.80	4.16	4.16	3.79	3.72
	80	0	4.94	4.96	4.96	4.96	4.96	4.96
		5	5.06	5.62	5.61	5.47	5.58	5.32
		10	5.25	5.85	5.68	5.62	5.60	5.48
		15	5.14	5.38	5.30	5.21	5.18	5.08
		22	4.54	5.17	4.92	4.77	4.62	4.69
		29	3.93	4.78	4.64	4.33	4.07	4.30
30	50	0	4.74	4.62	4.62	4.62	4.62	4.62
		5	4.63	4.86	4.72	4.77	4.75	4.93
		10	5.23	5.47	5.25	5.29	5.28	5.26
		15	5.12	5.22	5.12	5.14	5.02	5.12
		22	4.05	4.16	4.81	4.38	4.25	4.70
		26	3.08	3.89	2.98	2.83	3.67	3.08
		33	1.90	2.70	2.57	2.49	2.00	2.92
		42	1.72	2.00	2.00	2.13	1.79	1.79
	65	0	4.94	5.12	5.12	5.12	5.12	5.12
		2	6.08	6.39	6.28	6.16	6.13	5.96
		5	6.28	6.61	6.32	6.49	6.24	6.32
		10	5.75	6.46	6.40	6.25	6.15	6.24
		15	5.59	6.38	6.42	6.08	5.99	6.13
		19	4.90	6.31	6.43	5.91	5.74	5.93
		26	4.40	5.87	5.39	5.43	5.35	5.08
		33	3.90	5.48	5.00	4.67	4.87	4.64
		43	3.75	5.08	4.60	3.90	4.38	4.20
		48	3.55	4.47	3.99	3.87	3.88	3.85
		53	2.90	3.91	3.38	3.20	3.38	3.55

Table A2 Continued

Temp (°C)	RH (%)	Time (days)	Sucuks					
			A1	B1	B4	B7	B8	B9
30	80	0	5.54	5.58	5.58	5.58	5.58	5.58
		5	5.96	6.18	6.18	6.00	6.17	6.15
		10	5.97	6.29	6.29	6.30	6.32	6.43
		15	5.83	6.26	6.26	6.14	6.28	5.99
		22	5.80	6.20	6.20	6.22	6.17	5.85
		29	5.67	6.16	6.16	6.09	6.02	5.92
40	50	0	3.83	3.83	3.83	3.83	3.83	3.83
		5	4.75	5.32	4.86	4.84	4.94	4.60
		15	4.60	5.02	4.96	4.84	4.91	4.70
		22	4.29	4.81	4.48	4.58	4.40	4.38
		36	2.59	2.93	3.01	3.32	3.01	2.79
	65	0	4.65	4.88	4.88	4.88	4.88	4.88
		5	6.10	6.32	6.08	6.08	6.05	5.94
		10	5.37	6.07	5.90	5.72	5.53	5.90
		15	5.37	5.99	5.79	5.43	5.40	5.61
		19	5.20	5.90	5.68	5.43	5.42	5.32
		22	5.04	5.78	5.43	5.19	5.10	5.04
		26	4.77	5.66	5.18	4.95	4.88	4.76
		33	4.48	5.37	4.89	4.52	4.56	4.57
		48	4.19	5.08	4.60	4.08	4.24	4.37
		54	3.60	4.48	3.91	3.70	3.74	3.98
	80	0	5.41	5.49	5.49	5.49	5.49	5.49
		5	6.45	7.03	6.58	6.57	6.58	6.50
		15	5.92	6.69	6.35	6.50	6.27	6.13
		22	5.40	6.51	6.02	6.00	6.01	5.79
		29	5.10	5.98	5.45	5.53	5.45	5.40

Table A3 Changes of mold and yeast count (log CFU/g) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
20	50	0	3.90	3.90	3.90	3.90	3.90	3.90	3.90	3.90	3.90	3.90	3.90	3.90
		5	4.42	3.40	3.35	4.32	3.65	3.45	4.22	3.40	3.40	4.15	3.65	3.30
		8	3.60	3.18	3.00	3.55	3.34	3.15	3.52	3.25	3.10	3.30	3.00	3.00
		12	3.31	2.98	2.70	3.18	3.00	3.00	3.18	3.00	2.70	3.00	2.60	2.55
		15	3.25	2.82	2.55	3.09	2.85	2.80	3.09	2.85	2.50	2.85	2.50	2.42
		19	3.18	2.65	2.40	3.00	2.70	2.60	2.99	2.70	2.30	2.70	2.40	2.30
		26	3.00	2.40	2.00	2.70	2.55	2.11	2.66	2.48	2.00	2.40	2.00	2.00
	65	0	4.85	4.85	4.85	4.85	4.85	4.85	4.85	4.85	4.85	4.85	4.85	4.85
		5	5.68	5.26	4.77	5.52	4.94	4.50	5.31	5.00	4.47	5.24	4.84	4.38
		10	5.22	5.16	4.48	5.03	4.85	3.61	5.04	4.19	4.08	5.06	3.61	3.30
		15	4.93	4.75	4.18	4.88	4.17	3.57	4.67	3.71	3.40	4.61	3.35	2.79
		19	4.63	4.33	3.35	4.30	3.48	3.18	4.18	3.22	3.06	3.76	3.08	2.27
		22	4.14	3.78	3.25	3.98	3.24	3.02	3.46	2.80	2.70	2.90	2.30	2.20
		26	3.56	3.08	2.60	3.40	3.00	2.60	3.04	2.30	2.06	2.60	2.11	1.90
		33	3.31	2.65	2.30	2.87	2.43	2.18	2.64	2.18	1.88	2.46	1.98	1.51
	80	0	4.40	4.40	4.40	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31
		5	5.06	4.55	3.94	4.53	4.38	4.24	4.47	4.36	4.00	4.17	4.00	3.79
		10	4.82	4.30	3.84	4.25	4.18	3.99	4.43	4.24	4.09	4.04	3.92	3.65
		15	4.58	4.06	3.73	4.40	4.11	3.98	3.97	3.97	3.93	3.92	3.84	3.50
		22	4.24	4.03	3.94	4.23	4.16	4.07	4.21	4.09	3.89	4.17	4.01	3.49
		29	4.15	4.05	3.78	3.89	3.89	3.78	3.97	3.87	3.59	3.93	3.78	3.45
30	50	0	4.54	4.54	4.54	4.54	4.54	4.54	4.54	4.54	4.54	4.54	4.54	4.54
		5	5.08	4.66	4.56	5.09	4.58	4.50	4.99	4.56	4.49	4.91	4.51	4.48
		10	4.68	4.60	4.53	4.66	4.60	4.31	4.61	4.52	4.46	4.49	4.45	4.41
		15	4.51	4.44	4.34	4.48	4.44	4.06	4.49	4.31	4.24	4.40	4.34	3.99
		22	4.46	4.29	4.02	4.42	4.24	4.00	4.42	4.00	4.22	4.34	4.17	3.88
		27	4.41	4.11	3.88	4.28	4.11	3.70	4.18	3.88	3.80	4.14	4.01	3.66
		34	4.15	3.71	3.40	4.10	3.70	3.40	4.00	3.57	3.40	3.78	3.51	3.34
		42	4.00	3.51	3.00	3.65	3.48	3.30	3.60	3.41	3.21	3.54	3.24	2.70
	65	0	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29
		2	5.04	4.78	4.30	4.85	4.54	4.49	4.81	4.27	4.21	4.74	4.26	4.00
		6	4.76	4.40	4.18	4.68	4.42	4.30	4.61	4.18	4.11	4.74	4.00	3.91
		10	4.58	4.24	4.00	4.40	4.23	4.18	4.30	4.11	4.04	4.55	3.89	3.70
		15	4.39	4.18	3.95	4.33	4.00	4.00	4.28	3.87	3.84	4.10	3.74	3.55
		19	4.27	4.02	3.83	4.26	3.90	3.74	4.12	3.78	3.67	4.02	3.70	3.40
		28	4.13	3.94	3.70	4.07	3.81	3.70	3.95	3.70	3.51	3.81	3.54	3.40
		43	3.93	3.85	3.51	3.84	3.80	3.65	3.81	3.60	3.34	3.74	3.41	3.11
		53	3.88	3.60	3.37	3.72	3.65	3.40	3.70	3.51	3.00	3.70	3.30	2.70
	80	0	4.90	4.90	4.90	4.81	4.81	4.81	4.81	4.81	4.81	4.81	4.81	4.81
		1	5.57	5.11	5.04	5.44	5.13	5.00	5.39	5.22	5.16	5.35	5.18	4.48
		5	5.24	4.81	4.81	5.21	5.05	4.90	5.15	5.16	4.86	5.08	4.98	4.40
		10	5.11	4.73	4.35	5.04	4.88	4.78	4.98	4.74	4.65	4.74	4.65	4.33
		15	4.93	4.70	4.30	4.84	4.73	4.56	4.78	4.68	4.48	4.55	4.51	4.00
		22	4.81	4.56	4.18	4.69	4.65	4.40	4.76	4.65	4.30	4.48	4.41	3.76
		29	4.54	4.18	3.88	4.48	4.40	4.30	4.54	4.34	4.16	4.29	4.30	3.70

Table A3 Continued

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
			Sucuks											
40	50	0	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95
		5	4.56	4.16	3.88	4.48	3.87	4.00	4.25	4.20	3.65	4.20	3.68	3.55
		15	4.03	3.83	3.68	3.93	3.79	3.79	3.96	3.65	3.35	3.85	3.50	3.33
		22	3.33	2.98	2.85	3.24	2.93	2.80	3.23	3.00	2.77	3.00	2.83	2.59
	65	0	3.31	3.31	3.31	3.31	3.31	3.31	3.31	3.31	3.31	3.31	3.31	3.31
		5	4.31	4.07	3.85	4.25	4.02	3.65	4.15	4.25	3.62	3.76	3.73	3.68
		10	3.93	3.76	3.69	3.75	3.73	3.40	3.84	3.79	3.52	3.79	3.55	3.19
		15	3.68	3.58	3.51	3.63	3.40	3.13	3.68	3.46	3.24	3.58	3.16	3.11
		19	3.62	3.39	3.33	3.53	3.00	2.86	3.43	3.13	2.96	3.32	2.76	3.04
		26	3.32	3.23	2.82	3.19	3.03	3.02	3.03	2.92	2.80	3.05	2.77	2.57
		48	3.11	2.83	2.45	3.01	2.73	2.66	2.92	2.79	2.46	2.86	2.72	2.30
		54	2.96	2.61	2.22	2.80	2.68	2.52	2.73	2.75	2.34	2.53	2.49	2.03
	80	0	3.90	3.90	3.90	3.81	3.81	3.81	3.81	3.81	3.81	3.81	3.81	3.81
		5	4.93	4.40	4.09	4.80	4.40	3.98	4.55	4.41	3.97	4.54	4.37	3.97
		15	4.61	4.17	3.88	4.52	4.00	3.84	4.31	4.20	3.81	4.27	3.88	3.76
		22	4.27	4.00	3.70	4.21	3.90	3.60	4.27	4.18	3.51	4.18	3.70	3.41
		29	4.22	3.84	3.64	4.13	3.82	3.54	4.01	3.74	3.24	3.84	3.65	3.18

Table A4 Changes of pH in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp	RH	Time	Sucuks											
(°C)	(%)	(days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
20	50	0	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78
		5	4.62	4.82	4.92	4.52	4.70	4.78	4.56	4.64	4.75	4.60	4.72	4.92
		8	4.53	4.74	4.96	4.51	4.62	4.90	4.62	4.73	4.79	4.62	4.78	4.86
		12	4.70	5.10	5.02	4.63	4.72	4.73	4.68	4.78	4.86	4.68	4.84	4.88
		15	4.83	5.12	5.05	4.71	4.66	4.79	4.71	4.85	4.84	4.76	4.79	4.92
		19	4.96	5.13	5.07	4.78	4.60	4.84	4.73	4.92	4.82	4.83	4.73	4.96
		26	4.96	5.06	4.96	4.79	4.89	5.06	4.87	4.97	4.86	4.88	4.88	4.93
		33	4.76	5.18	5.20	4.80	4.88	5.05	4.96	5.02	5.06	4.90	4.90	4.95
		39	4.93	5.33	5.45	5.10	5.14	5.18	5.10	5.09	5.28	5.01	4.97	5.24
		48	4.95	5.16	5.42	5.16	5.11	5.20	5.15	5.15	5.37	5.12	5.17	5.29
		60	4.97	5.47	5.52	5.27	5.22	5.25	5.12	5.19	5.31	5.08	5.21	5.34
	65	0	5.72	5.72	5.72	5.72	5.72	5.72	5.72	5.72	5.72	5.72	5.72	5.72
		5	4.87	4.86	5.09	4.80	5.01	4.98	4.81	4.79	5.11	4.87	4.92	5.02
		10	4.76	5.15	5.16	4.89	4.96	5.08	4.95	4.88	5.11	5.00	4.93	5.05
		15	4.94	5.18	5.20	5.01	5.00	5.14	4.95	5.09	5.17	5.07	5.16	5.19
		19	5.07	5.30	5.25	5.14	5.04	5.17	5.04	5.14	5.25	5.18	5.25	5.20
		22	5.08	5.32	5.26	5.17	5.19	5.26	5.08	5.20	5.27	5.19	5.28	5.20
		26	5.13	5.35	5.42	5.20	5.20	5.27	5.11	5.21	5.30	5.28	5.29	5.29
		33	5.14	5.35	5.44	5.20	5.22	5.27	5.14	5.22	5.33	5.29	5.31	5.30
		46	5.18	5.37	5.46	5.26	5.22	5.27	5.15	5.24	5.33	5.31	5.32	5.38
		54	5.22	5.38	5.46	5.32	5.22	5.28	5.20	5.28	5.36	5.32	5.32	5.40
		61	5.25	5.41	5.50	5.33	5.24	5.37	5.26	5.28	5.39	5.35	5.35	5.41
	80	0	5.67	5.67	5.67	5.67	5.67	5.67	5.67	5.67	5.67	5.67	5.67	5.67
		5	4.66	4.88	4.98	4.68	4.81	4.89	4.64	4.71	4.85	4.82	4.82	4.89
		10	4.62	4.76	4.89	4.69	4.79	4.91	4.69	4.73	4.80	4.76	4.82	4.87
		15	4.59	4.64	4.81	4.70	4.78	4.93	4.74	4.76	4.76	4.71	4.83	4.84
		22	4.75	4.83	4.96	4.82	4.91	5.01	4.79	4.92	4.97	4.72	4.92	5.03
		29	4.88	4.87	5.11	4.93	4.95	5.17	4.87	4.95	5.09	4.79	4.91	5.07
30	50	0	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78	5.78
		5	5.22	5.32	5.36	5.26	5.29	5.35	5.26	5.28	5.37	5.28	5.38	5.42
		10	4.62	4.81	4.93	4.79	4.79	4.74	4.83	4.70	4.83	4.78	4.69	4.67
		15	4.86	4.94	5.10	4.85	4.85	4.93	4.75	4.86	5.03	4.92	4.91	5.03
		22	4.78	4.96	5.13	4.83	4.91	5.00	4.84	4.85	4.90	4.73	4.82	4.77
		26	4.90	4.88	4.95	4.97	4.89	5.06	4.76	4.77	4.99	4.73	4.81	4.83
		33	4.98	4.86	5.20	5.14	5.11	5.35	4.98	4.98	5.18	4.96	5.08	5.11
		42	5.04	5.11	5.25	5.23	5.27	5.26	5.37	5.14	5.34	5.24	5.13	5.23
		51	5.25	5.28	5.45	5.40	5.45	5.54	5.45	5.37	5.48	5.34	5.39	5.40
	65	0	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75	5.75
		2	4.64	4.81	4.84	4.84	4.82	5.06	4.86	4.98	4.84	4.88	5.03	5.13
		5	4.75	4.86	5.02	4.94	4.94	5.07	4.90	5.03	5.10	4.95	5.11	5.14
		10	4.85	4.99	5.06	4.96	5.06	5.11	4.93	5.07	5.15	5.12	5.15	5.19
		15	4.87	5.05	5.07	5.02	5.11	5.16	5.03	5.13	5.17	5.16	5.17	5.21
		19	4.93	5.05	5.13	5.08	5.16	5.21	5.05	5.15	5.21	5.19	5.18	5.30
		26	4.95	5.16	5.14	5.17	5.17	5.24	5.12	5.20	5.26	5.21	5.24	5.40
		33	4.99	5.20	5.19	5.31	5.19	5.34	5.16	5.23	5.31	5.28	5.26	5.40
		43	5.05	5.28	5.20	5.32	5.20	5.38	5.24	5.26	5.31	5.35	5.33	5.42
		48	5.05	5.30	5.25	5.39	5.33	5.42	5.35	5.31	5.34	5.36	5.36	5.58
		53	5.11	5.38	5.37	5.52	5.38	5.58	5.44	5.34	5.45	5.49	5.41	5.69

Table A4 Continued

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
30	80	0	5.82	5.82	5.82	5.82	5.82	5.82	5.82	5.82	5.82	5.82	5.82	5.82
		1	4.99	4.99	4.99	4.99	4.99	4.99	4.99	4.99	4.99	4.99	4.99	4.99
		5	4.58	4.79	4.95	4.52	4.59	4.69	4.38	4.44	4.63	4.68	4.61	4.70
		10	4.53	4.75	5.00	4.76	4.97	4.86	4.87	4.90	5.10	4.75	4.98	5.08
		15	4.53	4.74	5.10	4.85	4.97	5.17	4.84	5.02	5.03	4.88	5.14	5.17
		22	4.27	4.70	4.91	4.55	4.80	4.80	4.58	4.86	4.94	4.41	4.88	5.05
		29	4.53	4.71	5.13	4.64	4.81	5.04	4.67	4.85	5.07	4.49	4.79	5.02
40	50	0	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62
		5	4.66	4.75	4.88	4.83	4.93	4.96	4.82	4.90	4.98	4.86	4.90	5.02
		15	4.72	4.80	4.83	4.86	4.87	4.99	4.74	4.86	4.97	4.86	5.00	5.09
		22	4.79	4.83	4.91	4.99	5.10	5.13	4.86	4.90	5.09	4.93	5.02	5.11
		36	4.99	5.00	5.06	5.07	5.10	5.26	5.11	5.09	5.22	5.05	5.10	5.19
	65	0	5.79	5.79	5.79	5.79	5.79	5.79	5.79	5.79	5.79	5.79	5.79	5.79
		5	4.84	4.90	4.86	4.71	4.83	4.94	4.69	4.95	4.97	4.87	4.92	4.95
		10	4.86	5.07	5.03	4.93	5.03	5.11	4.97	5.07	5.17	5.07	5.10	5.17
		15	4.78	5.01	4.99	4.85	4.94	5.01	4.87	4.97	5.08	5.01	5.07	5.11
		19	4.84	5.06	5.03	4.86	4.94	5.02	4.87	4.98	5.10	5.05	5.08	5.14
		22	4.90	5.11	5.05	4.99	5.02	5.06	4.97	4.99	5.11	5.07	5.09	5.15
		26	4.93	5.18	5.12	5.08	5.03	5.10	5.06	5.05	5.12	5.13	5.10	5.16
		33	5.05	5.25	5.14	5.11	5.10	5.20	5.10	5.12	5.18	5.17	5.13	5.26
		48	5.12	5.27	5.25	5.23	5.17	5.25	5.15	5.19	5.24	5.26	5.27	5.27
		54	5.19	5.28	5.37	5.34	5.23	5.39	5.23	5.26	5.35	5.35	5.40	5.45
	80	0	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62	5.62
		5	4.74	4.97	5.01	4.85	5.02	5.09	4.90	4.97	5.07	4.95	5.02	5.09
		15	5.14	5.03	5.02	5.04	5.08	5.19	5.14	4.99	4.98	5.03	5.01	5.00
		22	5.43	5.16	5.20	5.28	5.23	5.42	5.20	5.18	5.20	5.23	5.16	5.20
		29	5.43	5.24	5.28	5.41	5.28	5.49	5.28	5.24	5.31	5.28	5.24	5.32

Table A5 Changes of TBA values (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp	RH	Time	Sucuks											
(°C)	(%)	(days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
20	50	0	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
		5	1.19	0.98	0.76	1.03	0.85	0.76	1.09	0.76	0.71	0.90	0.85	0.69
		8	1.99	1.43	1.18	1.64	1.66	1.27	1.85	1.43	1.26	1.48	1.35	1.13
		12	2.56	2.10	1.59	2.57	2.05	2.00	2.29	2.18	2.05	2.29	1.90	1.52
		15	2.30	2.07	1.77	2.21	2.08	1.99	2.29	2.12	1.91	2.28	1.93	1.52
		19	2.29	2.03	1.94	1.99	1.98	1.84	2.21	2.05	1.82	2.11	1.96	1.51
		26	2.20	2.08	1.74	1.82	1.81	1.70	1.64	1.54	1.44	1.83	1.48	1.28
		33	1.93	1.80	1.61	1.62	1.64	1.66	1.79	1.56	1.19	1.64	1.78	1.22
		39	2.30	1.95	1.52	1.78	1.65	1.52	2.13	1.75	1.40	2.07	1.77	1.24
		48	1.96	1.90	1.52	1.91	1.85	1.66	1.91	1.73	1.37	1.93	1.65	1.30
		60	2.41	2.04	1.60	2.36	1.71	1.69	2.11	1.65	1.62	1.82	1.81	1.55
	65	0	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
		5	2.04	1.51	1.50	1.83	1.65	1.27	1.49	1.40	1.27	1.49	1.19	1.13
		10	1.76	1.27	1.13	1.47	1.34	1.51	1.51	1.35	1.31	1.65	1.70	1.13
		15	1.81	1.25	1.23	1.41	1.32	1.45	1.50	1.37	1.26	1.66	1.61	1.07
		19	1.62	1.22	1.33	1.35	1.29	1.38	1.49	1.38	1.22	1.55	1.51	1.00
		22	1.56	1.29	1.27	1.43	1.37	1.31	1.50	1.44	1.27	1.56	1.41	1.02
		26	1.65	1.46	1.30	1.61	1.54	1.41	1.60	1.60	1.34	1.60	1.41	1.14
		33	1.59	1.49	1.19	1.53	1.46	1.35	1.53	1.29	1.26	1.40	1.31	1.16
		48	1.65	1.36	1.18	1.53	1.53	1.36	1.45	1.31	1.23	1.31	1.39	1.15
		54	1.69	1.51	1.32	1.48	1.42	1.38	1.51	1.44	1.32	1.48	1.41	1.31
		61	1.75	1.62	1.40	1.49	1.50	1.45	1.55	1.45	1.43	1.49	1.44	1.36
	80	0	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59
		5	1.95	1.87	1.75	1.74	1.72	1.63	1.72	1.69	1.67	1.73	1.70	1.65
		10	2.02	1.96	1.91	2.00	1.95	1.86	1.93	1.88	1.87	1.90	1.85	1.76
		15	2.01	1.74	1.67	2.02	1.83	1.77	1.92	1.75	1.69	1.87	1.78	1.53
		22	2.12	1.87	1.89	2.05	2.01	1.94	2.04	1.99	1.92	1.95	1.90	1.72
		29	2.30	2.12	2.05	2.18	2.15	2.05	2.16	2.13	1.97	2.09	2.03	1.95
30	50	0	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
		5	1.69	1.47	1.27	1.57	1.46	1.35	1.61	1.55	1.43	1.47	1.46	1.18
		10	1.84	1.63	1.51	1.34	1.76	1.30	1.73	1.72	1.27	1.31	1.54	1.09
		15	1.66	1.37	1.19	1.64	1.50	1.48	1.65	1.58	1.49	1.67	1.33	1.03
		22	1.91	1.73	1.57	1.82	1.70	1.52	1.72	1.64	1.54	1.65	1.59	1.50
		26	2.08	1.73	1.69	1.69	1.64	1.62	1.88	1.74	1.69	1.72	1.55	1.47
		33	1.87	1.43	1.37	1.65	1.36	1.22	1.73	1.71	1.27	1.54	1.46	1.19
		42	1.96	1.51	1.45	1.78	1.62	1.59	1.75	1.61	1.49	1.59	1.53	1.43
		51	2.35	2.00	1.77	2.20	1.87	1.71	1.93	1.77	1.50	1.82	1.69	1.31
	65	0	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
		5	1.43	1.21	1.09	1.38	1.34	1.21	1.40	1.33	1.24	1.11	1.18	1.04
		10	2.05	2.01	1.74	1.94	1.90	1.84	1.90	1.89	1.87	2.05	2.00	1.78
		15	1.95	1.80	1.71	1.80	1.79	1.76	1.70	1.89	1.74	1.87	1.75	1.62
		19	1.91	1.58	1.67	1.64	1.72	1.53	1.58	1.93	1.57	1.69	1.45	1.45
		26	1.78	1.50	1.49	1.76	1.46	1.49	1.50	1.73	1.48	1.72	1.49	1.34
		33	1.75	1.60	1.52	1.63	1.55	1.45	1.61	1.65	1.50	1.72	1.57	1.31
		43	1.71	1.71	1.32	1.75	1.62	1.56	1.70	1.57	1.53	1.72	1.36	1.34
		48	1.81	1.73	1.57	1.81	1.72	1.59	1.74	1.67	1.49	1.80	1.49	1.28
		53	2.28	1.94	1.51	2.11	1.89	1.61	2.05	1.71	1.55	2.06	1.81	1.18

Table A5 Continued

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
			Sucuks											
30	80	0	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
		5	2.26	2.19	1.94	2.09	2.03	1.97	2.04	1.98	1.90	2.13	2.06	1.88
		10	2.31	2.26	2.25	2.29	2.24	2.14	2.30	2.12	2.03	2.11	2.07	2.07
		15	2.62	2.48	2.29	2.61	2.37	2.14	2.59	2.28	1.96	2.26	2.23	1.76
		22	2.32	2.18	2.08	2.24	2.12	1.97	2.18	2.08	1.98	2.09	2.05	1.94
		29	2.53	2.43	2.03	2.48	2.38	1.98	2.39	2.09	1.94	2.31	2.08	1.83
40	50	0	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
		5	2.06	1.86	1.81	1.95	1.86	1.86	1.98	1.90	1.85	1.98	1.98	1.86
		15	1.96	1.76	1.71	1.88	1.71	1.74	1.96	1.81	1.71	1.85	1.85	1.61
		22	2.04	1.91	1.85	1.96	1.91	1.94	1.91	1.94	1.82	1.96	1.91	1.82
		36	2.18	2.09	1.90	2.15	2.08	2.01	2.04	2.02	1.92	2.01	1.99	1.88
	65	0	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
		5	2.32	1.46	1.43	1.96	1.66	1.44	1.58	1.40	1.37	1.31	1.12	1.02
		10	2.24	1.91	1.77	1.88	1.82	1.73	2.05	1.89	1.65	2.14	1.91	1.73
		15	2.11	1.66	1.66	1.72	1.77	1.67	1.89	1.71	1.75	2.01	1.72	1.56
		19	2.20	1.72	1.84	1.96	1.92	1.81	2.02	1.9	1.76	2.17	1.91	1.65
		22	2.02	1.82	1.82	1.89	1.85	1.74	1.95	1.87	1.69	1.89	1.87	1.56
		26	2.01	1.72	1.48	1.82	1.77	1.66	1.92	1.58	1.47	1.84	1.79	1.46
		33	1.99	1.73	1.56	1.85	1.77	1.69	1.88	1.56	1.47	1.83	1.69	1.44
		48	1.95	1.66	1.53	1.92	1.81	1.72	1.89	1.65	1.46	1.82	1.58	1.35
		54	2.09	1.71	1.68	1.84	1.72	1.42	1.86	1.83	1.60	1.84	1.75	1.18
	80	0	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
		5	2.03	1.73	1.38	1.89	1.58	1.47	1.58	1.58	1.38	1.47	1.47	1.30
		15	1.94	1.32	1.32	1.88	1.32	1.56	1.83	1.68	1.43	1.67	1.50	1.25
		22	2.60	1.52	1.74	2.26	1.95	1.47	1.85	1.92	1.81	1.85	1.81	1.15
		29	2.93	2.50	1.97	2.59	2.19	1.97	2.37	2.32	2.41	2.32	2.24	1.76

Table A6 Changes of nitrosomyoglobin conversion (%) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp	RH	Time	Sucuks											
(°C)	(%)	(days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50
		5	27.03	28.32	31.27	36.60	30.64	45.46	37.91	44.29	37.09	39.44	45.15	47.20
		8	42.25	52.50	58.75	58.90	52.00	60.00	69.50	69.00	69.50	81.00	80.50	81.50
		12	50.00	60.00	68.00	77.00	70.00	75.00	80.00	78.00	72.00	83.00	82.00	82.50
	50	15	45.23	54.23	61.11	63.65	58.57	62.63	67.04	67.64	64.60	76.16	64.71	69.41
		19	40.45	48.46	54.21	50.29	47.13	50.25	54.07	53.28	57.20	69.32	57.42	57.32
		26	41.58	48.87	55.66	50.66	46.07	42.83	52.52	49.37	57.21	57.99	54.03	57.28
		33	39.15	43.62	46.12	40.69	43.91	43.97	51.30	47.66	50.28	57.08	52.94	56.75
		39	35.68	40.04	42.58	39.96	42.31	40.11	49.57	42.25	42.25	56.00	50.90	47.50
		48	34.48	38.60	41.17	39.64	40.30	39.18	43.17	38.18	40.91	54.67	46.06	46.36
		0	20.20	20.20	20.20	20.20	20.20	20.20	20.20	20.20	20.20	20.20	20.20	20.20
		5	41.79	46.19	55.65	51.94	51.27	54.56	52.52	51.72	54.56	53.23	52.03	52.03
		10	52.57	66.05	79.04	53.03	58.27	65.55	83.01	77.80	68.04	85.04	83.37	83.12
20		15	51.79	65.03	75.72	52.09	57.35	64.89	79.86	76.43	65.08	82.30	81.24	80.22
		19	51.00	64.00	72.39	51.14	56.42	64.22	76.70	75.05	62.11	81.22	79.44	79.56
	65	22	45.78	52.93	63.06	49.43	52.64	58.28	71.61	66.46	61.66	78.33	75.23	74.22
		26	47.90	57.51	61.18	47.51	50.78	56.01	67.98	60.50	55.27	71.87	66.88	65.78
		33	43.98	49.80	52.08	50.82	47.01	52.03	65.22	55.12	53.22	68.40	62.42	60.12
		48	38.00	44.23	49.85	40.10	42.88	48.88	51.69	48.80	42.20	60.02	53.69	44.52
		54	29.78	35.85	49.52	30.27	33.29	36.05	40.50	33.59	35.40	48.75	42.53	37.68
		61	22.00	29.39	34.23	26.72	32.82	27.98	31.52	30.30	23.85	35.67	32.56	28.48
		0	20.77	20.77	20.77	20.77	20.77	20.77	20.77	20.77	20.77	20.77	20.77	20.77
		5	62.21	65.71	73.53	68.56	73.80	75.68	71.91	73.70	74.72	68.16	71.12	75.55
	80	10	54.35	60.85	64.55	62.63	66.39	64.40	66.84	65.99	65.94	65.83	66.03	68.93
		15	52.50	53.99	55.58	56.71	58.99	53.12	61.77	58.28	57.17	63.51	60.93	64.32
		22	50.06	52.43	61.64	62.70	61.81	61.53	66.75	62.81	64.79	62.59	60.15	64.26
		29	42.14	51.02	52.15	50.52	49.95	54.81	53.91	59.10	61.58	59.19	61.17	54.31
		0	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88
		5	47.90	62.58	63.96	55.69	55.94	65.84	65.49	63.10	54.38	71.35	67.94	58.39
		10	50.56	61.61	63.84	53.47	57.93	53.17	53.05	53.60	54.03	58.45	55.85	55.10
	50	15	43.90	54.84	52.99	56.58	48.91	51.63	48.95	47.70	54.05	56.19	51.58	50.59
		22	39.17	47.07	56.77	47.87	44.31	45.75	40.54	51.38	51.18	51.63	47.97	54.95
		26	36.49	45.50	49.49	49.39	39.92	47.14	51.07	50.29	53.03	50.32	51.94	52.29
		33	33.45	38.50	42.61	40.31	37.07	42.44	52.58	45.41	44.90	52.81	51.40	50.27
		42	30.21	40.60	39.19	41.11	37.45	38.28	40.09	31.87	36.86	51.07	43.41	45.00
		0	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88	23.88
30		2	58.81	73.97	83.00	81.30	81.46	80.30	86.51	80.30	87.32	87.97	82.97	85.32
		5	68.89	75.88	81.90	85.15	83.98	71.87	85.53	83.51	77.30	85.23	79.69	82.29
		10	63.46	58.46	79.12	73.20	68.57	62.39	80.63	64.43	59.51	80.94	64.06	78.67
		15	51.24	54.68	63.03	65.78	61.64	50.31	65.02	57.65	53.71	72.50	52.75	66.67
	65	19	41.79	47.24	55.17	53.27	52.62	47.39	61.01	50.88	52.01	66.88	48.29	58.35
		26	40.32	46.08	47.20	52.12	50.92	43.01	51.98	48.68	51.96	63.57	47.49	57.25
		33	39.21	44.92	45.12	44.41	43.74	40.62	50.97	44.11	39.01	55.96	44.02	54.63
		43	36.88	42.27	39.52	41.99	40.20	40.36	47.57	43.35	38.85	54.67	42.22	54.21
		48	31.39	40.58	39.23	41.14	40.19	38.23	46.96	41.11	38.10	52.21	38.27	50.07
		53	25.90	30.26	33.92	38.89	36.66	33.33	42.95	38.86	37.35	46.07	32.51	45.92

Table A6 Continued

Temp	RH	Time	Sucuks											
(°C)	(%)	(days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92
		5	70.80	73.37	73.41	86.01	75.77	83.27	71.97	69.59	82.98	87.79	71.02	72.53
30	80	10	59.97	66.20	67.37	69.90	62.78	62.37	71.32	65.27	70.10	73.36	69.64	70.02
		15	58.11	66.39	66.62	68.24	65.27	61.05	67.98	64.56	64.19	69.58	62.56	61.71
		22	52.69	56.78	63.02	63.39	62.90	57.04	64.16	62.84	60.80	67.28	58.38	57.95
		29	47.20	52.65	59.43	57.59	54.52	54.04	58.13	59.92	54.48	61.78	54.98	57.21
	50	0	15.56	15.56	15.56	15.56	15.56	15.56	15.56	15.56	15.56	15.56	15.56	15.56
		5	66.85	68.34	74.56	81.15	75.99	71.49	85.65	81.95	82.88	86.24	82.18	83.89
		15	65.04	65.14	75.09	74.46	73.13	68.64	74.99	71.74	70.76	75.47	72.89	75.50
		22	59.26	61.80	65.35	63.43	61.74	61.82	70.99	64.84	63.63	66.38	60.60	64.10
		36	49.28	50.45	53.81	57.89	59.03	57.50	62.84	56.71	53.91	62.23	57.01	59.52
		0	22.04	22.04	22.04	22.04	22.04	22.04	22.04	22.04	22.04	22.04	22.04	22.04
		5	53.73	55.62	61.48	79.24	68.23	63.62	72.03	70.96	76.36	80.76	75.13	74.85
		10	50.37	54.04	56.01	60.15	55.24	53.22	66.98	59.81	65.04	69.12	64.68	64.64
		15	50.17	52.46	55.78	58.02	51.94	51.53	64.00	58.67	63.71	64.72	64.23	63.21
40	65	19	49.42	51.04	53.04	56.89	48.64	49.44	62.48	58.66	61.79	61.53	61.36	63.19
		22	47.44	50.14	50.07	53.63	47.77	49.32	61.01	57.62	59.87	59.42	57.59	61.79
		26	46.89	50.10	48.25	51.57	47.03	49.22	58.78	56.39	55.93	58.34	55.86	61.74
		33	43.88	47.02	47.50	49.50	46.61	49.20	56.56	51.26	53.67	58.18	53.67	60.63
		48	40.87	41.78	40.48	48.59	42.91	44.58	52.24	46.03	47.11	56.76	53.30	57.16
		54	34.05	37.54	40.38	43.56	38.38	41.58	50.65	43.85	40.98	49.60	43.10	52.57
		0	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92	24.92
		5	76.49	77.97	80.00	86.00	80.00	79.00	87.00	85.57	82.00	87.65	86.18	83.00
	80	15	62.57	67.74	69.34	76.95	70.35	69.50	82.00	77.99	74.30	82.13	78.00	79.85
		22	46.57	69.66	77.05	72.36	69.36	67.66	71.60	71.29	70.33	72.30	73.67	73.18
		29	45.71	48.59	49.69	56.70	54.24	54.44	60.28	58.62	60.41	67.67	63.95	63.99

Table A7 Changes of residual nitrite level (ppm) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp	RH	Time	Sucuks											
(°C)	(%)	(days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
20	50	0	2.05	75.00	150.00	2.05	2.05	2.05	75.00	75.00	75.00	150.00	150.00	150.00
		5	2.77	13.56	34.50	2.61	2.05	2.29	8.37	8.45	8.77	20.50	28.40	23.70
		8	0.95	8.87	12.74	2.92	0.79	0.87	3.63	1.34	1.34	1.66	2.77	4.03
		15	3.84	5.14	5.77	2.85	2.06	2.02	2.96	3.24	2.77	3.64	4.63	3.77
		26	6.72	9.40	9.16	2.77	3.32	3.16	5.29	5.14	4.19	5.61	6.48	7.51
		33	8.14	16.75	17.30	4.74	4.74	5.53	4.90	4.74	5.37	6.64	6.87	6.32
		39	6.87	15.56	19.75	9.96	11.69	12.64	11.85	12.09	14.06	9.09	8.22	9.09
		48	5.14	15.88	17.14	12.01	12.80	12.64	11.85	12.09	12.25	10.90	11.93	14.22
	65	0	3.00	75.00	150.00	3.43	3.94	3.44	75.00	75.00	75.00	150.00	150.00	150.00
		5	5.45	14.94	31.24	4.03	4.08	4.40	9.59	8.39	8.03	11.70	11.04	9.93
		10	10.80	12.00	18.20	6.82	6.40	7.26	6.28	6.74	8.12	15.18	15.16	14.54
		15	10.78	9.64	13.58	5.24	4.62	5.46	6.30	6.62	7.62	12.36	11.58	11.62
		19	10.74	7.26	11.92	3.64	2.84	3.64	6.32	6.48	7.12	8.78	8.00	8.70
		22	8.53	8.05	10.46	3.80	3.16	4.66	5.37	7.66	5.61	8.97	7.71	8.06
		26	6.32	8.84	9.16	3.96	3.48	5.68	4.42	8.84	4.10	8.15	7.42	7.42
		33	5.06	5.06	9.32	3.60	4.42	3.20	3.96	3.16	4.10	3.16	8.70	6.64
		48	3.96	3.32	12.32	3.32	3.16	3.16	5.06	5.84	5.84	5.06	5.38	8.84
		54	3.66	4.90	11.38	5.84	5.54	5.22	3.96	4.42	4.74	3.96	6.32	7.58
		61	2.80	3.80	6.96	3.80	5.22	6.96	3.48	3.16	3.16	3.32	3.48	3.32
	80	0	0.79	75.00	150.00	0.79	0.79	0.79	75.00	75.00	75.00	150.00	150.00	150.00
		5	1.15	10.85	27.16	1.45	1.39	1.69	7.68	7.55	7.36	13.12	10.88	13.25
		10	2.42	10.13	14.36	2.21	2.21	2.30	7.67	7.92	7.22	11.43	9.90	11.43
		15	3.25	6.90	10.57	5.18	5.43	4.83	8.89	9.31	7.91	7.46	8.16	6.97
		22	2.72	2.94	9.53	2.54	8.19	1.31	3.29	3.33	2.66	2.91	3.14	2.66
30	50	0	0.85	75.00	150.00	0.85	0.85	0.85	75.00	75.00	75.00	150.00	150.00	150.00
		10	7.25	20.05	30.45	8.55	9.40	11.95	18.35	17.05	17.50	14.95	16.65	10.25
		15	8.10	9.40	15.40	8.10	7.65	7.25	10.70	12.80	8.10	11.05	12.80	8.10
		26	9.50	10.23	12.32	9.45	8.14	7.12	13.98	14.51	10.32	15.32	14.51	7.32
		33	2.15	4.25	12.55	1.75	1.75	1.75	5.15	3.85	3.40	6.45	5.55	5.55
	65	0	0.85	75.00	150.00	0.85	0.85	0.85	75.00	75.00	75.00	150.00	150.00	150.00
		5	7.68	18.97	35.13	10.23	12.81	12.81	8.19	10.23	4.86	10.23	10.50	13.32
		15	8.70	8.34	17.68	11.52	9.72	8.82	6.66	8.07	4.47	7.68	7.56	8.97
		26	9.72	7.68	14.23	12.81	6.63	4.86	5.13	5.91	4.08	5.13	4.59	4.59
	80	0	0.85	75.00	150.00	0.85	0.85	0.85	75.00	75.00	75.00	150.00	150.00	150.00
		5	5.16	10.24	29.56	5.44	5.80	6.84	10.24	11.64	12.60	12.96	13.64	14.36
		10	5.44	10.60	15.96	7.52	8.56	8.20	10.60	10.60	10.92	10.92	12.28	11.28
		15	9.88	11.28	11.88	6.84	5.80	3.40	10.24	10.92	9.88	8.56	9.56	8.20
		22	8.56	6.12	10.84	6.84	8.96	4.12	8.20	6.84	5.44	9.20	8.84	6.84

Table A7 Continued

Temp	RH	Time	Sucuks											
(°C)	(%)	(days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	0.95	75.00	150.00	0.95	0.95	0.95	75.00	75.00	75.00	150.00	150.00	150.00
		5	1.21	20.78	38.53	1.51	1.61	1.90	14.76	13.68	13.90	27.60	21.79	27.98
	50	15	3.22	5.96	10.13	4.70	5.36	5.13	6.39	6.39	7.88	6.84	7.67	7.04
		22	3.40	6.62	7.47	4.28	3.62	2.14	6.62	7.27	6.84	5.36	5.96	5.13
		36	4.91	6.19	7.04	4.01	3.11	2.57	6.39	6.62	6.19	5.76	5.54	4.28
		0	5.87	75.00	150.00	7.33	4.04	6.74	75.00	75.00	75.00	150.00	150.00	150.00
		5	4.69	10.63	27.69	4.31	4.82	5.01	7.27	8.92	7.52	12.93	12.76	14.42
40	65	15	4.19	7.35	10.14	3.94	3.55	3.81	4.77	6.33	4.76	5.31	4.92	5.75
		22	3.95	5.71	5.37	3.75	2.91	3.21	3.52	5.04	3.38	4.51	4.00	4.42
		26	3.70	4.07	8.60	3.56	2.27	2.60	2.27	3.74	1.99	3.70	3.08	3.08
		33	2.78	3.05	6.45	2.67	1.70	1.95	1.70	2.81	1.49	2.78	2.31	2.31
		0	0.95	75.00	150.00	0.95	0.95	0.95	75.00	75.00	75.00	150.00	150.00	150.00
		5	1.32	23.18	38.53	1.97	1.72	2.16	15.58	14.88	14.16	27.88	22.32	27.82
	80	15	2.22	3.24	6.54	1.66	2.10	2.35	3.24	4.20	2.68	2.74	2.93	2.48
		22	1.97	2.99	5.18	2.16	1.72	0.95	2.99	3.43	2.74	2.10	2.54	2.35
		29	3.49	2.48	4.40	2.16	1.77	1.18	3.24	2.99	3.49	2.80	3.05	2.16

Table A8 Changes of histamine concentration (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
20	50	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	279.32	189.15	38.62	187.40	165.42	150.64	103.84	45.91	36.14	23.95	26.70	24.45
		15	154.12	65.24	32.27	113.74	118.86	108.60	105.15	58.34	31.33	9.53	15.65	15.20
		19	108.13	58.65	18.88	94.80	62.85	62.14	71.68	28.88	40.93	7.88	15.48	14.04
		33	81.20	25.21	15.63	62.35	36.67	3.56	7.37	24.18	29.68	6.88	13.17	1.91
		48	26.26	0.00	11.69	20.27	16.14	0.85	3.92	0.00	0.00	0.00	0.00	0.00
		60	13.82	0.00	0.00	12.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	65	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	95.15	77.89	39.76	94.02	39.66	44.43	28.31	35.48	36.76	28.57	26.48	25.88
		10	158.42	73.46	65.97	139.03	59.49	22.00	100.34	68.12	39.79	76.44	48.06	59.38
		15	107.98	59.05	39.78	84.49	68.14	14.17	73.12	46.83	25.99	47.06	36.85	37.36
		22	76.14	66.67	24.55	52.64	47.70	9.44	54.76	33.29	17.57	29.78	28.74	24.24
		33	76.79	57.55	13.59	29.94	44.65	6.34	45.91	25.54	12.20	17.68	25.64	15.33
		48	22.11	17.24	11.71	12.48	3.00	2.48	17.35	5.17	3.12	12.46	5.18	5.95
		61	16.57	11.00	0.00	0.00	0.00	0.00	2.00	0.10	0.30	0.00	0.00	0.00
	80	0	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88	5.88
		5	228.02	145.90	97.18	163.90	151.91	85.61	158.29	105.13	158.82	114.21	89.14	60.08
		15	121.20	103.10	44.32	113.57	100.78	72.28	90.86	87.98	79.32	90.19	51.73	44.56
		22	106.07	87.60	41.75	86.80	87.74	45.95	72.91	56.80	51.11	69.94	41.52	41.00
		29	92.28	70.10	31.60	47.09	52.33	38.02	49.32	44.74	44.11	56.49	34.20	26.50
30	50	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	213.66	157.59	130.88	190.98	148.63	145.60	143.65	157.99	157.89	134.48	126.91	134.92
		15	178.01	132.12	129.00	146.72	140.43	129.41	126.30	140.76	135.15	140.45	114.40	118.83
		26	176.75	123.23	113.45	142.07	135.58	129.15	125.12	135.98	127.59	138.47	111.93	115.11
		33	170.68	113.61	108.38	138.33	109.59	124.02	112.56	108.64	112.06	125.07	121.56	105.97
		42	96.30	88.92	88.39	71.72	81.17	57.83	87.14	74.26	71.20	83.11	102.52	47.94
		51	43.74	42.58	32.41	30.98	35.21	29.46	37.42	29.76	26.63	30.85	30.11	24.56
	65	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	149.60	57.84	62.64	71.39	146.09	116.90	41.34	56.23	51.49	46.07	39.18	40.75
		10	212.76	124.85	106.02	76.07	173.68	77.85	39.17	111.77	56.24	92.84	77.09	53.71
		15	175.86	99.39	49.13	57.37	98.25	40.20	40.18	38.75	30.55	47.70	43.77	37.47
		26	156.89	84.90	40.68	34.67	66.35	32.51	31.55	32.61	29.80	33.76	37.06	14.44
		33	127.23	74.91	26.15	22.96	33.93	20.91	30.50	21.65	16.65	20.56	23.52	11.94
		48	135.58	87.30	19.44	13.00	11.65	18.27	18.58	17.17	5.78	13.50	17.02	18.11
		53	85.95	62.22	16.80	1.95	0.00	4.47	12.20	6.71	0.00	16.59	9.58	0.00
	80	0	3.39	3.39	3.39	3.39	3.39	3.39	3.39	3.39	3.39	3.39	3.39	3.39
		5	271.16	169.54	152.78	230.33	135.85	142.60	217.44	164.33	142.10	136.79	130.46	123.53
		15	292.66	262.24	233.33	229.50	187.05	137.63	260.08	242.57	213.57	242.28	220.96	144.56
		22	171.84	117.30	129.86	161.05	137.69	102.10	158.27	147.75	129.10	127.25	135.83	90.89
		29	75.98	74.68	44.63	66.83	58.55	45.59	71.04	66.52	56.51	23.94	63.20	12.05

Table A8 Continued

Temp (°C)	RH (%)	Time (days)	Sucuks											
			A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31	4.31
		5	243.29	212.91	107.05	188.12	142.32	113.04	108.06	53.21	62.47	67.78	43.10	27.34
	50	15	207.30	175.94	105.31	198.82	120.10	109.40	165.42	146.07	93.59	145.69	94.58	55.28
		22	102.77	55.08	97.34	90.30	86.93	54.24	73.93	85.47	104.47	96.33	60.15	35.69
		36	73.63	40.18	25.79	74.25	61.52	45.70	50.79	51.51	46.42	60.46	26.63	21.05
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	152.75	97.41	74.71	87.70	91.78	77.72	54.37	34.78	67.97	51.49	51.23	50.58
		10	153.87	135.96	130.22	151.55	87.79	94.17	128.25	120.38	105.08	104.42	115.65	94.78
40	65	15	93.17	76.85	60.84	73.63	63.48	62.54	91.98	68.04	47.82	60.21	56.41	47.31
		22	92.24	62.52	38.88	60.85	58.62	39.29	75.33	44.14	40.82	49.96	42.20	32.91
		33	80.45	56.17	21.89	55.83	62.19	40.21	67.93	46.10	18.81	46.09	33.38	22.71
		48	78.92	52.91	11.78	35.74	47.18	14.28	51.62	31.75	16.24	28.91	23.49	10.31
		0	7.93	7.93	7.93	7.93	7.93	7.93	7.93	7.93	7.93	7.93	7.93	7.93
		5	144.22	116.40	84.60	84.05	101.28	107.84	117.41	127.98	109.89	50.76	54.90	56.23
	80	15	269.73	201.66	148.42	227.72	202.27	154.73	181.48	161.66	169.36	110.58	92.86	110.88
		22	154.72	138.74	85.48	93.57	79.92	115.59	97.98	97.17	116.80	65.37	51.72	71.69
		29	145.96	130.44	77.96	68.91	70.65	97.70	96.59	89.13	93.56	60.84	59.90	63.24

Table A9 Changes of tyramine concentration (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	56.56	56.56	56.56	56.56	56.56	56.56	56.56	56.56	56.56	56.56	56.56	56.56
		5	445.71	330.67	282.50	426.13	351.34	316.97	394.27	347.80	302.45	380.79	251.83	225.82
		15	350.77	249.74	189.76	306.35	195.41	155.20	289.34	257.14	230.38	207.07	194.77	132.04
	50	19	278.14	175.21	167.69	223.93	193.83	131.32	202.65	162.01	153.91	179.17	130.13	119.93
		33	194.05	143.02	133.86	177.48	151.14	88.83	148.07	125.52	103.51	131.79	77.53	69.80
		48	181.59	133.77	98.10	170.24	139.36	87.91	163.68	122.66	117.06	140.89	66.99	46.69
		60	160.77	95.19	45.00	155.17	67.53	43.60	131.55	68.10	55.00	93.09	56.37	25.00
		0	47.38	47.38	47.38	47.38	47.38	47.38	47.38	47.38	47.38	47.38	47.38	47.38
		5	406.64	329.69	327.44	369.04	283.16	272.20	367.18	361.31	355.34	379.16	326.53	212.00
20		10	367.12	328.65	327.29	348.32	238.73	216.80	347.39	344.45	341.47	353.38	325.47	140.48
	65	15	361.01	342.65	258.43	318.38	157.01	146.35	246.67	253.83	288.01	275.48	251.81	96.41
		22	315.38	316.26	219.32	307.06	121.63	124.64	231.97	245.26	212.31	221.40	179.21	78.59
		33	259.94	189.72	126.16	213.27	99.14	113.79	195.91	99.15	32.47	190.03	60.77	7.57
		48	186.10	49.00	21.00	148.28	64.00	38.81	111.36	30.00	5.65	63.64	20.69	1.58
		61	41.98	11.23	7.77	39.27	4.15	0.46	39.00	0.00	0.00	11.24	0.00	0.00
		0	50.53	50.53	50.53	50.53	50.53	50.53	50.53	50.53	50.53	50.53	50.53	50.53
		5	396.02	367.99	342.53	371.86	361.95	332.20	369.71	355.23	351.84	352.85	314.96	283.16
	80	15	311.06	280.22	260.07	290.57	241.87	261.30	264.64	255.57	244.69	263.74	241.03	240.49
		22	286.54	246.79	214.49	228.60	217.47	201.45	223.51	219.68	207.51	215.73	217.12	190.11
		29	252.13	205.07	165.09	169.38	163.05	156.45	172.44	182.60	158.80	162.06	158.30	152.59
		0	67.87	67.87	67.87	67.87	67.87	67.87	67.87	67.87	67.87	67.87	67.87	67.87
		5	446.43	356.75	310.68	419.04	311.17	344.51	412.10	283.13	330.12	286.59	305.22	300.92
		15	386.43	326.75	317.51	382.10	281.17	314.51	359.04	253.13	280.68	256.59	275.22	270.92
	50	26	378.96	275.46	300.12	326.27	267.73	246.05	275.48	249.48	239.25	280.42	295.56	226.72
		33	348.96	270.97	287.51	296.27	245.46	216.05	245.48	219.48	209.25	250.42	265.56	196.72
		42	319.25	237.73	128.79	231.50	186.29	140.37	200.79	144.70	107.68	176.02	146.98	93.34
		51	195.55	123.02	120.15	144.65	113.63	115.04	137.49	125.93	92.71	141.66	124.39	56.82
		0	45.12	45.12	45.12	45.12	45.12	45.12	45.12	45.12	45.12	45.12	45.12	45.12
		5	495.25	436.25	378.08	472.89	433.51	379.19	458.50	430.58	403.94	433.58	425.31	362.29
30		10	463.85	375.75	303.37	453.34	389.62	346.97	455.63	372.42	354.72	408.13	338.20	297.65
	65	15	461.74	345.74	291.20	428.64	368.36	314.75	420.06	341.24	305.51	372.25	287.74	245.89
		26	428.23	328.58	284.74	381.61	338.58	308.80	380.36	310.06	299.02	368.74	277.55	213.85
		33	337.18	284.92	224.37	319.67	291.76	293.14	287.43	254.87	206.48	264.35	237.27	188.42
		48	247.36	219.70	184.00	231.11	221.47	214.10	213.25	181.71	140.11	179.97	166.91	144.32
		53	174.29	147.57	120.92	152.36	136.75	133.58	146.13	86.58	90.94	162.36	80.68	70.68
		0	45.57	45.57	45.57	45.57	45.57	45.57	45.57	45.57	45.57	45.57	45.57	45.57
		5	363.17	287.71	311.50	262.30	319.06	305.41	308.17	299.90	255.34	262.50	327.56	250.67
	80	15	331.52	265.49	307.78	235.58	262.99	200.28	274.67	289.04	242.90	215.66	289.24	196.53
		22	299.86	263.30	304.07	208.88	206.93	170.48	241.17	278.18	230.47	168.82	250.91	152.76
		29	243.28	206.01	164.56	185.38	180.50	142.39	153.82	188.00	171.11	145.54	172.21	85.56

Table A9 Continued

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	30.92	30.92	30.92	30.92	30.92	30.92	30.92	30.92	30.92	30.92	30.92	30.92
		5	442.24	346.77	305.80	389.37	343.14	322.75	389.52	312.13	306.78	389.10	352.06	314.94
	50	15	280.83	237.95	190.91	260.70	246.40	213.90	256.70	217.40	210.68	238.38	237.92	183.75
		22	214.52	187.03	149.37	189.18	157.13	122.53	174.20	151.56	117.69	155.68	139.52	110.97
		36	126.98	109.29	98.12	108.60	98.13	82.30	106.98	89.00	68.89	106.80	83.80	66.27
		0	58.80	58.80	58.80	58.80	58.80	58.80	58.80	58.80	58.80	58.80	58.80	58.80
		5	152.30	152.61	146.94	145.57	153.01	121.13	158.28	136.27	149.64	155.75	138.39	139.65
		10	490.19	362.97	294.17	453.45	430.10	354.83	430.84	275.60	192.16	346.95	324.61	185.42
40	65	15	380.51	312.20	276.40	345.03	336.04	215.44	294.38	249.44	157.04	285.70	299.14	154.95
		22	328.49	210.19	187.16	260.12	205.13	169.87	260.78	208.65	149.76	253.62	220.43	116.99
		33	249.27	180.96	161.67	173.87	151.14	146.73	172.16	150.36	106.77	152.17	146.92	85.58
		48	205.94	149.31	114.24	125.14	96.88	84.87	119.72	88.12	66.92	81.73	67.30	51.73
		0	54.96	54.96	54.96	54.96	54.96	54.96	54.96	54.96	54.96	54.96	54.96	54.96
		5	334.85	283.85	264.03	308.58	260.53	259.59	274.28	263.71	242.72	287.33	277.98	229.50
	80	15	256.92	223.81	217.96	245.03	204.65	183.00	228.28	213.85	185.49	221.94	199.35	175.58
		22	196.97	189.78	164.13	195.10	177.22	166.04	191.27	170.08	159.39	188.38	176.11	133.83
		29	178.88	153.22	138.03	173.89	132.38	124.64	175.54	155.90	135.45	157.54	151.53	122.44

Table A10 Changes of putrescine concentration (mg/kg) in sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
		0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		5	396.23	345.34	239.08	342.68	266.04	214.26	317.31	296.58	150.93	285.11	262.33	37.53
		15	341.15	301.28	129.54	273.36	260.38	101.33	259.62	282.86	89.82	234.97	200.90	20.38
	50	19	248.13	206.64	84.78	215.24	141.07	100.90	205.53	168.83	48.14	169.87	139.89	10.14
		33	179.65	146.57	76.87	176.27	104.87	86.65	157.38	129.83	30.54	113.18	85.69	4.97
		48	145.63	126.10	43.48	119.45	43.48	2.51	99.02	67.33	6.03	33.84	49.78	0.00
		60	90.99	0.00	0.00	44.58	0.00	0.00	1.08	0.00	0.00	1.80	0.00	0.00
		0	7.63	7.63	7.63	7.63	7.63	7.63	7.63	7.63	7.63	7.63	7.63	7.63
		5	256.33	206.12	112.90	188.89	171.00	162.24	186.95	152.36	141.53	198.54	141.33	116.70
20		10	205.14	166.23	107.41	101.10	102.00	85.58	93.43	98.72	97.05	111.07	96.65	79.80
	65	15	160.30	126.68	85.73	71.79	79.00	58.48	70.67	64.03	71.88	71.35	64.58	49.64
		22	127.30	87.12	74.04	61.00	66.00	46.14	57.90	40.12	60.53	55.49	53.61	22.78
		33	64.28	45.77	52.14	47.50	44.00	36.00	44.36	29.35	44.00	38.00	36.71	22.42
		48	46.76	28.00	23.63	38.21	34.66	29.27	39.00	18.45	31.00	32.80	28.33	19.47
		61	38.81	13.50	10.00	23.56	11.17	20.05	25.14	12.01	10.96	21.63	22.51	11.77
		0	14.76	8.86	8.86	14.76	14.76	14.76	14.76	14.76	14.76	14.76	14.76	14.76
		5	239.58	115.81	128.46	177.42	146.75	121.81	152.44	128.01	126.10	146.34	126.95	124.45
	80	15	248.39	167.90	111.85	213.88	130.14	112.44	152.98	125.87	113.16	125.35	111.42	101.81
		22	202.46	125.51	85.63	140.67	110.00	78.92	112.76	74.02	94.54	119.98	90.31	71.44
		29	135.93	97.98	76.13	109.23	78.12	71.71	74.85	71.87	74.14	78.58	91.00	63.05
		0	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23	11.23
		5	134.46	118.37	110.92	116.55	121.29	115.32	118.84	115.92	117.09	114.52	116.13	123.40
		15	231.96	217.76	188.68	211.18	212.61	197.24	211.65	209.19	209.44	193.07	214.53	208.59
	50	26	167.49	128.98	139.25	139.86	144.23	144.20	127.71	127.59	132.16	139.35	137.69	134.11
		33	148.39	126.23	130.26	132.99	134.66	133.36	120.02	124.35	130.35	134.37	125.08	126.03
		42	138.05	118.85	121.27	129.17	125.10	122.51	119.19	120.85	128.54	129.38	112.47	117.96
		51	133.47	108.72	129.84	126.13	112.55	123.75	118.36	117.34	116.01	113.99	106.44	101.56
		0	36.71	36.71	36.71	36.71	36.71	36.71	36.71	36.71	36.71	36.71	36.71	36.71
		5	357.30	252.31	303.96	290.32	317.16	290.56	295.59	319.51	289.43	321.47	235.59	200.58
30		10	324.63	248.10	232.71	283.69	290.29	282.78	269.81	276.77	220.47	234.20	206.76	157.05
	65	15	291.96	187.87	182.07	243.82	273.77	275.00	265.73	271.06	158.38	188.30	136.17	130.53
		26	271.90	167.99	161.45	214.51	263.43	237.70	221.37	222.60	128.39	141.01	116.41	121.13
		33	240.91	115.45	103.98	203.95	198.77	153.41	199.50	135.08	95.48	140.24	113.52	84.20
		48	136.66	106.51	96.50	125.33	90.25	66.25	114.84	84.04	82.57	94.90	91.68	82.22
		53	104.62	91.10	70.03	91.92	66.53	64.19	88.03	74.53	65.65	78.25	70.75	58.89
		0	30.36	30.36	30.36	30.36	30.36	30.36	30.36	30.36	30.36	30.36	30.36	30.36
		5	392.32	364.92	279.66	362.57	340.35	320.50	338.46	327.01	302.10	313.28	311.37	137.98
	80	15	325.18	265.93	247.37	272.81	258.11	247.93	274.55	247.17	215.56	263.26	196.27	129.51
		22	248.05	211.01	200.79	229.51	190.51	177.02	188.06	177.33	160.84	179.27	147.74	95.47
		29	166.21	152.21	149.83	155.13	128.00	112.76	142.21	127.56	119.02	130.07	113.70	62.97

Table A10 Continued

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
			Sucuks											
		0	38.34	38.34	38.34	38.34	38.34	38.34	38.34	38.34	38.34	38.34	38.34	38.34
		5	231.64	124.88	72.48	178.04	103.02	91.97	132.43	83.31	90.01	92.43	84.90	61.68
	50	15	367.89	324.18	270.06	357.03	284.09	259.59	317.45	279.37	248.92	302.88	253.73	239.93
		22	273.19	254.67	208.71	252.00	169.23	170.69	234.91	171.37	166.40	198.43	176.05	152.64
		36	168.69	102.38	97.36	129.79	104.37	96.97	115.35	113.37	91.45	137.33	108.37	83.88
		0	49.60	49.60	49.60	49.60	49.60	49.60	49.60	49.60	49.60	49.60	49.60	49.60
		5	391.05	317.51	313.40	364.92	315.20	320.38	388.66	336.56	297.34	378.25	325.28	266.29
		10	335.68	283.16	209.19	305.10	271.78	258.65	286.71	296.04	302.80	290.54	189.29	178.61
40	65	15	297.22	231.17	171.87	273.71	266.10	214.62	225.62	236.04	234.99	187.91	118.98	127.89
		22	202.84	165.75	129.71	136.36	161.83	144.08	169.12	124.67	118.14	129.54	60.70	59.60
		33	144.33	57.56	80.81	111.49	55.25	40.40	48.44	46.07	46.58	64.20	48.67	23.24
		48	88.78	35.20	28.64	35.07	23.64	20.29	17.05	29.68	40.08	28.48	22.38	12.72
		0	28.44	28.44	28.44	28.44	28.44	28.44	28.44	28.44	28.44	28.44	28.44	28.44
		5	377.25	274.92	310.67	352.67	343.19	228.60	265.66	241.38	235.73	347.51	310.08	221.06
	80	15	331.42	206.41	278.51	310.09	257.16	235.93	180.89	217.50	202.06	234.06	227.85	169.35
		22	284.32	183.18	173.95	219.32	167.17	192.23	163.94	179.66	149.25	201.35	159.80	111.81
		29	179.46	142.50	138.20	149.24	97.27	135.55	142.65	128.50	106.45	151.73	120.08	84.15

Table A11 Changes of overall sensory quality of sucuks (A1-B9) during the ripening and storage periods (20, 30 and 40 °C with 50, 65 and 80 % RH)

Temp (°C)	RH (%)	Time (days)	A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
			Sucuks											
		5	4.00	5.25	5.50	5.25	5.50	5.75	5.50	5.50	6.00	5.25	6.25	6.25
		8	5.00	5.75	7.00	6.50	7.00	7.25	6.75	7.50	7.50	7.00	7.50	7.75
		12	5.25	7.00	7.75	7.00	8.00	7.75	7.75	8.25	8.75	8.00	9.00	9.00
		15	5.25	7.00	7.63	6.63	7.63	7.75	7.38	7.88	8.25	7.88	8.75	8.88
	50	19	5.25	7.00	7.50	6.25	7.25	7.75	7.00	7.50	7.75	7.75	8.50	8.75
		26	4.75	6.50	7.50	5.75	7.25	7.25	6.50	7.00	7.00	7.00	8.25	8.00
		33	4.75	6.50	7.00	5.25	7.25	6.75	6.25	6.50	6.25	6.50	8.00	7.75
		39	4.25	5.50	6.50	4.75	7.50	6.50	5.75	5.50	6.00	6.00	6.25	6.75
		48	3.75	4.25	5.25	4.25	6.50	6.50	5.50	4.75	6.00	6.25	6.50	7.00
		60	2.50	3.50	4.25	3.50	5.50	5.75	3.50	4.50	5.00	5.50	6.00	6.25
20		5	4.00	5.25	5.50	5.25	5.50	5.75	5.50	5.50	6.00	5.25	6.25	6.25
		15	4.50	6.25	6.50	6.00	6.50	6.75	7.25	7.50	7.75	7.50	7.75	8.00
	65	19	3.00	4.75	6.50	5.50	7.25	7.75	7.25	8.25	8.75	7.50	8.75	9.00
		22	2.88	4.50	5.88	5.00	6.38	6.63	6.25	7.38	7.63	6.38	7.88	8.13
		48	2.75	4.25	5.25	4.50	5.50	5.50	5.25	6.50	6.50	5.25	7.00	7.25
		61	2.50	3.50	4.75	4.00	4.75	6.00	4.25	5.00	6.00	5.25	6.50	6.75
		5	5.40	6.60	7.20	6.00	6.46	7.36	6.60	6.76	7.66	6.16	8.10	7.66
		10	6.38	7.58	8.03	7.20	7.51	7.96	7.50	8.18	8.26	7.21	8.70	8.56
	80	15	7.36	8.56	8.86	8.40	8.56	8.56	8.40	9.60	8.86	8.26	9.30	9.46
		22	6.00	7.36	7.96	7.80	8.40	8.26	7.80	8.70	9.16	8.26	8.56	8.86
		29	4.96	6.16	6.76	6.30	7.06	7.20	5.86	7.50	7.20	5.86	7.20	7.20

Table A11 Continued

Temp (°C)	RH (%)	Time (days)	Sucuks A1	A2	A3	B1	B2	B3	B4	B5	B6	B7	B8	B9
30	50	5	3.50	4.25	4.25	3.75	5.00	5.00	4.25	4.75	5.00	4.25	5.50	5.50
		10	4.50	5.75	5.75	5.25	6.75	6.75	6.75	7.50	7.25	7.50	8.00	7.25
		15	6.00	7.00	7.75	7.25	8.25	7.75	7.75	8.25	8.75	8.00	8.75	8.75
		22	5.25	7.75	8.00	6.75	8.25	8.25	7.50	8.25	8.00	7.75	8.25	8.50
		26	4.75	6.75	7.50	6.00	7.00	7.00	7.25	8.00	7.00	7.00	8.75	8.50
		33	4.00	5.50	6.00	4.75	5.75	6.00	6.00	6.75	7.00	6.50	7.50	7.75
		42	3.75	4.75	5.50	4.50	5.25	5.25	6.00	6.00	6.50	6.75	6.50	7.50
		51	3.25	4.50	4.50	4.25	4.50	5.25	4.50	5.25	5.75	6.00	6.00	6.50
	65	5	3.75	5.50	6.75	5.00	6.75	6.00	6.75	6.25	7.25	7.00	7.50	7.75
		10	5.00	6.50	7.00	6.25	7.00	7.50	7.75	7.50	7.75	7.75	8.50	9.00
		15	5.13	6.75	7.38	6.38	7.38	7.75	7.38	8.13	8.13	7.75	8.50	9.00
		19	5.25	7.00	7.75	6.50	7.75	8.00	7.00	8.75	8.50	7.75	8.50	9.00
		26	4.50	6.75	7.00	5.75	6.75	7.00	6.50	7.50	7.25	6.75	8.25	8.25
		33	4.13	6.38	6.63	5.63	6.50	6.88	6.25	7.38	7.00	6.88	7.75	7.88
		43	3.75	6.00	6.25	5.50	6.25	6.75	6.00	7.25	6.75	7.00	7.25	7.50
		48	3.50	5.50	6.00	5.13	5.88	6.38	5.75	6.50	6.63	6.63	6.88	7.13
		53	3.25	5.00	5.75	4.75	5.50	6.00	5.50	5.75	6.50	6.25	6.50	6.75
	80	5	4.75	6.00	7.00	5.25	6.00	6.50	5.25	5.50	7.00	5.75	7.75	7.50
		10	6.25	7.00	8.25	6.75	7.75	7.50	7.25	7.75	7.50	7.25	8.25	9.00
		15	7.00	8.00	8.50	7.25	7.50	8.00	7.50	9.00	8.50	7.75	8.25	8.50
		22	6.00	7.00	7.50	7.25	8.00	7.50	7.00	8.25	8.75	8.00	8.00	8.00
		29	5.25	6.50	7.25	6.00	7.00	7.25	5.75	7.75	7.25	6.75	7.25	7.50
40	50	5	5.25	5.50	6.00	6.25	7.00	7.00	6.50	7.50	7.25	5.25	7.00	7.25
		15	5.50	6.25	6.75	6.25	7.25	8.00	6.75	7.50	7.25	7.25	7.50	8.75
		22	3.75	5.00	5.00	5.00	4.50	4.50	4.00	5.25	5.50	4.75	5.50	5.75
		36	3.00	4.00	4.50	4.00	4.50	4.75	4.25	4.00	5.00	4.25	5.00	5.25
	65	5	3.75	5.50	6.75	5.00	6.75	6.00	6.75	6.25	7.25	7.00	7.50	7.75
		10	5.00	6.50	7.00	6.25	7.00	7.50	7.75	7.50	7.75	7.75	8.50	9.00
		15	5.13	6.75	7.38	6.38	7.38	7.75	7.38	8.13	8.13	7.75	8.50	9.00
		19	4.13	5.88	7.13	6.00	7.50	7.88	7.13	8.50	8.63	7.63	8.63	9.00
		22	3.69	5.51	6.44	5.50	6.69	7.01	6.38	7.69	7.63	6.88	7.88	8.19
		33	3.47	5.32	6.10	5.25	6.29	6.57	6.01	7.29	7.13	6.51	7.51	7.79
		48	3.25	5.13	5.75	5.00	5.88	6.13	5.63	6.88	6.63	6.13	7.13	7.38
	80	5	6.25	7.00	7.00	6.75	6.75	7.75	7.75	7.75	7.75	6.50	7.75	7.25
		15	7.25	8.25	8.25	8.75	8.75	8.25	8.50	9.00	8.25	8.00	9.25	9.25
		22	5.00	6.25	6.75	6.75	7.00	7.25	7.00	7.25	7.50	6.75	7.25	7.75
		29	5.00	5.75	6.00	6.50	6.75	6.75	6.00	6.75	6.75	5.00	6.75	6.50

Table A12 Results of sucuks collected from butchers and local markets

Sample	APC log CFU/g	LAB log CFU/g	Mold log CFU/g	pH	TBA (mg/kg)	Nitrosomyoglobin conversion (%)	Residual Nitrite (ppm)	Overall sensory quality
Trad	5.00	7.80	4.48	5.00	0.98	54.85	1.90	8.00
Trad	4.70	8.02	5.00	4.90	0.69	37.26	1.19	6.50
Trad	6.76	5.30	5.08	5.13	0.98	78.78	1.98	4.00
Trad	5.00	5.00	4.38	5.34	0.78	34.87	3.00	6.83
Trad	4.70	5.00	4.67	4.75	1.07	95.69	1.19	4.25
Trad	5.40	7.04	4.96	4.66	1.39	58.62	1.26	6.75
Trad	7.25	8.26	4.99	5.14	1.12	29.49	2.61	6.50
Trad	5.00	7.06	4.48	5.69	0.49	32.50	2.45	4.75
Trad	5.00	7.93	3.60	5.23	0.59	86.15	1.58	7.15
Trad	6.30	4.70	3.70	5.53	2.87	61.49	2.36	7.35
Trad	6.15	5.00	4.06	5.04	1.18	83.48	1.26	3.03
Trad	4.70	6.49	4.51	4.97	0.44	68.24	0.95	7.43
Trad	5.18	5.00	4.61	4.82	0.62	67.04	0.40	5.23
Trad	5.00	7.45	3.95	5.60	1.47	73.09	1.11	7.00
Trad	6.08	5.74	4.88	5.36	1.64	33.73	1.82	6.00
Trad	6.60	7.76	4.04	5.57	0.74	59.89	2.13	4.75
Trad	4.70	5.00	4.00	4.77	2.59	53.18	2.45	6.00
Trad	5.48	7.20	5.18	5.40	0.44	28.26	1.42	7.15
Trad	6.85	4.70	4.81	4.91	1.60	63.13	2.69	8.00
Trad	5.40	4.93	3.85	5.34	1.74	28.94	1.27	7.35
Trad	7.65	5.18	4.19	5.19	0.90	93.79	3.00	6.50
Trad	6.79	4.70	4.32	4.22	1.81	44.71	1.64	3.68
Trad	7.82	5.00	4.58	5.61	1.14	23.66	5.54	3.68
Trad	7.19	4.70	3.81	5.62	1.80	52.86	1.45	7.88
Trad	7.53	7.51	5.30	5.21	2.71	30.76	2.09	5.25
Firm	6.78	8.15	4.38	6.04	0.80	57.68	26.86	4.00
Firm	5.00	5.18	3.48	6.36	1.18	54.53	14.30	3.50
Firm	5.54	6.15	3.70	5.31	0.83	41.28	4.74	2.75
Firm	4.70	4.70	3.95	4.96	0.40	66.32	3.87	6.75
Firm	5.54	6.93	4.80	4.90	0.56	45.25	9.64	3.50
Firm	6.48	8.00	4.27	6.04	0.51	94.66	11.22	5.75
Firm	6.30	8.40	4.48	5.04	1.12	88.76	27.65	6.00
Firm	5.00	4.70	3.70	6.10	1.33	44.40	5.45	8.80
Firm	5.70	5.00	5.18	5.46	1.13	20.59	7.27	4.40
Firm	7.11	4.70	4.15	6.12	0.51	64.02	21.17	6.88
Firm	6.87	7.24	5.05	5.80	0.03	62.41	26.63	4.68
Firm	4.70	7.17	3.65	5.84	1.32	50.57	3.08	7.98
Firm	7.39	5.86	3.54	6.18	0.89	94.73	13.35	6.05
Firm	6.56	4.18	3.65	5.06	0.85	35.00	9.80	3.85
Firm	6.46	7.45	3.85	5.88	0.77	33.04	4.82	5.23
Firm	6.82	7.22	4.51	5.55	1.02	73.87	7.03	7.20
Firm	5.00	6.40	4.87	5.87	1.56	82.43	1.82	3.85
Firm	5.70	4.70	4.50	4.81	0.96	65.39	6.08	6.33
Firm	6.60	4.93	3.95	5.74	2.35	56.01	1.11	6.60
Firm	5.30	4.70	4.60	6.18	0.93	71.58	2.77	4.75
Firm	7.17	5.91	4.30	5.86	0.46	46.51	4.98	8.50
Firm	5.00	4.90	4.35	5.98	1.67	66.52	5.22	5.50
Firm	5.60	7.23	4.30	6.41	0.47	68.04	7.19	7.50
Firm	5.48	7.89	3.48	5.30	1.06	31.85	7.22	3.50
Firm	5.00	6.98	4.00	5.87	0.82	64.83	19.12	3.25

Table A12 Continued

Sample	APC log CFU/g	LAB log CFU/g	Mold log CFU/g	pH	TBA (mg/kg)	Nitrosomyoglobin conversion (%)	Residual Nitrite (ppm)	Overall sensory quality
Firm	5.70	5.18	4.33	5.57	0.51	84.78	5.37	5.00
Firm	5.70	7.29	4.68	5.68	0.56	41.00	3.79	4.75
Firm	6.48	7.66	4.00	6.09	1.22	37.38	3.95	7.00
Firm	4.70	4.70	4.65	6.23	2.04	25.23	3.56	7.75
Firm	4.70	7.21	4.00	5.39	1.85	35.25	15.01	6.50
Firm	6.40	8.19	4.16	5.38	3.01	87.44	19.67	6.25
Firm	6.30	5.74	4.40	5.08	2.66	73.78	2.05	2.75
Firm	5.18	7.66	3.30	6.07	1.92	42.94	3.08	7.25
Firm	5.18	7.02	3.48	5.92	1.74	27.98	4.27	5.00
Firm	4.70	5.00	3.48	5.12	1.30	31.83	2.61	5.25
Firm	5.70	7.65	4.66	5.11	1.85	75.20	4.66	6.25
Firm	4.70	8.04	4.23	4.83	1.55	52.64	2.13	4.50
Firm	5.00	5.00	5.23	5.80	3.20	66.44	6.48	6.50
Firm	5.00	5.00	4.16	4.53	1.24	67.13	3.00	5.25
Firm	7.67	7.47	5.31	4.94	2.11	70.15	6.48	6.50
Firm	5.70	5.00	5.15	4.66	2.71	41.46	7.54	8.00
Firm	5.00	5.25	4.46	4.66	3.31	64.78	18.09	6.75
Firm	6.35	5.00	3.74	4.84	1.20	15.43	3.54	4.99
Firm	5.18	4.70	4.11	5.48	1.05	38.32	15.36	8.93
Firm	6.88	4.70	4.57	5.67	2.36	48.34	11.27	5.78
Firm	5.70	4.70	3.88	5.39	3.34	24.16	7.00	4.99
Firm	5.40	5.30	4.98	5.94	1.41	47.01	3.18	8.14
Firm	5.30	4.70	4.40	5.58	2.98	40.55	5.72	6.83
Firm	6.60	4.70	4.00	5.81	1.63	70.79	6.00	6.56
Firm	7.35	5.00	4.18	5.78	2.73	47.10	8.31	7.61
Firm	7.39	4.70	3.93	5.04	1.74	32.75	11.27	5.51
Firm	7.67	5.40	4.54	5.64	2.08	51.61	21.99	5.25
Firm	4.70	4.70	4.62	5.30	1.09	26.28	6.18	5.51
Firm	6.04	5.74	3.95	5.52	2.26	56.44	4.36	6.56
Firm	5.54	6.03	4.41	5.54	2.95	85.58	4.54	4.72
Firm	5.00	5.18	4.95	6.04	2.93	45.05	4.09	6.30
Firm	5.88	4.70	4.32	5.62	3.18	65.74	17.26	6.30
Firm	6.48	4.70	3.95	5.86	2.74	77.83	22.62	8.40
Firm	7.43	5.18	4.18	5.01	1.20	32.64	3.54	6.83
Firm	7.56	4.70	3.40	5.77	0.86	57.32	8.18	6.83
Firm	7.68	4.70	4.80	5.74	1.06	84.48	4.91	5.51
Firm	6.23	5.48	4.53	5.57	0.59	40.48	5.36	8.40
Firm	5.65	5.75	5.53	5.25	0.83	29.40	2.45	7.09
Firm	4.70	7.04	4.30	5.65	0.65	69.61	12.09	6.25
Firm	5.18	8.23	4.66	4.90	0.72	62.51	10.27	7.25
Firm	5.00	4.70	4.94	4.94	1.20	27.66	13.35	5.50
Firm	6.74	7.70	3.70	5.07	0.51	69.11	28.60	7.70
Firm	7.13	5.25	4.31	5.03	0.77	28.56	20.07	4.40
Firm	5.70	5.30	4.10	5.29	0.69	66.81	7.11	6.60
Firm	6.78	4.70	3.70	5.83	1.58	65.17	8.27	2.89
Firm	7.06	7.43	3.98	4.87	0.81	67.18	9.80	3.50
Firm	5.70	6.22	3.95	4.88	2.36	57.20	7.11	5.25
Firm	4.70	5.00	4.08	4.67	2.88	41.40	9.64	6.00
Firm	5.00	5.30	4.22	4.60	1.25	31.60	8.09	3.41
Firm	6.00	4.70	4.04	4.90	1.57	78.65	4.50	7.00

Table A12 continued

Sample	TA	PA	PUT	Cd	HA	Dia	Ser	TYR	Spd	Sm
Trad	0.00	12.65	94.14	0.00	47.30	0.00	0.00	67.19	0.00	0.00
Trad	0.00	8.22	6.70	0.00	80.98	0.00	0.00	11.35	0.00	4.37
Trad	5.77	0.00	375.44	0.00	483.01	2.12	5.07	81.11	0.00	5.05
Trad	18.69	4.43	434.83	0.00	231.52	2.36	0.00	188.66	0.00	11.46
Trad	0.00	4.26	5.95	0.00	7.90	2.43	3.33	8.59	0.00	7.82
Trad	0.00	0.00	32.53	0.00	16.84	7.91	1.23	8.03	0.00	21.62
Trad	0.00	0.00	25.20	16.23	0.00	2.40	0.00	94.88	0.00	6.73
Trad	0.00	4.90	6.84	0.00	9.08	2.80	3.83	9.88	0.00	9.00
Trad	0.00	0.66	12.84	0.00	14.65	1.15	2.55	6.96	0.00	1.45
Trad	0.00	0.00	7.22	3.46	4.67	6.79	2.71	12.29	0.00	7.31
Trad	0.00	19.68	0.00	0.00	4.33	4.82	3.92	2.66	0.00	6.53
Trad	27.46	16.34	0.00	4.00	1.31	1.26	87.73	4.31	0.00	3.19
Trad	40.46	18.43	0.00	0.00	11.19	1.74	14.13	5.57	0.00	0.00
Trad	27.02	75.81	0.00	0.00	10.11	1.33	14.67	8.83	0.00	0.00
Trad	20.61	0.00	10.92	0.00	14.06	2.04	1.88	11.35	0.00	3.31
Trad	32.55	28.78	0.00	0.00	6.16	1.54	6.87	0.00	0.00	0.00
Trad	0.00	16.10	0.00	0.00	3.35	2.15	6.76	1.82	0.00	19.27
Trad	8.24	1.48	392.23	0.00	176.22	1.16	0.00	154.10	0.00	7.94
Trad	0.00	97.67	0.00	5.78	1.99	2.35	0.00	122.06	0.00	13.61
Trad	0.00	5.95	11.05	0.00	10.46	3.23	2.48	9.85	0.00	35.84
Trad	0.00	27.74	0.00	8.89	2.00	5.49	0.00	10.45	0.00	36.08
Trad	0.25	0.88	69.26	0.00	36.80	1.77	1.43	64.52	0.00	5.48
Trad	18.87	0.00	551.62	0.00	724.24	2.13	13.09	244.41	0.00	9.25
Trad	0.00	12.40	2.44	0.00	10.58	2.67	3.83	3.95	0.00	23.85
Trad	0.29	1.01	79.64	0.00	42.33	2.04	1.65	74.20	0.00	6.30
Firm	0.00	0.00	24.47	0.00	9.57	4.22	0.00	3.23	0.00	15.16
Firm	0.00	5.77	4.38	0.00	28.40	3.39	1.07	3.54	8.42	16.21
Firm	0.00	1.43	537.89	0.00	27.87	2.24	1.75	167.65	0.00	8.57
Firm	0.00	0.00	21.91	14.11	0.00	2.09	0.00	82.50	0.00	5.85
Firm	11.33	3.51	420.15	0.00	395.81	0.00	1.83	155.96	0.00	8.20
Firm	0.00	6.15	4.34	0.00	68.70	1.38	0.00	32.85	0.00	4.79
Firm	0.00	6.47	43.10	0.00	110.68	2.91	2.88	61.88	0.00	6.72
Firm	19.22	2.02	986.97	0.00	363.99	0.00	0.00	476.10	0.00	0.00
Firm	0.00	14.55	108.26	0.00	54.39	0.00	0.00	77.27	0.00	0.00
Firm	0.00	9.45	7.70	0.00	93.13	0.00	0.00	13.05	0.00	5.02
Firm	6.64	0.00	431.76	0.00	555.46	2.44	5.83	93.28	0.00	5.80
Firm	21.49	5.09	500.06	0.00	266.25	2.72	0.00	216.95	0.00	13.17
Firm	0.00	0.00	28.14	0.00	11.01	4.86	0.00	3.71	0.00	17.43
Firm	0.00	6.64	5.04	0.00	32.66	3.89	1.23	4.08	9.68	18.64
Firm	0.00	1.65	618.57	0.00	32.05	2.58	2.02	192.80	0.00	9.86
Firm	0.00	0.00	37.41	0.00	19.37	9.09	1.41	9.23	0.00	24.86
Firm	13.02	4.03	483.17	0.00	455.18	0.00	2.11	179.36	0.00	9.43
Firm	0.00	7.07	4.99	0.00	79.01	1.59	0.00	37.78	0.00	5.51
Firm	0.00	7.44	49.57	0.00	127.28	3.35	3.31	71.17	0.00	7.72
Firm	22.11	2.32	1135.01	0.00	418.59	0.00	0.00	547.51	0.00	0.00

Table A12 Continued

Sample	TA	PA	PUT	Cd	HA	Dia	Ser	TYR	Spd	Sm
Firm	27.60	4.66	0.00	0.00	15.52	3.46	2.55	8.55	0.00	3.21
Firm	15.21	0.00	0.00	0.00	13.95	1.74	7.14	7.59	0.00	0.00
Firm	48.73	2.26	0.00	0.00	7.06	0.00	72.08	5.48	0.00	0.00
Firm	23.49	65.92	0.00	0.00	8.79	1.16	12.76	7.68	0.00	0.00
Firm	17.93	0.00	9.50	0.00	12.22	1.78	1.63	9.87	0.00	2.88
Firm	19.13	0.00	70.27	0.00	7.94	1.38	1.14	7.81	0.00	0.00
Firm	27.66	9.56	0.00	0.00	13.02	1.01	1.27	4.95	0.00	1.28
Firm	19.49	0.00	9.55	0.00	11.83	1.42	1.97	9.36	0.00	2.56
Firm	28.31	25.02	0.00	0.00	5.36	1.34	5.97	0.00	0.00	0.00
Firm	53.52	0.00	20.29	0.00	12.64	1.64	22.28	10.81	0.00	2.27
Firm	0.00	0.76	14.77	1.48	16.85	1.32	2.94	8.00	0.00	1.67
Firm	0.00	0.00	8.31	3.97	5.37	7.81	3.12	14.13	0.00	8.41
Firm	0.00	22.63	0.00	0.00	4.98	5.54	4.50	3.06	0.00	7.51
Firm	31.74	5.36	0.00	0.00	17.85	3.98	2.93	9.83	0.00	3.70
Firm	17.49	0.00	0.00	0.00	16.04	2.00	8.21	8.73	0.00	0.00
Firm	56.04	2.60	0.00	0.00	8.12	0.00	82.90	6.30	0.00	0.00
Firm	31.58	18.79	0.00	4.60	1.51	1.45	100.89	4.96	0.00	3.66
Firm	46.53	21.19	0.00	0.00	12.87	2.00	16.25	6.41	0.00	0.00
Firm	22.00	0.00	80.81	0.00	9.13	1.59	1.31	8.98	0.00	0.00
Firm	31.80	10.99	0.00	0.00	14.97	1.17	1.47	5.70	0.00	1.47
Firm	22.42	0.00	10.98	0.00	13.61	1.63	2.26	10.76	0.00	2.94
Firm	61.55	0.00	23.33	0.00	14.54	1.88	25.62	12.43	0.00	2.61
Firm	16.41	0.00	479.67	0.00	629.78	1.86	11.38	212.53	0.00	8.05
Firm	9.14	2.62	112.03	0.00	267.01	3.09	9.87	193.02	0.00	9.34
Firm	2.27	7.60	3.97	0.00	8.27	3.17	6.73	3.02	0.00	21.14
Firm	0.00	4.35	12.59	0.00	14.79	2.38	2.90	71.55	0.00	12.62
Firm	0.00	13.34	5.60	0.00	2.92	2.58	3.52	2.40	0.00	17.53
Firm	2.56	0.00	526.20	0.00	827.42	0.82	0.00	188.62	0.00	4.99
Firm	0.00	10.79	2.12	0.00	9.20	2.32	3.33	3.44	0.00	20.74
Firm	26.45	0.00	26.43	0.00	1.52	6.62	0.00	5.45	0.00	18.79
Firm	1.06	3.07	154.08	0.00	19.30	2.43	3.81	113.86	0.00	11.51
Firm	110.36	0.00	799.08	0.00	478.16	0.83	3.17	275.01	0.00	8.07
Firm	10.51	3.02	128.83	0.00	307.07	3.55	11.35	221.97	0.00	10.74
Firm	2.61	8.74	4.57	0.00	9.51	3.65	7.74	3.47	0.00	24.31
Firm	0.00	5.00	14.48	0.00	17.01	2.74	3.33	82.29	0.00	14.51
Firm	0.00	15.34	0.00	6.43	3.36	2.97	4.05	2.76	0.00	20.16
Firm	0.00	18.52	0.00	0.00	3.85	2.47	7.78	2.09	0.00	22.16
Firm	9.47	1.70	451.06	0.00	202.65	1.33	0.00	177.21	0.00	9.13
Firm	2.95	0.00	605.13	0.00	951.53	0.94	0.00	216.92	0.00	5.74
Firm	30.41	0.00	30.40	3.04	1.75	7.61	0.00	6.27	0.00	21.61
Firm	0.00	112.32	0.00	6.65	2.28	2.70	0.00	140.36	0.00	15.65
Firm	0.00	6.84	12.70	0.00	12.03	3.71	2.85	11.33	0.00	41.22
Firm	1.22	3.53	177.19	0.00	22.20	2.80	4.38	130.94	0.00	13.24
Firm	126.91	0.00	918.94	0.00	549.89	0.95	3.64	316.26	0.00	9.28
Firm	0.00	31.90	10.20	1.02	2.30	6.31	0.00	12.02	0.00	41.49

VITA

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