ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

EFFECT OF PRE-CONCENTRATION TECHNIQUE ON THE EXTRACTION OF VOLATILE COMPOUNDS IN BEEF AND CHANGES IN QUALITY PARAMETERS UNDER DYNAMIC STORAGE CONDITIONS

Ph.D. THESIS

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İSTANBUL TEKNİK ÜNİVERSİTESİ★ FEN BİLİMLERİ ENSTİTÜSÜ

ÇİĞ ETTEN UÇUCU BİLEŞENLERİN EKSTRAKSİYONUNDA ÖN KONSANTRASYON TEKNİĞİNİN ETKİSİ VE DİNAMİK MUHAFAZA ŞARTLARI ALTINDA KALİTE PARAMETRELERİNİN DEĞİŞİMİ

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MAYIS 2018

Aylin MET ÖZYURT, a Ph.D. student of İTU Graduate School of Science Engineering and Technology student ID 506092504, successfully defended the thesis/dissertation entitled "EFFECT OF PRE-CONCENTRATION TECHNIQUE ON THE EXTRACTION OF VOLATILE COMPOUNDS IN BEEF AND CHANGES IN QUALITY PARAMETERS UNDER DYNAMIC STORAGE CONDITIONS", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my beloved family,

FOREWORD

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Aylin MET ÖZYURT
(Food Engineer, M.Sc)

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ABREVIATIONS

APC : Aerobic plate count
ANOVA : Analysis of variance
CAR : Carboxen
CASO : Casein-peptone soymeal-peptone
GC : Gas chromatography
DED : Direct extraction device
DHS : Dynamic headspace
DVB : Divinylbenzene
FAIMS : Field asymetric ion mobility spectrometry
FAO : Food and agricultural organisation
HS : Headspace
LAB : Lactic acid bacteria
MAP : Modified atmosphere packaging
MDA : Malondialdehyde
MS : Mass spectrometry
PCA : Principal component analyses
PE : Prediction error
PMDS : Polydimethylsiloxane
PID : Photon ionization dedector
PLS-R : Partial least square regression
PRESS : Predicted residual sum of squares
PTR : Proton transfer reaction
SDE : Steam distillation extraction
SPME : Solid phase microextraction
SSO : Spesific spoilage organism
TBARS : Thiobarbutyric acid reactive species
TVC : Total viable count
TD : Thermal desorber
VP : Vacuum packaging
VOC : Volatile organic compound

SYMBOLS

$\mathbf{A_{spiked\ sample}}$	**:** Peak Area of Target Analyte in the Spiked Sample
$\mathbf{A_{unspiked\ sample}}$	**:** Peak Area of Target Analyte in the Unspiked Sample
$\mathbf{A_{spike}}$	**:** Peak Area of the Spike Added Target Analyte
AU	**:** Absorbance Unit
a*	**:** Redness
cfu	**:** Colony Forming Unit
b*	**:** Yellowness
L*	**:** Lightness
p	**:** Probability Value
ppb	**:** Part Per Billion
R-Sq	**:** Square of the Correlation Coefficient
R	**:** Correlation Coefficient
RSD%	**:** Relative Standard Deviation
$\mathbf{t_{open}}$	**:** Total Door Open Time
$\mathbf{n_{cycle}}$	**:** Door Open-Close Cycle
T	**:** Storage Temperature (°C)
t	**:** Time (min.)
X-variance	**:** The Amount of Variance in the Predictors that is Explained by the Model

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EFFECT OF PRE-CONCENTRATION TECHNIQUE ON THE EXTRACTION OF VOLATILE COMPOUNDS IN BEEF AND CHANGES IN QUALITY PARAMETERS UNDER DYNAMIC STORAGE CONDITIONS

SUMMARY

Fresh raw meat present excellent nutrient composition for microorganisms and chemical/biochemical reactions. Meat approximately contains 75.0% water, 18.5% protein, 3.0% lipids, 1.0% carbohydrate and 1.0% inorganic material. Therefore, raw meat is one of the most perishable foods if it is stored under improper storage conditions. Microbial growth and enzymatic oxidation reactions are the main two mechanisms responsible for spoilage of meats. Rancidity and browning of red color are seen due to the lipid oxidation. Microbial activity on spoilage is more dominant than oxidation reactions for fresh beef. Glucose is the first substrate used in the metabolic pathway; lactate and amino acids are metabolized as second and third energy sources. As a consequence, of spoilage reactions, undesirable metabolic by-products are produced. Off-odor and gas formation, souring, slime formation on the surface, discoloration, pH changes and decomposition of the structural components are observed after microbial growth.

Microbial spoilage in meat product causes production of various volatile organic compounds (VOCs) such as aldehydes, ketones, alcohol, esters, fatty acids, sulphur compounds, aliphatic and aromatic compounds. Besides spoilage, these compounds play an important role for the production of off-odors and off-flavors. These undesired changes make the detection of VOCs an obligatory. Some common techniques are currently being used for the determination of VOCs in meat and meat products. These techniques are gas chromatography-mass spectrometry (GC-MS), gas chromatography-photon ionization dedector (GC-PID), selected ion flow tube mass spectrometry (SIFT-MS), proton transfer reaction-mass spectrometry (PTR-MS) and electronic nose etc. Dynamic headspace (DHS) and solid phase microextraction (SPME) are most commonly used pre-concentration methods for detection of VOCs because of their simplicity and rapidness. They can identify very wide range of volatile compounds.

This thesis consists of two main sections. In the first section of the study, it was focused on the effect of loading factor (sample amount: total volume ratio) in the container where meat samples stored by comparing the two pre-concentration techniques: Headspace (HS)-Solid Phase Microextraction (SPME) and gas flushing on Tenax tube. GC-MS and thermal desorber (TD)-GC-MS systems were used for further analysis of volatile organic compounds. The interaction of volatiles in headspace with adsorbent fiber is dependent on the amount of product in a specified volume due to the changes in the partial pressure and volatility of analytes. Additionally, differentiation quality

of different adsorbent is possible due to the variety in chemical structure of volatiles. Three different loading factors such as; 0.025 kg/L, 0.05 kg/L and 0.10 kg/L were studied. Both of two preconcentration techniques identified similar volatile organic compounds in the group of alcohols, aldehydes and ketones compounds. Loading factor affected the extraction efficiency both on fiber and Tenax significantly. However, while amines were measured only with Tenax, ethyl acetate was only detected with SPME method. Optimum loading factor for the best efficient extraction method depended on the type of the volatile compound. In general, loading factor of 0.05 kg/L was regarded as the most efficient extraction factor for both pre-concentration techniques for most of the volatiles and according to the total peak areas. It was noticed that the competitiveness was more existing in SPME due to the stationary conditions. Related with this, humid headspace may be a problem for SPME especially at high sample amounts. Higher recovery of analytes was obtained by thermal desorber-tenax sampling for all studied loading factor due to the forced flushing of the all headspace. The average recoveries with gas flushing on Tenax were between 70%-110% accepted as suitable for further analysis. It was continued with gas flushing on Tenax tube sampling technique coupled with TD-GC-MS identification system for the second part of the study and loading factor was selected as 0.05 kg/L based on the findings. The application of Tenax sampling method for volatile analyses of meat can be extensively studied in future works.

In the second part of the study, the volatile organic compound profile released from beef meat under the effect of isothermal and non-isothermal cold storage conditions of two temperatures, simulating the domestic conditions were investigated. Non-isothermal conditions such as 15 min. and 30 min. intervals between cooler door open-close cycles at two temperatures (0°C and 4°C) were studied. Fresh beef samples stored under aerobic conditions were monitored by analytical methods, and several volatile compounds found to be possible chemical indicators. Temperature fluctuations affected the intensity of some indicative volatiles significantly, parallel to off-odor development. Two different non-isothermal conditions mostly differentiated at 0°C storage temperature. Especially, 2,3-butandione, 3-hydroxy-2-butanone and 1-pentanol could be used as indicative volatiles for different cold storage conditions according to the statistical analysis.

For qualitative analysis of data, principal component analysis (PCA) classification method was applied to all GC-MS data by using sensory scores as predictors. 2,3-butandione, 3-hydroxy-2-butanone, 1-pentanol, 2-pentanone, 3-methyl-1-butanal were classified as common indicators of spoilage both for 0°C and 4°C conditions in PCA analysis. Discriminant analysis was also used for classification performance of GC-MS data on shelf life prediction as fresh, semi-fresh and spoiled state. Discriminant analysis were in consistence with PCA results.

For further quantitative analysis, partial least square regression (PLS-R) prediction models were applied for estimation of all quality parameters by using GC-MS volatile compounds data. *Pseudomonas* spp. counts were predicted well with GC-MS data using PLS-R model compared to other analytic parameters. Based on the PLS-R prediction model, predicted errors (log) distributed homogenously between 0.5 and 1.5 log (accepted range) with %70.83 (accepted percentage should be above 70% for good prediction) of all data both for 0°C and 4°C storage temperatures for *Pseudomonas* spp. counts.

Correlations between all quality parameters gave also a good approach for the identification of indicators. According to pearson correlation coefficients, hexanal did not show any relationship with thiobarbutyric acid (TBA) value unexpectedly both for 0°C and 4°C. Hue angle (°) together with TBA value showed very high correlations around 0.9 with ester compounds that formed at the end of the storage period. There was a strong negative correlation between overall acceptability scores and 2-butandione, 3-hydroxy-2-butanone and 1-pentanol volatiles with high coefficients of -0.761, -0.911 and -0.830, respectively for 4°C storage condition. For 0°C storage temperature, there was a strong correlation of overall acceptability with 2-butandione, 3-hydroxy-2-butanone, 2-heptanone, 1-pentanol, 3-methyl-1-butanol, 1-octen-3-ol, 2-ethyl-1-hexanol and 1-hexanol volatiles with high coefficients around 0.7-0.9. It shows that overall acceptability is mainly affected by microbial changes. Since no change was observed in hue angle parameter at 0°C, no linear relationship was observed with any of the volatile compounds.

Qualitative and quantitative analysis of volatiles are needed for developing freshness/odour sensors in order to give advice about meat quality non-invasively in consumer level and to ensure both quality and safety. On the other hand, decreasing food waste is an indirect result of these sensors. The application of this study can support the “freshness sensor” development for domestic use and be extended to different conditions in future studies. Also for the food industry, freshness and safety of fresh meat are usually considered as the most important issues. Therefore, characterization and validation of the volatiles may be replaced with the traditional methods and these methods could be used as a new rapid tool for assessment of frehness and microbial spoilage of meats.

ÇİĞ ETTEN UÇUCU BİLEŞENLERİN EKSTRAKSİYONUNDA ÖN KONSANTRASYON TEKNİĞİNİN ETKİSİ VE DİNAMİK MUHAFAZA ŞARTLARI ALTINDA KALİTE PARAMETRELERİNİN DEĞİŞİMİ

ÖZET

Çiğ kırmızı et, mikroorganizmalar ve kimyasal/biyokimyasal reaksiyonlar için uygun bir besin kompozisyonuna sahiptir. Et bileşimi yaklaşık olarak %75.0 su, %18.5 protein, %3.0 yağ, %1.0 karbonhidratlardan ve geri kalan eser oranda da inorganik bileşenlerden oluşmaktadır. Bu sebeple, uygun muhafaza koşulları altında korunmayan çiğ etler çok hızlı bozulabilir ürünler arasında yer almaktadır. Mikrobiyal gelişim ve enzimatik oksidasyon reaksiyonları etin bozulmasından sorumlu iki ana mekanizmadır. Yağ oksidasyonu sebebiyle acılaşma ve kırmızı renkte kahverengileşme meydana gelmekle birlikte, mikrobiyal aktivitenin bozulma üzerindeki payının oksidasyondan daha baskın olduğu söylenebilmektedir. Glukoz, metabolik izyolu üzerinde kullanılan ilk substrat madde olup, laktat ve amino asitler ikincil ve üçüncül enerji kaynakları olarak kullanılmaktadır. Substrat maddelerin mikrobiyal aktivite ile kullanılması sonucu bazı ön maddeler istenmeyen metabolitlere dönüştürülmektedir. Kötü koku ve gaz oluşumu, ekşime, yapışkan yüzey oluşumu, renk değişimi, pH değişimi ve yapısal bileşenlerin bozulması gibi problemler mikrobiyal gelişim sonrası meydana gelen değişikliklerdir.

Etin tazelik seviyesini hızlı bir şekilde tayin etmek, tüketiciler için zor olabilmektedir. Bu noktada, taze ürünün atık olarak atılması veya tersi bir şekilde taze olmayan etin kullanımı söz konusu olabilmektedir. Ev tipi soğutuculardaki sıcaklık set değerleri, soğutucu kapısını açıp kapama alışkanlıklarına göre tüketici davranışları çeşitlilik göstermekle birlikte, kötü kullanımdan kaynaklı sıcaklık dalgalanmaları altında ette kalite kayıpları kaçınılmazdır. Etin tazelik durumunun sensör gibi hızlı ve hasarsız yöntemler ile belirlenerek tüketiciyi bilgilendirmek, günümüzde artan alternatif araştırma alanları arasındadır. Ortamdaki uçucu bileşenlerin güvenilir bir şekilde ölçülmesi, kesinliği ve doğruluğu yüksek sensörlerin geliştirilmesi için önemlidir. Sensörlerin geliştirilmesi evresinde evsel kullanım şartlarını da simüle edebilmek amacıyla hem stabil saklama sıcaklığı hem de sıcaklık dalgalanmasının yüksek olduğu şartların incelenmesi gereklidir. Ek olarak, uçucu bileşenlerin ön-konsantrasyon basamağında etkin olarak ekstrakte edilmesi de farklı gıda yapıları için araştırılmalıdır.

Et ve et ürünlerinde mikrobiyal bozulma sonucunda çeşitli metabolitler meydana gelmekle birlikte bunların önemli bir bölümünü aldehitler, ketonlar, alkoller, esterler, yağ asitleri, sülfürlü bileşikler, alifatik ve aromatik uçucu organik bileşenler oluşturmaktadır. Bu metabolitlerin oluşturduğu kötü koku ve aroma değişimi sebebiyle uçucu organik bileşenlerin de bir kalite göstergesi olarak kullanılması önemli hale gelmektedir. Et ve et ürünlerinde uçucu organik bileşenlerin belirlenmesi

için yaygın kullanılan teknikler bulunmaktadır. Bu teknikler; gaz kromatografisi-kütle spektroskopisi (GC-MS), gaz kromatografisi-foton iyonizasyon dedektörü (GC-PID), seçilmiş iyon akış tübü kütle spektrometresi (SIFT-MS), proton transferi reaksiyon kütle spektrometresi (PTR-MS) ve elektronik burun olarak sıralanabilmektedir. Dinamik tepeboşluğu (DHS) ve katı faz mikroekstraksiyonu (SPME) ise en yaygın araştırılan ön-konsantrasyon teknikleri olup, geniş spektrumda uçucu bileşenleri tanımlayabilmektedir.

Bu tez çalışması iki ana kısımdan oluşmaktadır. Çalışmanın ilk kısmında, kontrollü bir hacim içerisinde, 4ºC'de muhafaza edilen kırmızı etten açığa çıkan uçucu bileşenlerin, iki farklı ön-konsantrasyon tekniği ile üç farklı yük faktöründe (örnek miktarı: tepe boşluğu hacmi) incelenmesi hedeflenmiştir. Çalışma kapsamında irdelenen iki farklı ön-konsantrasyon yöntemi: 1) Vial içinde tepeboşluğu-katı faz mikroekstraksiyonu tekniği, 2) Kontrol hacminde Tenax tüp üzerine gaz ile süpürme tekniği. Tenax tüp üzerine gaz ile süpürme tekniği bu tez kapsamında et uçucuları için ilk defa denenen bir yöntemdir. Bu ön konsantrasyon basamakları ile bütünleşik olarak, uçucu bileşen analizleri için sırasıyla GC-MS ve Isıl Ayrıştırmalı-GC-MS sistemleri kullanılmıştır. Etin muhafaza edildiği kontrol hacminin tepe boşluğunda biriken uçucu bileşenler ile adsorbe edici maddenin etkileşimi, maddenin kısmi basınç ve uçuculuk özelliklerindeki değişimlere bağlı olduğu için ürün miktarından etkilenmektedir. Ek olarak, farklı adsorban maddelerin ayırt etme kapasitesi uçucu bileşiğin yapısından kaynaklanan değişkenliklerden de etkilenmektedir. Her iki ön-konsantrasyon basamağı için etkisi incelenen üç farklı yük faktörü; 0.025 kg/L, 0.05 kg/L ve 0.10 kg/L olarak seçilmiştir.

Her iki ön-konsantrasyon tekniği de alkol, aldehit ve keton gruplarından benzer yapılarda uçucu organik bileşikleri tepeboşluğundan ekstrakte edebilmiştir. Yükleme faktörü hem katı faz hem de Tenax adsorbantları için ekstraksiyon etkinliğini önemli seviyede etkilemiştir. Bununla birlikte, aminler sadece Tenax ile ölçülürken, ester gruplarından etil asetat sadece katı faz mikroekstraksiyon (SPME) yöntemi ile tespit edilebilmiştir. En iyi ekstraksiyon için optimum yükleme faktörü uçucu bileşenin türüne göre değişmekle birlikte, her iki ön-konsantrasyon tekniği için de genel olarak 0.05 kg/L'lik yükleme faktörü, toplam pik alanlarına göre de değerlendirildiğinde en etkili ekstraksiyonu sağlamıştır. Katı faz mikroekstraksiyon yönteminde durgun denge şartları söz konusu olduğu için uçucu bileşenler arasındaki rekabetin daha belirgin olduğu gözlenmiştir. Buna bağlı olarak da yüksek nem içerikli tepe boşluğu koşullarında katı faz mikroekstraksiyonunun, yüksek yük faktörlerinde ekstraksiyon veriminde azalmaya sebep olduğu düşünülmektedir. Gaz ile Tenax tüp üzerine süpürme tekniğinde zorlanmış taşınımın etkili olduğu ve tanımlanan uçucu analitlerin geri kazanımının daha yüksek olduğu belirlenmiştir. Geri kazanım analizlerine göre, Tenax yönteminde %70-%110 aralığında geri kazanım elde edilerek, daha sonraki aşamada bu yöntemin kabul edilebilir bir yöntem olarak kullanılabileceği belirlenmiştir. Uçucu bileşenlerin tespit edilen toplam pik alanları kıyaslandığında, katı faz mikroekstraksiyon tekniği ile 0.05 kg/L yük faktörü için ~290x10^6 AU pik alanı elde edilirken, Tenax tüpü üzerine gaz süpürme tekniği ile aynı yük faktörü için ~390x10^6 AU ile daha yüksek pik alanı elde edilmiştir. Çalışmanın ilk basamağında elde edilen bulgulara göre, tezin ikinci aşamasındaki uçucu bileşen analizleri için gaz ile Tenax tüp üzerine süpürme ön-konsantrasyon basamağı ile birleştirilmiş ısıl ayrıştırmalı-GC-MS analiz sisteminde 0.05 kg/l yük faktörü ile devam edilmiştirr. Tenax tüpler ile ekstraksiyon yönteminin gelecekteki çalışmalarda farklı gıda

maddeleri için detaylı olarak araştırılacak yöntemler arasında yerini alacağı düşünülmektedir.

Çalışmanın ikinci aşamasında, ev tipi soğutucu kullanım profilini simüle edebilecek iki farklı muhafaza sıcaklığında (ortalama sıcaklık 0°C ve 4°C) izotermal ve izotermal olmayan (dinamik) koşulların yaratılarak, çiğ ette bozulmaya bağlı olarak ortaya çıkan uçucu bileşiklerin karakteristiğinin incelenmesi ve diğer kalite metrikleri ile ilişkilendirilmesi hedeflenmiştir. İzotermal olmayan koşulların etkisi iki farklı dinamik sıcaklık profili kullanılarak incelenmiştir. İki izotermal olmayan koşula ait şartlar; 1) soğutucu kapı açma-kapama sıklığı 15 dakika, soğutucu kapısı açık kalma süresi 15 saniye, 2) soğutucu kapı açma-kapama sıklığı 30 dakika, soğutucu kapısı açık kalma süresi 15 saniye olarak ayarlanmıştır. Veri analizleri ve verilerin sınıflandırılma aşamaları için temel bileşen analizi (PCA), diskriminant analizi, kısmi en küçük kareler regresyonu (PLS-R), genel lineer model (GLM) ve pearson korelasyonu analizlerinden faydalanılmıştır.

0˚C muhafaza sıcaklıklığında, genel tüketilebilirlik değerlendirmesine göre tüm koşullar için 4˚C koşullarına göre daha uzun raf ömrü değerleri elde edilmiştir. 4˚C, ve 0˚C izotermal olmayan koşullarda genel tüketilebilirlik değerleri, izotermal koşullara göre sırasıyla 24 saat ve 72 saat sonra yüksek oranda düşüş göstermiştir. 7 günlük muhafaza süresi içerisinde 0˚C şartlarında uçucu bileşenler, bozulma sürecinin yavaşlatılması sebebiyle 4˚C şartlarına kıyasla daha az sayıda ölçülmüştür. Ek olarak, iki izotermal olmayan koşul arasındaki farklılık 0˚C koşullarında daha belirgin olarak ayırt edilebilmiştir. 2,3-butandion, 3-hidroksi-2-butanon ve 1-pentanol bozulma periyodunda önemli seviyede artış göstermiş olup, dinamik muhafaza koşullarında da belirgin olarak değişimleri izlenebilmiştir. Temel bileşen analizi (PCA) sınıflandırma tekniğine göre; 2,3-butandion, 3-hidroksi-2-butanon, 1-pentanol, 2-pentanon ve 3-metil-1-butanal, 0˚C ve 4˚C muhafaza şartları için ortak bozulma indikatörü olarak değerlendirilebilmektedir. Diskriminant analiz tekniğinin, GC-MS verileri ile etteki taze, yarı-taze ve bozulmuş durumlarının sınıflandırılması performansında temel bileşen analizleri ile uyumlu göstergeler sunduğu belirlenmiştir.

Kısmi en küçük kareler regresyonu (PLS-R) tahminleme modeli ile uçucu bileşenlerin zamana bağlı değişim verileri kullanılarak, diğer kalite metriklerini (mikrobiyolojik, duyusal, renk ve oksidasyon ölçümleri) tahminleme gücü değerlendirilmiştir. *Pseudomonas spp*. sayısının PLS-R modeli kullanılarak GC-MS verileri ile tahminleme performansının diğer metriklere kıyasla daha yüksek olduğu belirlenmiştir. *Pseudomonas spp*. sayısı için PLS-R tahminleme modeline göre, tahminlenen hata (log), 0ºC ve 4ºC koşulları için mimimum ve maksimum olarak belirtilen 0.5 ve 1.5 log sınır değerleri arasında homojen olarak dağılım göstermiştir. Tüm datanın %70.83 oranında sınır değerler arasında kaldığı ve bu oranın kabul edilebilir düzeyde olduğu tespit edilmiştir. Belirtilen koşullar altında, ette *Pseudomonas spp*. sayısı uçucu bileşen analizi ile tahmin edilebilmektedir. Diğer kalite metrikleri için yapılan PLS-R modeli analizlerine göre tahminleme kalitesinin yeterince yüksek olmadığı gözlenmiştir. Pearson korelasyon katsayısı hesaplamalarına göre, 0ºC ve 4ºC koşulları için hekzanal'ın, tiyobarbutirik asit (TBA) değerleri ile herhangi bir ilişkisi olmadığı beklenmedik bir sonuç olarak elde edilmiştir.

Hue açısı (°) ve TBA değerlerinin, genellikle muhafaza sonunda ortaya çıkan ester bileşenleri ile 0.900 katsayı değerinde yüksek pozitif korelasyon gösterdiği belirlenmiştir. 4ºC muhafaza koşulları için 2-butandione, 3-hydroxy-2-butanone ve 1-pentanol uçucu bileşikleri ile genel tüketilebilirlik değerleri arasında çok güçlü negatif

korelasyon tespit edilmiştir. Bu korelasyon katsayıları sırası ile -0.761, -0.911 ve -0.830'dir. 0°C muhafaza şartlarında ise genel tüketilebilirlik puanları ile 2-butandione, 3-hidroksi-2-butanon, 2-heptanon, 1-pentanol, 3-metil-1-butanol, 1-okten-3-ol, 2-etil-1-hekzanol ve 1-hekzanol uçucuları arasında katsatyısı -0.700 - (-)0.900 arasında değişen yüksek negatif korelasyon belirlenmiştir. Bu uçucu bileşenlerin genellikle mikrobiyal gelişim kaynaklı olduğu düşünüldüğünde, genel tüketilebilirlikteki değişimlerin temel olarak mikrobiyal değişim kaynaklı olduğu söylenebilmektedir. Hue açısı parametresinde istatistiksel olarak önemli bir değişim gözlenmediği için herhangi bir uçucu bileşen ile ilişkisi kurulamamıştır.

Bu tez çalışmasına konu olan araştırmanın kapsamı, farklı et veya gıda türleri için başlangıç pH seviyesi, yağ oranı, orijini gibi farklı iç faktörlerin ve farklı sıcaklık aralıkları ve ambalajlama yöntemleri gibi dış faktörlerin etkisi de dahil edilerek yaygınlaştırılabilir. Bu çalışmanın benzer araştırma analojisi, bozulmanın hızlı tayin edilmesi amaçlı kullanılacak spektroskopik, kolorimetrik ve çeşitli sensör ölçüm teknikleri için de kurulabileceği düşünülmektedir.

1. INTRODUCTION

Meat and meat products are highly nutritious foods regarding their high content of protein, essential amino acids, minerals and vitamins. Due to their appropriate conditions for microorganisms and oxidation, they are also very susceptible to spoilage (Jay et al, 2003). The spoilage time of meat can be defined as reaching of bacteria to a maximum acceptable bacterial level or formation of an unacceptable off-odour/off-flavor or appearance (Chiofalo and Lo Presti, 2012). As a consequence of spoilage reactions, different metabolic by-products are produced. These metabolic by-products result with undesirable colour, odoriferous volatiles and textural changes on meat (Chiofalo and Lo Presti, 2012).

There are several sampling techniques to determine volatile organic compounds released from meat during spoilage. Solvent extraction, simultaneous steam distillation–extraction, static and dynamic headspace and solid phase microextraction (SPME) are commonly preferred techniques (Chiofalo and Lo Presti, 2012). SPME is a novel approach in sample preconcentration based on the partition of the volatile between fiber and the matrix (Xu et al, 2016). The concentration of the volatile compound on the fiber may be affected by the chemical properties of the analytes, equilibrium time, experimental conditions and concentration of the analytes (Martos and Pawliszyn, 1997; Agelopoulos and Pickett, 1998). Air sampling using Tenax with thermal desorption may serve high performance for extraction of many volatile organic compounds. Sorbent tube sampling can offer high recovery of polar compounds (Peng and Batterman 2000). While SPME is the passive preconcentration sampling technique, whereas gas flushing on Tenax tube with Thermal Desorption (TD) is the dynamic technique with forcing all headspace to absorbent material in a controlled volume.

Some parameters like analyte properties, concentration, equilibrium temperature and time were investigated in recent years to increase the efficiency of extraction (Pawliszyn, 1997; Gorecki et al, 1998; Zhu et al, 2003; Risticevic et al, 2009; Soria et al, 2015). However, studies on sample amount: total volume ratio (loading factor)

effect are very limited and not fully understood due to the interactions and competitiveness between volatile compounds in the complex food matrix. Up to date, there is no related research focused on meat volatiles.

In raw meat, different types of volatile metabolites are formed due to the spoilage reactions. Therefore, assessment of meat freshness is very important for consumers in order to use or discard to prevent intoxications/ poisonings and also to avoid food waste. Moreover, decrease in meat quality is inevitable during storage due to causing factors such as temperature setting and door open-close cycle of refrigerator which result with huge fluctuations in temperature. Identifying volatiles are essential for developing odor sensors to give advice about meat freshness for consumers. Therefore, this study was conducted to investigate the spoilage-cause odor compounds of raw beef stored at constant and fluctuating cold storage conditions in domestic refrigerators.

Storage temperatures below the optimum range for microbial growth have preventive effects on meat spoilage (Zhou et al, 2010). The effective monitoring of time and temperature conditions of meats during transporation and storage is critical for safety and quality (Nychas, 2008). Especially, fluctuating temperatures may stimulate the growth of microorganism and accelerate quality degradations in meat (Zhu et al, 2004). Several researches related with microbial growth, and quality degradation have been carried out to perform the significant effect of low temperature, temperature fluctuations or temperature abuses during handling of different cold stored meats (Li and Torres, 1993; Baranyi et al, 1995; Taoukis et al, 1999; Huang, 2003; Kotsoumanis et al, 2006; McMeekin et al, 2006; Gospavic et al, 2008; Margeirsson et al, 2012). However, there is no directly related research focused on meat volatiles under changing temperatures including refrigerator door open-close cycle.

In this thesis, it was aimed to investigate the volatile organic compounds of raw beef meat preserved at isothermal and non-isothermal cold storage conditions in domestic refrigerators at two different storage temperatures (0°C and 4°C) in order to find out the relationships with other quality parameters such as lipid oxidation, surface color, pH, sensory evaluation and microbiological assesments. GC-MS data that was obtained for different conditions were classified with Principal Component Analysis (PCA) and volatile organic compound data were subjected to factorial discriminant analysis for the classifying quality as fresh, semi-fresh and spoiled meat. For quantitative analysis, partial least squares regression (PLS-R) model was used to

predict the lipid oxidation, hue angle, pH value, microbiological counts by using GC-MS data. Before this above-mentioned study, different product loading amount for a control volume loading factor effect was investigated for two volatile sampling techniques such as 1) Solid phase microextraction and 2) Gas flushing on Tenax tube with Thermal Desorption (TD) to investigate the interactions and relationship between sample amount, type of analyte and extraction method. The outputs of this study were used in the second part of study.

Development of spoilage odour sensors are widely developing area to increase public health and maintain non-invasive quality inspection in consumer level and increase food safety awareness. Identification of the volatiles and their levels are needed for developing freshness/odour sensors for sensing meat quality non-invasively. To develop an odour/freshness sensor in domestic level, measurement of volatiles is critical in terms of correct sensing under different storage handlings such as amount of fresh product and volume of storage box in refrigerator. Therefore, it is believed that the findings of this research will serve data for the development and design of freshness/odor sensors systems working on volatile compounds.

The schematic outline of this Ph.D. thesis is presented in Figure 1.1.

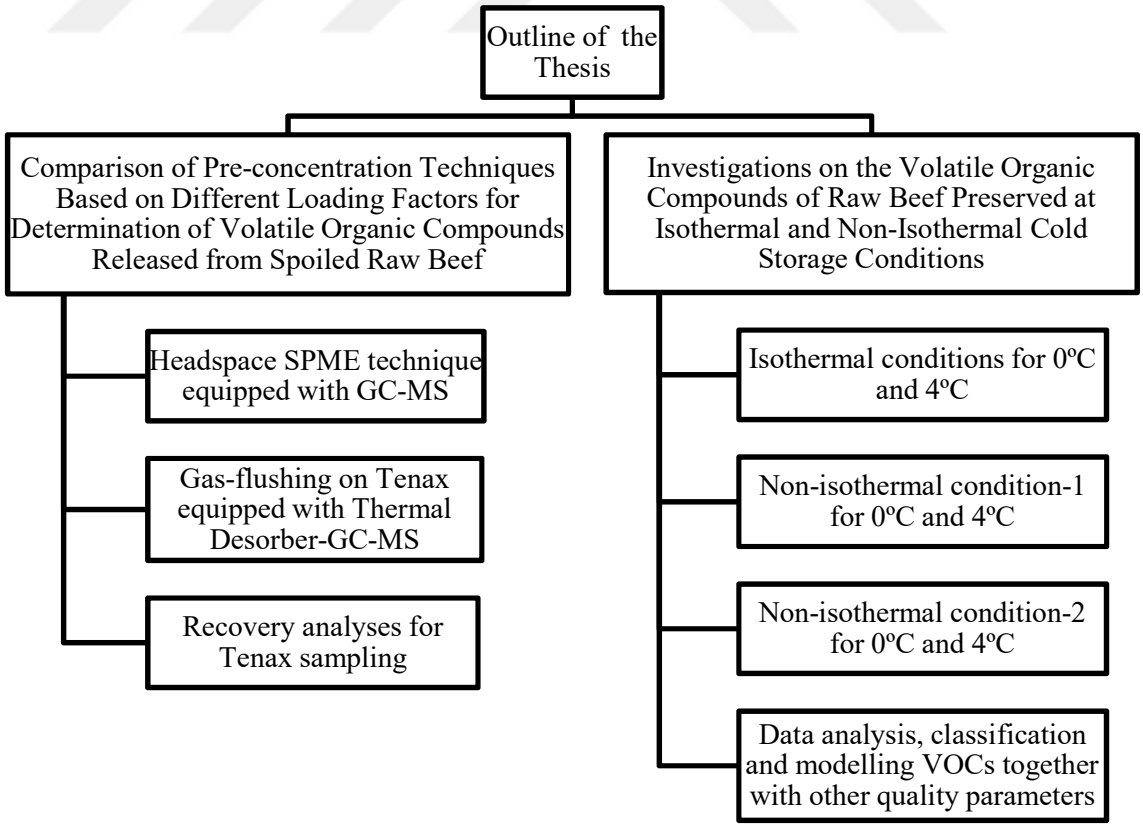


Figure 1.1 : Schematic outline of this Ph.D. thesis.

2. LITERATURE REVIEW

2.1 Meat Consumption in the World

In many parts of the world, meat makes up a considerable portion of a typical diet. It contributes protein, minerals, vitamins and fat intake in daily diet and these nutrients are important for their beneficial effects on our well-being. However, some components of meat, such as saturated fats, can cause negative health consequences. The contribution of meat and meat products is important for consumption of energy. According to global value and consumption of meat ni average, the daily intake per person is up to 110 g (De Smet and Vossen, 2016).

In FAO (2010) report, it has been reported that difficulties were present in estimating comparison of meat consumption in different countries because of missing data based on consumption of meat and level of meat export-import. Individual/household incomes affect the consumption of meat. When underdeveloped countries are compared with rich countries, a huge difference could be seen based on their budgets. Differences is due to social, economic, political, religious, and local factors.

Average total consumption of meat is 108 g and 72 g per day in The Great Britain. These values are 168 g and 107 g in the Ireland. Also, daily consumption of meat might vary according to gender. In Germany, total daily consumption of meat is 154 g for men, whereas it is 84 g for women. Greece has the lowest meat consumption in Europe. On the other hand, the highest value consumption of meat belongs to Spain in Europe (Grujic, 2015).

2.2 Composition and Health Effects of Meat

Meat includes fat, connective tissue, muscle, bone and water. Composition of meat can change depending on the size, breed, species, age, feeding type, and environmental conditions. A typical muscle consists of around 75% water, 20 protein, 3 fat and 2% soluble non-protein substances. Proteins are the major component of the dry matter of lean meat (Briggs and Schweigert, 1990).

Meat is an important source of protein for human body. Nine of the amino acids present in proteins are essential (or semi-essential) because the human body can not synthesize them from other compounds, and therefore must be taken by our daily diet. Meat contains generally high levels of the major essential amino acids, lysine, total sulfur amino acids, threonine, and tryptophan (Pellett and Young, 1990). The other important compound of meat is fat. Fat supplies very rich dietary source of energy and supplies essential fatty acids and precursors of compounds that regulate several physiological functions and helps to absorb A, D, E and K vitamins. Furthermore, fat is the most compact energy store of the body, it supplies fixation and protection of the organs and provides fatty acids which act as structural element of cell membranes (Colmenero, 2000).

Beside the macronutrients protein and fat, meat also consists of minerals, trace elements, vitamins and cholesterol. Meat includes iron, zinc and selenium which are important for human body because they cannot be synthesized in sufficient amount. For human nutrition, B vitamin is an important vitamin group supplied by meat. B_6, B_{12} and B_2 are the vitamins that can be found mostly in meat. Cholesterol is another component that is essential for human body. It should be taken in sufficient amounts for cell membranes and lipoproteins, and it is also working as a precursor for steroid hormones and bile acids (Maurice et al, 1994).

Due to nutritious and complex composition of fresh meat, it is one of the most perishable foods, and is also ideal for the growth of a wide range of spoilage bacteria. If appropriate food safety, food preservation and food storage techniques are not applied, meat become unappetizing, poisonous or infectious. Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which originate from the animal itself, or by the people handling the meat.

The chemical composition of typical meat muscle given in Table 2.1.

Table 2.1 : Chemical composition of meat muscle (modified from Argyri (2010)).

Components	Weight (%)
Water	75.0
Protein	19.0
Myofibrillar	11.5
Sarcoplasmic	5.5
Connective tissue	2.0
Lipid	2.5
Carbohydrate and lactic acid	1.2
Lactic acid	0.90
Glucose-6-phosphate	0.15
Gylcogen	0.10
Glycose and glycolytic intermediates	0.05
Soluble non-protein compounds (Nitrogenous and inorganics)	2.3
Vitamin	Traces

2.3 Quality Parameters of Fresh Meat

Fresh meat quality varies upon consumers in different countries. Consumer preferences are directly related to appearance, smell, taste and quality of meat. In addition, fresh meat quality components can be defined based on basic and scientific factors such as nutrients, pigments, water retention capacity, softness, hydration, aroma, deterioration and contamination (Troy and Kerry, 2010).

Factors such as meat color, amount of fat, meat texture determine meat appearance. Appearance quality traits (AQT) cause the consumers strongly prefer meat during purchase. The softness, flavor and hydration are gathered under the name of eating quality traits (EQT). Meat reliability, nutritional value, animal health, price, product presentation, origin and brand are introduced as reliance quality traits (RQT). AQT and EQT are known as internal factors related to meat and RQT is known as external factors (Joo et al, 2010).

Color acts as the most noticeable change during meat spoilage. The most important parameters are provided by myoglobin, which gives color to meat. Immediately after the meat is cut off, myoglobin, which contacts with oxygen, is converted into

oxymyoglobin, allowing the meat to regain its last bright red color. As the animal get older, an increase in the concentration of myoglobin is observed, which gives the meat a dark red appearance. The meat of bull (more hemoglobin), cattle and sheep (more myoglobin), is dark red. However, some type of animals such as old cows and oxes have light red meat color. Factors such as type and gender of animals indicates the color of meat (Faustman et al, 2010).

The most important feature that is required in the structure of meat is juiciness and tenderness. These texture properties depend on animal sex, age, gender, etc. (Park et al, 2002). It is expected that the meat will break up easily and leave no residue between the teeth. It is not desired to be too flexible or too hard. If the age of the animal is young, the meat obtained is tender. Thus, the amount of connective tissue is less in young animals than in older animals. Also after cutting, meat is hard during rigor mortis. Meat in rigor mortis should be stored at certain temperature and time (0- 4°C, 3-4 days), for the dissolution of rigor mortis in order to be tender (Ali et al, 2008).

Volatile compounds (aldehydes, ketones, esters, alcohols, benzene compounds, furans, lactones) and non-volatile compounds that are formed in meat identify taste and flavor of meat products. Under normal conditions, raw meat smells blood and taste is sweet-salty (Joo et al, 2010). By heat treatments, specific taste and odor of meat are obtained.

2.4 Deterioration Process of Meat and Affecting Factors

Shelf life is the length of time that a commodity may be stored without becoming unsuitable for use, consumption, or sale. The reason for not consuming is due to the development of microorganisms and undesirable chemical reactions. Shelf life depends on bacteria level, type of bacteria and the population. Shelf life is affected by some factors which affect microbial population and activation such as temperature, atmosphere, pH and salt concentration.

Various conditions influence the microbiological status of carcass meat such as the physiological state of the animal during the slaughtering process, contamination at the slaughtering site, temperature of the environment and post-cutting storage and distribution (Nychas and Drosinos, 2014).

The surface area of meat is another parameter for the deterioration. If the surface area of the meat is too high, there is a difficulty in maintaining the quality, which is caused

by the contact with oxygen and the increase of microbial spoilage, increase in oxidation reactions (Perez et al, 2008).

If slaughtering process does not take place under controlled conditions, gram-positive, gram-negative bacteria and yeast infections can occur on the surface of the meat and cause serious problems. Contamination occurs either from the environment or from the intestine of the animal. *Enterobacteriaceae* members and psychotropic bacteria have been reported as the source of contamination from slaughtering area. The number of bacteria commonly encountered in red and poultry meat decreases with the cooling conditions.

The microbial growth occurs in meat as a result of improper storage conditions and insufficient packaging procedure. Microbial quality in raw meat is also affected by several factors such as the physical condition of the animal at the time of slaughtering, meat processing, transfer and storage. Low microbial quality in meat causes reactions such as fat oxidation and autolytic enzymatic reactions with nutrients and inappropriate pH conditions in the meat (Casaburi et al, 2015)

When food spoilage occurs, microbial growth generally varies depending on environmental conditions such as food processing, storage, and distribution. Environmental factors are grouped under five main topics. These are;

- Factors that are inherent in the meat (pH, water activity, natural antimicrobial components, Eh, redox capacity, nutrient composition)
- Food processing factors
- External parameters (temperature, relative humidity, gas atmosphere composition)
- Indigenous factors (inhibitory properties of other organisms)
- Interactions of all betting parameters with each other

Growth of microorganisms can be identified for different atmospheres and packaging conditions. For example, under cold temperatures, high relative humidity and aerobic storage conditions, *Pseudomanas spp*. is the main deterioration source in meat. Gram-positive bacteria (LAB and *Brochothrix thermosphacta*) are reported as the main microorganism in modified atmospheric packaging with high content of oxygen in cold storage conditions (Nychas and Drosinos, 2014).

Jay et al. (2003) listed the microorganisms in the raw meats and they are shown in Table 2.2.

Table 2.2 : Isolated microorganisms from raw meat (Jay et al, 2003).

Bacteria	Isolation Frequency	Mold and Yeast	Isolation Frequency
Acinetobacter	XX	*Alternaria*	X
Aeromonas	XX	*Aspergillus*	X
Alcaligenes	X	*Cladosporium*	XX
Bacillus	X	*Fusarium*	X
Brochotrix	X	*Geotrichum*	XX
Citrobacter	X	*Mucor*	XX
Clostridium	X	*Penicillium*	X
Corynebacterium	X	*Rhizopus*	XX
Enterobacter	X	*Sporotrichum*	XX
Enterococcus	X	*Thamnidium*	XX
Escherichia	XX	*Candida*	XX
Flavobacterium	X	*Cryptococcus*	X
Kuthia	X	*Debaryomyces*	X
Lactococcus	X	*Hansenula*	X
Lactobacillus	X		
Leuconostoc	X		
Listeria	X		
Micrococcus	X		
Moraxella	XX		
Pseudomonas	XX		
Psyhcrobacter	XX		
Salmonella	X		
Serratia	X		
Staphylococcus	X		
Weissella	X		

XX: Frequently identified microorganism, X: Identified microorganism

Three important species of the *Pseudomonas* that form in the aerobic cold storage conditions are *P. fragi, P. fluorescens* and *P. lundensis*. When the number of colony forming units exceeds 7 log cfu/g, they form undesired taste components in meat. The deterioration of the meat stored under modified or vacuum atmospheric conditions is less than in aerobic conditions. Growth of gram negative bacteria is difficult because of the differences in the levels of oxygen and carbon dioxide in storage conditions frequently used in provide long shelf life. The main microorganisms causing

degradation are *Lactobacillus sakei, Leuconostoc* and *Weissella viridescens* (Nychas and Drosinos, 2014).

According to some studies, microorganism growth is not observed at temperatures below freezing (especially below -8°C) (Joo et al, 2010). Another important case is the type and size of the degradation state depends on the amount of bacterial substrates in the meat. Most of the microorganisms prefer to grow even though the meat is kept in different conditions such as aerobic, vacuum or modified atmospheric conditions. In the case of decrease in glucose, microorganisms consume amino acids and other nitrogen components, which result with secondary metabolic reactions (Nychas and Drosinos, 2014).

After slauhtghering process, meat is generally contaminated up to 2-4 log cfu/g level (Irkın et al, 2011). Eventhough the positive relationship between spoilage and microbial level is known, the threshold microorganism levels are not found in literature clearly. Irkın et al. (2011) stated that the TVC limit was generally accepted as 6.7, 7 or 8 log cfu/g for spoilage in literature. Berruga et al. (2005) accepted the 6-7 log cfu/g of TVC level as criticial for spoilage. Some researchers found out that when *Pseudomonas* spp. level reached to 7-8 log cfu/g, off-odors and slime formation is occured in meat (Gill and Newton, 1977; Olaoye and Ntuen, 2011). For Koutsoumanis et al. (2006), this level is accepted as 7 log cfu/g. In the study of Ellis and Goodacre (2001), when the TVC level increased to 8 log cfu/g levels ammonia, dimetil sulphide, diacetil were measured as off-odor compounds.

Pseudomonas spp. in the aerobic conditions, *Brochothrix thermospacta* in the aerobic and anaerobic conditions, lactic acid bacteria and *Shewanella putrefaciens* for the vacuum and modified atmosphere conditions are reported as dominant microorganisms (Doulgeraki et al, 2012; Ercolini et al, 2006). Detected and isolated bacteria groups from raw meat during storage at different packaging conditions are given in Table 2.3 (Casaburi et al, 2015).

Table 2.3 : Bacteria genera that are isolated from meat for different storage conditions (Casaburi et al, 2015).

Gram-positive bacteria	Storage Conditions			Gram-negative bacteria	Storage Conditions		
	Air	Modified Atmosphere	Vacuum		Air	Modified Atmosphere	Vacuum
Bacillus	+		+	*Achromobacter*	+		
Brochotrix	+	+	+	*Acinetobacter*	+	+	+
Carnobacterium	+	+	+	*Aeromonas*	+		+
Corynebacterium	+		+	*Alcaligenes*	+	+	+
Clostridium			+	*Alteromonas*	+	+	+
Enterococcus	+	+		*Camplylobacter*	+		
Kokuria	+			*Chromobacterium*	+		
Kurthia	+			*Citrobacter*	+	+	
Lactobacillus	+	+	+	*Enterobacter*	+	+	
Lactococcus	+			*Echerischia*	+		
Leuconostoc	+	+	+	*Flavobacterium*	+		
Listeria	+	+		*Hafnia*	+	+	+
Microbacterium	+	+	+	*Klebsiella*	+		
Micrococcus	+	+		*Kluyvella*	+		
Paenibacillus	+			*Morexella*	+		
Staphylococcus	+	+	+	*Panteoa*	+		+
Streptococcus	+	+		*Proteus*	+	+	
Weissella	+	+	+	*Providencia*	+	+	+
				Pseudomonas	+	+	+
				Serratia	+	+	+
				Shewanella	+		
				Vibrio	+		
				Yersinia	+		+

2.5 Preservation Conditions

The rich nutritious food media of the meat makes it suitable for the development of microorganisms. Along with microbial growth, meat deterioration and the development of food-borne pathogens are developed. Thus, various methods have been applied to preserve the meat. The main purpose of different preservation methods is to eliminate the growth of microorganisms and slow down the physical, chemical and biochemical reactions as much as possible. Precise temperature control, modified atmospheric packaging and vacuum packing are the most familiar protection methods.

Modified atmosphere packaging for meat usually has a high oxygen ratio of 70-80%. It is not desirable because the level of carbon dioxide is below 15% and it does not prevent the growth of microorganisms. The ratio of carbon dioxide between 20-30% is preferred to increase shelf life of the meat (Lorenzo and Gomez, 2012).

A conducted research showed that, eventhough retailing with vacuum packaging resulted with undesirable appearance in meat due tooxidation in oils and reduction in proteins. Color stability was maintained throughout the storage period. In high-oxygen containing environment and meat that was covered with packaging material, the redness was reduced and the number of aerobic bacteria, TBARS value and carbonyl content lead to reduced oxygen content and reduced meat quality. In the case of modified atmosphere conditions with low oxygen and high carbon dioxide levels, oil oxidation and microbial growth have been delayed compared to high oxygen modified atmospheres. Sensory development, redness, and carbonyl contents were found to be the same in packaged meat with high oxygen modified atmosphere (Lorenzo and Gomez, 2012).

Each factor or combination of them affects the color, texture, smell, and taste of the meat. The storage of the meat can be examined in three categories. These can be categorized by meat temperature control, moisture control and inhibition processes (bactericidal, ionizing irradiation and packaging). To prevent spoilage, there are many protective methods that are applied to meat. These are classified as thermal, non-thermal, novel technologies and hurdle technologies (combined techniques) (Zhou et al, 2010).

Most common novel technologies include high hydrostatic pressure, modified atmosphere packaging, active packaging with use of natural antimicrobial compounds, chemical preservatives or bio-control agents like essential fats, chitosan, nisin and lysozyme to take the place of chemical protectors. Thermal ones are cooking, cooling, sous wide method, freezing and most common non-thermal technique is high pressure processing (HPP) (Zhou et al, 2010).

The hurdle technology involve combinatorial processes in which protection methods are combined, and is known as combinatorial protection. The most important barrier level (low or high) in food preservation is water activity, acidity, redox potential, preservatives, and competitor microorganisms. Together with the implementation of new non-thermal technologies, shelf life can be extended for weeks (Zhou et al, 2010).

Cold storage does not improve the current quality but it provides continuance of the quality as in the initial time. It is known that the most important external factor on the meat spoilage is the storage temperature (McDonald and Sun, 1999).

According to national standards (TS 6160; TS11566), recommended storage temperature is between 0-4°C. James et al. (2008) remarked that the increase in storage temperature even 1°C can result serious quality problems. James and James (2002) indicated that the 2-3°C temperature increase during storage may decrease half the shelf life of meat. Temperature level may affect both the spoilage characteristic and spoilage rate. While lower temperatures can supress the mesophilic microorganisms, pyscrotrophs may dominate on the media. The spoilage rate is slowed down in the cold conditions due to the slower growth of pyscrotrophs (Adams and Moss, 1995). Effect of cold temperatures on the spoilage rate depends on microorganism type (gram positives are more sensitive than gram-negatives), the growth phase of microorganisms (cells in the log phase is more sensitive than the cells in the stationary phase), cooling rate (high temperature differences can damage to cells more), and growing media (microorganisms are more durable in the complex media) (Adams and Moss, 1995).

It is known that temperature is the most important factor for preserving meat. Although many countries have set a maximum temperature limit, this rule has been violated. Meat distribution, retail storage and consumer-side storage at temperatures above 10°C are not suitable (Giannakourou et al, 2001).

The positive properties of packing at undesirable temperature conditions are reduced if it is packed even under modified atmosphere or vacuum. It is reported that temperature fluctuation and high temperature abuse may occur during storage and transfer of meat to the stores and retail sale (Zhu et al, 2004). In most countries, maximum temperature conditions for refrigerator conditions are regulated, but this is often dearranged (Koutsoumanis et al, 2006).

In the study performed by Koutsoumanis et al. (2006), the constant temperature near to general storage temperature of about 4°C and fluctuated temperature condition that simulate the home storage condition was compared. 19 units of thermal changes were observed during the meat preservation in the refrigerator at home. Total viable counts (TBC), lactic acid (LAB) and gram-negative bacteria were observed at storage

temperatures of 4.3, 8.1 and 15.5°C. The lag phase time (LPD), the maximal growth rate (μ_{max}) are given in Table 2.4 with standard error and specification coefficient. From Table 2.4, it is understood that the growth rate of lactic acid bacteria is higher than that of gram-negative bacteria. CO_2 use in MAP allows the growth of bacteria such as *Lactobacillus spp.* and *Leunostoc spp.* CO_2 concentration at high levels and under cold storage conditions help prevention of food pathogens than vacuum pouches (Ercolini et al, 2006).

The transition kinetics were applied to the obtained data to find the threshold time. The maximum acceptable times with each storage temperature are; 9.27±0.72 days at 4.3ºC; 5.71±0.63 days at 8.1ºC; 2.06±0.13 days at 15.5ºC. Threshold times were projected by Koutsoumanis et al. (2006) by modeling growth curves.

Table 2.4 : Lag phase duration and maximal growth rate (μ_{max}) obtained by the Gompertz equation of total bacteria counts (TBC), lactic acid bacteria (LAB) and Gram-negative bacteria in minced beef stored at 4.3, 8.1 and 15.5ºC (Koutsoumanis et al, 2006).

	T(°C)	Lag Phase Duration (days)	μ_{max}
TBC	4.3	6.62	0.31
	8.1	2.98	0.40
	15.5	1.19	0.84
LAB	4.3	6.34	0.26
	8.1	1.11	0.50
	15.5	0.15	1.31
Gram negative	4.3	6.03	0.16
	8.1	3.12	0.28
	15.5	1.24	0.60

It was indicated that the increase in the color index (hue angle) depends both on the storage temperature and the storage period. Higher the storage temperature higher the hue angle. This increase was explained by the accumulation of myoglobin oxidation and methmyoglobin over time (Mancini and Hunt, 2005). Hexanal and TBA is known as oil oxidation product in meat. TBA formation increase over time. At the highest storage temperature of 15.5°C, the fastest rate was found, while the slowest rate was observed at 4.3°C (Koutsoumanis et al, 2006).

Under fluctuating temperature conditions, *Clostridium perfringens* is another important microorganism observed in beef. Infection with *C. perfringens* in red meats is one of the most important problems. The thermostability of these microorganism spores is higher than that of foodborne pathogens such as *Salmonella* and *Listeria*. Even heat treatment or cooking is applied, they can survive on ham, canned beef and beef (Craven, 1980). *C. perfingens* can easily survive between 6 and 52°C. They can easily multiply on inevitable temperature increases during distribution, storage and retail operations. In order to prevent growth, food producers adopt the necessary precautions by taking into account the temperature and duration (Huang, 2002).

Considering microbiological development as a key study in terms of model comparisons, an increase from 30°C to 45°C has been seen to have a definite effect on the growth of microorganisms logarithmically. EGT (equivalent growth time) concept was used to easily see temperature fluctuations. Unlike other simple methods, the EGT method used does not show microbiological development only in isothermal conditions. The preferred method for monitoring temperature fluctuations has been achieved with working temperature variables (Huang, 2002).

Dainty and Mackey (1992) conducted a study about total viable counts and volatile compounds formed in commercially processed, raw chicken parts during storage at 4°C and 13°C and under simulated temperature abuse conditions. Bacterial contamination during cutting in poultry quality, processing effect, conditions and storage time, etc. cause an increase in bacteria count at pre-consumption stages. The variety of seasonal bacteria on the carcass makes it difficult to determine the storage quality. Microbial load counting or odor characterization is easier. When the aerobic plaque count reaches to 7-8 log cfu/g, consumption of chicken becomes inappropriate (Dainty and Mackey, 1992). When the number increases to this level, proteolysis and odor production occur. The surface smell is noticed by the fact that *Pseudomanas* and *Acinebacteria* population reaching to 6-7 log cfu/g (Cox et al, 1975).

In a study conducted by Senter et al. (2000), it was desired to establish an association between the volatile components in the chicken and the aerobic plaque count under a spesific temperature and time within the commercial processing and distribution chain. The average number of aerobic plaques (APC) was 4.08, 4.08, 3.96 and 3.52 log cfu, respectively, in the first day of each sample type. At the end of the 5^{th} day, the values obtained in chicken meat stored at 4°C were 6.57, 4.45, 7.31 and 8.38 log cfu in the

same order. In the samples stored at 13°C APC increased at a faster rate than expected. APC values obtained at the 2nd day were 7.36, 7.94, 8.87 and 8.19 $\log_{10}$ cob in boneless chest with skinless breast fillet, butt, wing and skin, respectively. Samples subjected to undesired temperatures (1 day at 4°C / 1 day at 4°C / 3 days at 13°C) are similar to APC at approximately 13°C for 2 days. The significant increase in the volatile increase in the chicken sections stored by applying the temperature fluctuation was greater than the samples analyzed in other conditions. An inconsistent relationship between microflora and volatile components was observed. Volatile compounds can be used as a means to determine the quality of the case in the event of temperature fluctuations, because the number of bacteria was at a level which causes degradation (Senter et al, 2000).

Growth of *Brochothrix thermosphacta* at changing temperature is another study about microbiological analysis on meat deterioration by changing temperatures (Baranyi et al, 1995). A dynamic model was developed at 5-25°C which estimated the change in microorganism count in the case of temperature decrease or increase gradually. As expected, the growth momentum continued to accelerate in sudden temperature increase. Sharp increase and decrease was seen in the growth curves.

Vacuum packed poultry meat was stored at 4, 8 and 12 ° C for up to 35 days after being subjected to different pressure conditions (400-600MPa) and retention times (1, 2 and 10 min.). The change in the total number of microorganisms in the applied pressure ranges was examined and the dominant microorganism was found as *Weissella viridescens*. The effect of temperature and retention time on microbial count within 35 days was investigated. It was observed that there was not much difference between storage at 8 and 12°C in samples with 1 and 2 minutes of exposure time of 600 MPa. As expected in the examples, the number of colony forming units was always higher in 8 and 12°C than those stored at 4°C. The number of colony forming units in the pressure-applied samples was always below 8.5 $\log_{10}$/g. The pressure was 600 MPa for 10 minutes and the sample was stored at 4°C. After exposure, it was found that the meat had at lowest microbial load for 35 days (Patterson et al, 2010).

Temperature abuse affects different meat types by different ways, and temperature abuse effects was studied on the quality of irradiated pork loins. Temperature abuse is a major problem in large scale distribution and storage of meat, especially in export situations. It is often encountered in such cases like the removal of meat from vessels

and the evacuation or the transfer of goods from trucks to retail sales. Temperature fluctuations cause meat degradation and changes in meat quality in the case of irradiation, transfer and post-storage (Labuza and Fu, 1995).

By irradiation, the DNA of the bacteria is damaged, but some bacteria found in the meat are multiplying by repairing their damage. This is especially true in case of temperature abuse (Lee et al, 1996). It has been found that temperature fluctuations accelerate the proliferation of *Listeria monocytogenes* in ready-to-eat turkey meat products (Bisha et al, 2003).

Temperature abuse accelerates enzymatic and chemical reactions that affect shelf life of meat in addition to microorganism proliferation. With elevated temperature, muscle proteins are converted into peptides with small molecular weight by protease activity (Gill, 1996). In addition, high temperatures accelerate oil oxidation.

In a study, meats exposed to an electron beam of 0, 1.5 or 2 kGy were subjected to 3 different temperature treatments: Placed in the refrigerator after irradiation in procedure 1. The treatment is allowed to stand at room temperature 3 hours before the refrigerator at 2. In treatment 3, samples stored at 4°C on three consecutive days were subjected to room temperature once a day. At the end of the experiment, it was noticed that temperature abuse did not cause color, oxidation and significant effect on the irradiated meat. In fact, temperature abuse has increased water retention capacity in meat due to the water that is likely to become hydrolyzed during high temperature. Temperature abuse had little effect on irradiated pork quality factors (Zhu et al, 2004).

In another study, the meat deterioration was investigated by means of potential NIR and MIR spectroscopy on the surface treated at different temperatures. The ground beef was packed with high oxygen modified atmosphere (30% CO_2 and 70% O_2) and stored at 3 different temperatures (4.3, 8.1 and 15.5°C). Spectra were collected from FT-NIR and FT-IR devices and the obtained data were applied to PCA. According to the results, the storage temperature of the modified atmospheric packing was found to be 6-7 days at 4.3°C, 8.1°C for 2-3 days at refrigerator conditions and less than 1 day at 15.5°C for temperature exploitation (Sinelli et al, 2010).

In a similar study parallel to this study, electronic nose color change, TBA decision, CO_2 production, volatile components were determined in ground beef. In the study,

shelf life was determined to be 9 days at 4.3°C, 3-4 days at 8.1°C and 2 days at 15.5°C (Limbo et al, 2010).

Temperature affects maximum deterministic growth rate of microorganisms and lag phase process. The primary model describes the time-course changes in the microbial population, while the secondary model describes the influence of environmental factors for the kinetic parameters (Whiting, 1995). For all these, development of a predictive model for spoilage of cooked cured meat products and its validation under constant and dynamic temperature storage conditions were investigated. For this purpose, sliced cooked meat was used. Models were adjusted to fixed and varying temperatures. The lag phase decreased with increasing temperature and the growth was accelerated. While the growth rate was increased as the temperature increased suddenly; the growth rate was slowed down as the temperature decreased suddenly. Another result is that the specific growth rate was found to increase with increasing temperature. At 4°C in the range of 0-12°C, specific growth rate was doubled. Physicochemical changes were observed under the storage conditions in the temperature range and it was found that the modified Gompertz model was used as the primary mode of effect on the microorganisms causing the deterioration of temperature (Mataragas et al, 2006).

Effect of temperature fluctuations were observed in pork meat that were kept for 12 hours at 0°C, 6 hours at 10°C, 6 hours at 15°C, and the change in microorganisms was observed at these conditions. If only the temperature effect was observed (except model conformity), all microorganisms increased their growth rates with temperature increase. The sudden rise in temperature increased the growth rate, while in the case of sudden falls, growth continued to slow down. As expected in pork, *Pseuodomonas* became the predominant microorganism and the most populated colony. *Enterobacteriaceae* showed slow growth over time (Koutsoumanis et al, 2006).

Cooling is an important factor to prolong shelf life of meat and prevent microbial contamination. In Spain, the application of cold treatment to most meerschaums is accomplished by transfering the meat to 0-2°C immediately after carcass processing. The study has been done to maintain meat quality at different temperature ranges. When the cooling temperature was low, the weight loss of the carcass reduced. Biochemical and structural changes in the meat is very important for meat quality within the first 24 hours after death. From this point of view, the softness of meat has

come to the question of which treatment application is provided. When the internal temperature of the carcass meat falls below 10°C and when the pH is high (pH>6.2), the cold shortening occurs within 10 hours and the meat hardens. In another research, the conventional method (24 hours at 2°C), ultra-speed (2°C for up to 24 hours at -20°C after 3.5 hours) and slow cooling (12°C for up to 7 hours, 2°C for up to 24 hours) were applied. The total viable count was lower than that of the other carcass meat in ultra-fast cooling application. All the losses that occurred in weight were at normal levels except for ultra-speed application. The higher weight loss due to ultra-speed cooling is based on the fact that the air velocity applied is different due to the high exit rate compared to other works. In all applications, weight loss is expected to be almost immediate.

No significant differences in water retention capacity, cooking loss and color were observed in all three applications. The shear force of the carcass under slow cooling was slightly lower than the others. As a result, the best storage condition in lambs was envisaged as 8 hours after death at 12°C. It did not affect weight loss and was found to be suitable in terms of sensory properties (Fernández and Vieira, 2012).

2.6 Volatile Organic Compounds Formed During Deterioration of Raw Meat

Fresh meat flavor is highly depends on the genotype, fat ratio and feeding type of the animal. The dominant flavour of raw meat is oily and it has methalic taste (Van Ruth, 2011). The main flavor of meat is formed by nitrogen based molecules like pyrazin by increase in pH and lipid degradation products. Especially, 9-hydroperoxyde and 11-hydroperoxyde autooxidation of fatty acids such as linoleic acid and arachidonic acid results with 2,4-decadienal, 2-nonenal, 1-octen-3-on, 2,4-nonadienal and 2-octenal formation which are the main compounds for the meat flavor (Calkins and Hodgen, 2007). Linares et al. (2007) listed the flavor compound found in fresh meat in their study. Off-odor compounds begin to accumulate during storage near to end of shelf life. Ercolini et al. (2009) stated that the most of the off-odor compounds are sourced from microbiological growth.

Microorganisms firstly use glucose for their growth mechanisms. If glucose level decrease in meat, lactate, gluconate, glucose-6-phosphate, pyruvate, ethanol, acetate, amino acids, and nucleotides are being used as substrate by meat microbiota (McMeekin, 1982). According to difference in usage of substrate, microorganism type

can be detected. The changes in amounts of glucose and lactate helps to predict type of spoilage type and degree. For example, *Pseudomanas spp.* consumes lactate, pyruvate, and gluconate in aerobic conditions after oxidation of glucose. However, it uses pyruvate, gluconate, acetate and aminoacids in anaerobic conditions. When *Pseudomanas spp.* use aminoacids, off odor sulphur compounds occur (Nychas, 2007). Consumption order of some substrates by different bacteria is given in Table 2.5.

Table 2.5 : Consumption order of some substrates by different types of bacteria (Nychas, 2007).

Compounds	*Pseudomonas* spp.	*Swanella putrafaciens*	*Brochotrix thermosphacta*	*Enterobacteriaceae*
Glucose	1*	1	1	1
Glucose-6-P	2			2
D-L Lactic acid	3	2		3
Pyruvate	4	3		
Glyconate	5	4		
Glyconate-6-P	6			
Propionate		5		
Ethanol		6		
Acetate		7		
Aminoacids	7	8	2	4
Kreatin	8			
Kreatinin	9			
Citrate	10			
Aspartate	11			
Glycomate	12*			
Ribose			3	
Glycerol			4	

*1: first preferred, 12: last preferred

Shelef (1977) studied the effect of glucose concentration on microbial growth of beef. It was indicated that gram-negative psychrophilic microorganism at glucose level below 2% starts to grow dominantly at cold conditions. After increasing the glucose level up to 2-10%, accumulation of acid due to the microorganism growth decreased the pH level from 5.8 to 5.0-5.2 and this decreasing trend continued until the complete consumption of glucose in beef.

Microbial growth and the consumption of nutrients from meat by microorganisms cause to form undesired metabolites. Volatile organic compounds (VOC) result with the formation of unpleasant odors during meat storage. Organically volatile compounds include generally alcohols, aldehydes, ketones, fatty acids, esters, sulfur compounds (Casaburi et al, 2015). Some of these compounds are important indicators of spoilage of meat. Previous studies reported that volatile organic compounds (VOCs)

and non-volatile compounds such as biogenic amines are primarily results of enzymatic decarboxylation of the amino acids by exogenous enzymes of bacterial origin. VOC relates with the microbiological and physicochemical profile of meat (Boothe & Arnold, 2002; Rajamäki et al, 2006; Balatsmania et al, 2007). Increased production of H_2S and sulphur-based, ammonia-based, methane-based, organic acid-based, mercaptan-based, acetone-based organic compounds and others important organic compounds are also the main limiting factors of meat quality (Lovestead and Bruno 2010). Increasing the amount of VOCs may lead to an increased health hazard for consumers by negative effects of carcinogenic compounds (Senter et al, 2000).

Acinetobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychobacter, Moraxellai Staphylcoccus, Micrococcus, Clostiridum, LAB and some members of *Enterobacteriaceae* are defined as specific spoilage organisms (SSO) that cause off-odor and off-flavor in meat. Most of them have high capability to grow in modified atmosphere packaging and air conditions rather than vacuum packaging (Dainty et al, 1983).

Volatile organic compounds can be classified as alcohols, aldehydes, ketones, esters, volatile fatty acids, sulphur compounds, aliphatic compounds, aromatic compounds, hydrocarbons, biogenic amines and esters. Desired flavor properties of fresh meat are bloody and mild salty. According to researches about VOCs in meat, volatile fatty acids and ketones cause cheesy, oily, lacteal flavor; aldehydes results in oily, grass flavor; alcohol and esters cause ethereal, fruity, sweet flavor; benzene, sulfur and terpene compounds lead to different flavors like cabbage and citrus fruits. For example, butanol from alcohols has fruity flavour whereas 1-hexanal has sweet, fruity, ethereal flavors (Moio et al, 2000). Alcohols are generally produced by *Pseudomonas spp.* and *Carnobacterium spp*. Alcohols pass many metabolic pathways. These can be summarized as proteolytic activity and amino acid metabolism, with methyl ketone reduction and reduction of aldehydes from oil oxidation (Garcia et al, 1991). The most common alcohols that are formed in the deteriorated meats are 3-methyl-1-butanol, 1-octen-3-ol, 2-ethyl-1-hexanol, 2, 3-butenediol, butanol, hexanol and 3-phenoxy-1-propanol (Insausti et al, 2002).

Aldehydes are formed by microorganisms: *Pseudomonas spp., Carnobacterium spp.* and *Enterobacteriacea*. Aldehydes are the result of the hydrolysis of triglycerides and the metabolism of fat. Generally, ethers are formed as aldehydes such as hexanal,

nonanal, heptanal, benzaldehyde and 3-methylbutanal (Casaburi et al, 2015). Aldehydes give vegetable-paint flavors to the food they are in (Ho and Chen, 1994).

Ketones can form in cold storage under MAP, VP and aerobic conditions. Some methyl ketones are the result of lipolytic activity, while others are the result of bacterial dehydrogenation of secondary alcohols or alkane degradation. Acetoin with a threshold value of 800 μg / L is the most common ketone variety (Forss, 1972). Ketone can be production due to the result of glucose catabolism by *Br. thermosphacta, Carnobacterium spp.* and *Lacobacillus spp.* growth (Ardö, 2006).

Esters are produced by *P. fragi*, the most important microorganism in aerobically preserved meats. The esterification reaction of the carboxylic acids and alcohols results with ester formation (Peterson and Chang, 1982). Ethanol is preferred for esterification reaction by microorganisms. Esters create fruity, spicy, oily, floral and orange flavor in meat (Stahnke, 1995b). The most common esters in deteriorated meats are ethyl acetate, ethyl butanoate, ethyl-3-methylbutanoate and ethyloctanoate (Dainty and Mickey, 1992).

Volatile fatty acid formation in meats are occurred by the hydrolysis of triglycerides and phospholipids. *Br. thermophacta* and *Carnobacterium spp*. are microorganisms that produce volatile fatty acids during storage of fresh meat. It is mentioned that the produced fatty acids in different conditions are acetic, butyral and hexanoic acid, while only under aerobic conditions, 2 and 3-methylbutanoic acid are being produced (Toldra, 1998).

Dimethylsulfide is the most important sulphur compound coming from the decomposition of sulphur-containing amino acids. This compound is followed by dimethyltrisulphide, dimethyldisulphide and methyl thioacetate. Sulphur compounds are the result of methionine metabolism produced by *Pseudomonas spp.* (Nychas et al, 2007).

Nychas et al. (2007) and Ercolini et al. (2009) presented possible precursors of some volatile compounds related with spoilage by different species of microorganisms under storage conditions (Table 2.6).

Table 2.6 : Some VOC end-products and their related precursers (Modified from Nychas et al. (2007) and Ercolini et al. (2009)).

End-products (VOCs)	Precursers
Sulfides	Cycteine, cystine, methionine
Dimethylsulfide	Methanethiol, methionine
Dimethyldisulfide	Methionine
Dimethyltrisulfide	Methionine, methanothiol
Methanethiol	Methionine
2-Methylbutanal	Iso-leucine
2-Methylpropanol	Valine
2-Methylbutanol	Iso-leucine
3-Methyl-1-butanol	Leucine
Ammonia	Amino acids
L-lactic acid	Glucose
D-lactic acid	Glucose
Acetic acid	Glucose, lactate, pyruvate
Formic acid	Glucose, acetic acid
Acetoin	Pyruvate, analine, diacetyl
Diacetyl	Pyruvate
Hexanal	Iso-leucine
Ethanol	Glucose
2,3-Butanediol	Diacetyl
2-Methylpropanol	Valine

Other compounds rather than alcohols, esters, ketones, volatile fatty acids, sulphur compounds, aldehydes, and other compounds which may occur in cold storage are aliphatic and aromatic hydrocarbons, terpenoids, phenols, lactones, and phthalates. Aliphatic hydrocarbons are the result of various fatty acid oxidation reactions (chemical auto-oxidation, oil degradation, etc.) (Montel et al, 1998). Some aliphatic and aromatic compounds are occured by chemical reactions of microbial metabolism.

Fruit-like, candy-like and cheese-like tastes form in the aerobic environment. Cheese-like taste is because of the formation of hydrogen sulphide by *Enterobacteriaceae.* In modified atmosphere, cheese-like and sour tastes are due to acetic acid and acetoin which are formed by *B. Thermosphacta* and lactic acid bacteria (Chiofalo ve Lo Presti, 2012). Some microbiologically caused undesired volatile compounds and their percieved odor definitions are listed in Table 2.7.

Table 2.7 : Common microbial cause meat spoilage volatiles and their odor definitions (Whitfield 2003).

Volatile Compounds	Odor Definition	Microorganisms
Diacetil, Acetoin	Butter, Cheesy	*Brochotrix thermosphacta*
3-Methyl butanol, 3-methyl propanol	Sweety, Fruity	*Lactobacillus spp.*
Ethyl acetate, Ethyl butanoate	Sweety, Fruity	*Pseudomonas spp., Pseudomonas fragi*
Ethyl octanoate, Ethyl hexanoate	Sweety, Fruity	*Moraxella spp.*
Ethyl 2-methyl butanoate, Ethyl 3-methyl butanoate	Fruity	*Brochotrix thermosphacta, Pseudomonas spp.*
Methanethiol, Dimethyl sulfide, Dimethyl trisulphide	Putrid, Sulphurous	*Enterobacteriaceae*
Aliphatic alcohols, aldehydes and aromatic hydrocarbons	Sour	*Acinetobacter*
2-methyl butanol, 3-methyl butanol, Hydrogen sulphide, Methanethiol, Dimethyl sulfide, Dimethyl trisulphide, methyl tioacetate	Putrid	*Serratia liquefaciens*

Regulations have been introduced in many countries because storage temperatures above 10°C cause deterioration of the meat (Doulgeraki et al, 2012). In some researches, the effect of storage conditions on microbial growth and the importance of the effect of volatile organic compounds and other metabolites on the sensory properties of the meat was mentioned (Casaburi et al, 2015).

Casaburi et al. (2014) studied the volatile organic compounds produced in bovine meat using HS-SPME-GC/MS technique. The meat samples were stored at 650, 482, 386 and 220 hours at 0, 5, 10 and 15°C, respectively, under modified atmosphere (40% CO_2, 30% O_2, 30% N_2) and atmospheric conditions. During the storage of the meat samples under MAP conditions, the alcohols may be selected from the group consisting of 1-penten-3-ol, pentanol, heptanol, 1-octen-3-ol, 2-octen-1-ol, octanol and aldehydes pentanal, hexanal, heptanal, octanal, trans-2-heptenal and trans-2-octenal.

In aerobic conditions 3-methyl-butanol and 2-methyl-butanol from alcohols and 3-methylbutanal and 2-methylbutanal from aldehydes increased during storage. While the rate of ethanol in cold storage was decreasing, the increase in high temperatures was also observed in two storage types. Gram negative bacteria, LAB and *B. thermosphacta* are known to be responsible for the production of alcohols and aldehydes (Casaburi et al, 2014).

Considering the aldehyde production, aldehydes such as 2-methyl-butanal, 3-methyl-butanal, hexanal, heptanal, octanal, nonanal and decanal are produced by *Pseudomonas fragi*. Other gram-negative bacteria can produce aldehydes such as 2-methyl butanal, 3-methyl butanal and heptanal. When the ketones were investigated, 2-butanone, 2-heptanone, 3-octanone, 2-octanone, 2-nonanone, 2,5-octanedion and 2,3-pentandion contents increased in all storage conditions while 2-butanone content decreased. Acetone and diacetyl contents have also increased in two storage conditions with increased temperature abuse, but the sample stored in the MAP condition in cold storage has decreased. The reason for the formation of ketones is known as various fatty acid oxidation reactions, chemical autooxidation, enzymatic α- or β-oxidation (Casaburi et al, 2014).

Contents of ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl lactate and ethyl hexanoate originated from esters increased at all conditions except aerobic at 0 and 5°C and MAP at 0°C (Casaburi et al, 2014). The formation of the esters may be effected by esterification of various alcohols and carboxylic acids as well as microbial esterase activity (Talon et al, 1998). The most important factor in the formation of ester is known as *Ps. Fragi* bacteria (Casaburi et al, 2014).

Pseudomonas spp., Sh. Putrefaciens, Moraxella, R. aquatilis, Carnobacterium spp., and *B. thermosphacta* can form esters under aerobic conditions, whereas *Carnobacterium spp., C. maltaromaticum, B. thermosphacta* or *S. proteamaculans* can form esters under MAP conditions (Nychas et al, 2007).

In aerobic environment, *Pseudomonas spp.* is the most effective microorganism in meat and it produces H_2S, volatile amines, esters and acetone which cause an undesirable odor. Metabolic effects of *Pseudomonas* were investigated in broth analyses. The compounds that have been formed are listed in Table 2.8. Metabolic events with the substrates used are; D-glucose breakdown, D-glucose breakdown with

L- and D-lactic acid, oxidation of glucose and glucose 6-phosphate (causing D-gluconate accumulation and 6-phosphogluconate increase) (Nychas et al, 2014).

Table 2.8 : Compounds formed by *Pseudomonas* during storage (Nychas et al, 2014).

Sulfur compounds	**Aliphatic hydrocarbons**
Sulfides, dimethylsulfide, dimethyldisulfite, methylmercaptan, methanethiol, hydrogen sulfide, dimethltrisulfide	Hexane 2,4-Dimethylheptone
Esters	**Aldehydes**
Methyl esters (acetate), Ethyl esters (acetate)	2-Methylbutanal
Ketones	**Alcohols**
Acetone, 2-butanone, acetoin/diacetyl	Methanol, ethanol 2-methylpropanol 2-methylbutanol 3-methylbutanol
Aromatic hydrocarbons	**Biogenic amines - Other compounds**
Diethyl benzene, trimethylbenzene, toluene	Cadaverine, ammonia, putrescine, methylamine, trimethylamine

Members of *Pseudomonas spp.* use glucose to break down glucose using the meat surface to grow. When the number of microorganisms reaches a high ratio (10^8 units/ cm^2), the glucose on the meat surface becomes unable to meet the demand for growth. In that case, *Pseudomonas spp.* use amino acids and nitrogen components as substrate for proteolytic initiation. As a result, malodorous amine, sulphides and sulfide esters occur (Nychas et al, 2014).

Lipid oxidation is another important quality change in meats, affecting the color, taste, odor and texture of foods. (Fernandez at al, 1997; Jacobsen, 1999; Ulu, 2004; Min and Ahn, 2005; Ross and Smith, 2006). Lipid oxidation is caused by enzymatic or non-enzymatic catalysis. Non-enzymatic mechanisms may include catalysis of hydrogen peroxide. Antioxidants like ascorbic acid, phenols and alfa tocopherols are used to prevent lipid oxidation because they form bond with oxygen on the surface. Unwanted taste compounds are also formed by enzymatic ways. Lipase and lipoxygenase enzymes are responsible for lipid deterioration as well. It is hard to separate this type of deterioration from microbial deterioration.

Lipid oxidation is a complex reaction which is the oxidation of unsaturated fatty acids in the presence of oxygen and free metal ions (Karabudak, 2003; Min and Ahn, 2005;

Ross and Smith, 2006). Oxidation depends on the environment conditions, free radicals, aldehydes and cholesterol oxidase can be produced as a result of lipid oxidations (Ferioli et al, 2010). Primary oxidation products are unstable and odorless hydroperoxides, secondary products are alkane, alkene, aldehydes, ketones, alcohols, esters, acids and hydrocarbons which are the results of hydroperoxide decompositon. The main responsible compounds of rancid and off-odor flavor in meat are aldehydes such as hexanal, octanal, nonanal and pentanal (Ross and Smith, 2006). Malondialdehyde is one of the water-soluble secondary oxidation product (Ross and Smith, 2006) and it is used as an oxidation index for meat products (Fernandez et al, 1996; Wang et al, 2002; Ulu, 2004). It was found out that there is a positive correlation between hexanal and TBA value due to the lipid oxidation process (Perez et al, 2008).

2.7 Determination of Volatile Organic Compounds in Meat

Microbial spoilage and consumption of nutritive compounds by microorganisms cause production of metabolites. Volatile organic compounds produce off odors during storage of meat. Typical volatile organic compounds that are mentioned above consists of alcohols, aldehydes, ketones, fatty acids, esters and sulfuric compounds (Casaburi et al, 2015). There are five different detection methods such as Gas Chromatography (GC), Selected Ion Flow Tube Mass Spectrometry (SIFT-MS), Proton Transfer Reaction- Mass Spectrometry (PTR-MS), electronic nose and other techniques for determination of VOCs.

2.7.1 Gas chromatography (GC)

Principle of gas chromatography method is based on the separation of organic compounds in a mixture. Analyte is volatilized with a carrier gas (mobile phase) and runs through in chromatography column. Inner surface of the columns is covered with a chemical film (stationary phase). During this movement, separation of molecules in analyte takes in different times. Time interval between injection and exit is called retention time. Retention time differences of molecules of analyte are based on polarity characteristics that aid to differentiate each molecule on the chromatogram (Chasteen, 2013).

For GC application, different sampling method and detector types are summarized below.

Volatile organic compounds analysis in liquid and solid food materials are analyzed using headspace methods effectively. Besides, this sampling function is used to detect residues contaminated from packaging materials.

Sampling methods for VOCs determination are classified into four main topics. These are Static Headspace (SHS), Dynamic Headspace (DHS), Solid Phase Microextraction (SPME) and Headspace Sorptive Extraction (HSE). Solvent extraction (SE) and Simultaneous Steam Distillation-Extraction (SDE) method can also be used in determination of VOCs in meat. Formed gases are injected into GC (Sandra et al, 2008).

2.7.1.1 Headspace sampling (HS) methods

Headspace sampling method aids in determination of automation degree and increase the sensitivity of the analyses. Also, it is a rapid analysis of VOCs and many data are obtained.

In HS method, interaction between solid and liquid samples is supplied. Volatile compounds are released into gaseous medium and GC analysis is completed. If extraction is completed at batch cycle, it is known as Static Headspace method (SHS). If extraction is done continuously, that is called Dynamic Headspace method (DHS) (Chiofalo and Lo Presti, 2012). In SHS method, solid or liquid sample is placed into vial and closed. After that, vial is heated for specific temperature and time. This heating application increases the concentration of gaseous phase of the targeted compounds. Although SHS method may seem ideal for determination of VOC in nature of meat, it is not preferred for animal sourced foods.

Dynamic headspace method has more advantages than SHS method for its advanced analytical sensitivity. In contrary to SHS method, DHS uses inert phase fluid for continuous extraction of volatile compounds and pre-concentration occurs inside of adsorbent or in cryogenic trap. Mostly known name of this application is ‘purge and trap’ method. Gases inside the trap are released with increasing temperature in the system. In DHS method, purge volume and extraction temperature must be optimized. Sampling design should be appropriate to the characteristics of the sample. There may be different choices of materials that have various size, heating stability and desorption properties for pre-concentration. Tenax, silica gel, chromosorb, graphitted carbon or carbon molecules are commonly used for DHS method (Ross and Smith, 2006). Most

popular adsorbent material for pre-concentration of DHS is Tenax trap. It is preferred for its properties of catching volatile compounds in wide range scale, high heating stability, low moisture absorption and longer shelf life (Soria et al, 2015).

2.7.1.2 Solid phase microextraction (SPME) sampling method

Headspace SPME-GC/MS is an inexpensive, simple, solvent-free, highly sensitive and well-suited method for many meat products (Bhattacharjee et al, 2011). There are two different mechanisms in SPME method. First step includes the separation of analytes between sample matrix and headspace, and the second step involves separation between headspace and fiber covering material. SPME is known as a balance technique. If high level of sensitivity is desired, temperature and mixing applications must be optimized (Sandra et al, 2008).

SPME method includes sample preparation, extraction and concentration. SPME has ability to extract molecules having different boiling points. Adsorbent covered inert injector is placed into surface of food material. Volatiles are adsorbed and collected by fiber having adsorbent material. Then, volatiles are released using injection channel with increasing temperature. Fiber selection is a major issue in SPME sampling method. Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) are the common fibers used for SPME. Dual fiber mixture can be chosen over single fiber for optimization of some meat products. SPME method is suitable not only for food products but also for drugs due to its sensitivity in analyses. However, difficulties in method validation and controlling temperature; damaging of fibers are the main disadvantages of SPME method (Chiofalo and Lo Presti, 2012).

2.7.1.3 Headspace sorptive extraction (HSE) sampling method

Headspace sorptive extraction is a novel method which is used for improvement of sorptive samples from headspace. Adsorptive phase is used at high level. Therefore, semi volatile organic compounds are analysed in an efficient way.

2.7.1.4 Solvent extraction (SE) and steam sistillation-extraction (SDE) sampling methods

Solvent extraction is a classical method used for separation of volatile compounds from food commodities. This method requires an extra concentration step which may cause sample loss. Solvent selection has an important role for an efficient separation.

Even though this method is simple and efficient, it is employed in the analysis of beverages rather than meat products (Freitas et al, 2012). However, SDE method is advantageous for its simplicity, lesser solvent requirement and effective separation of volatiles, it is not preferred commonly for its need of distillation, occurring of thermal decomposition, interaction between reactive compounds and wasting of samples having low boiling point (Sandra et al, 2008).

After sampling is completed, analytes are injected into GC and detected by using mass spectrometer (MS) or photon ionization detector (PID).

2.7.1.5 Studies on the determination of VOCs by GC-MS/PID

Insausti (2002) applied DHS method on beef samples from *longissimus dorsi* carcass by using Tenax as adsorbent. Helium was used as the mobile phase inside GC column with mass spectrometer whose flow rate 180 mL/min. Column temperature was held at 30°C for the first 5 minutes and increased to 220°C with a rate of 8°C/min. Sample was held at this temperature for 15 minutes. Injector temperature was kept at 250°C constant. There were 53 different volatile compounds that were detected by this method. Most of the detected volatile compounds were aldehydes and ketones (Insausti et al, 2002).

Zhao et al. (2010) used purge and trap method for sampling to detect volatile compounds in their study. In GC analysis, PID was used for detection. Its high-resolution separation power, low detection limit and enhanced sensitivity had contribution for the selection in the VOC analysis (Zhao et al, 2010).

Another study is the development of solid-phase microextraction methodology for analysis of headspace volatile compounds in simulated beef flavor. The suitable conditions for HS-SPME method was investigated in beef by applying various variables such as: Adsorption time (20-60 min.), adsorption temperature (30-60°C), addition of salt (0-6%) and fiber phase (65µm PDMS/DVB, 65 µm CW/DVB, 75 µm CAR/PDMS and 50/30 µm DVB/CAR/PDMS. The peak number as a result of the GC analysis was investigated for the effect of each factor and/or dual factor. It was observed that there was no effect of 6 % salt concentration on adsorption. The most convenient temperature, time and fiber types were found as 60°C, 60 min and 50/30 µm DVB/CAR/PDMS, respectively (Moon and Li-Chan, 2004).

Direct extraction of the fibers by SPME extraction of the volatiles is only carried out in liquid samples. Direct extraction equipment (DED) can be used for solid samples. However, the D-SPME (direct SPME) technique is problematic in solid specimens due to the fragile nature of the fiber. Nevertheless, it may be preferred in the analysis of volatile compounds in the solid sample (Perez et al, 2008)

In another study, SPME method was selected as sampling method to detect volatile compounds in meat carcasses from *longissimus dorsi* muscle of cattle. Four different fibers were selected as PDMS, PDMS-DVB, Car-PDMS, DVB/Car-PDMS. Helium was used as the mobile phase. Oven temperature was held at 40ºC for the first 10 minutes and increased to 200ºC with a rate of 5ºC/min. Temperature was kept constant at 250ºC. Mass spectrometer was used for the detection. By this analysis, the differences within the fiber types were identified and their effectiveness was discussed. As a result of this research, PDMS-DVB and DVB/Car-PDMS were found to be chosen as best fibers since 40 different volatile compounds were identified by these methods (Acevedo and Creixell, 2012).

Another study about volatile compounds is solid-phase micro extraction for determination of volatile compounds in the spoilage of raw ground beef (Perez et al, 2008)**.** The investigation of meat volatile components gives information about the characterization and deterioration of the product. In this study, the extraction procedure of the volatile components, the type of fiber and the method of analysis were investigated. To observe the changes in the volatile component profile, the samples were analyzed after being stored for 0.3 and 6 days at 4°C. SPME fibers were coated with 65 μm PDMS / DVB, 75 μm CAR / PDMS and 50/30 μm DVB / CAR / PDMS. The effect of addition of salt (0, 15 and 30 w / w) was also investigated. PDMS/DVB fiber provided low efficiency, while CAR/PDMS fiber and DVB/CAR/PDMS yielded high yields, respectively in the analysis of the volatile components. Addition of the salt did not increase the extraction rate of the volatile components. Table 2.9 shows the volatile component table obtained using HS-SPME and CAR/PDMS. It was emphasized that volatile compounds were present at low levels. Although a a significant increase was not observed for hexanal which is the most important deterioration factor; 3-hydroxy-2-butanone, 2,3-butanedione in ketones; 3-methyl-1-butanol and 1-hexanol in alcohols; butanoic acid and acetic acid in acids were found as representing gases. HS-SPME extraction results were found to be better than D-

SPME results. When raw meat was stored at the given ratios, butter flavor was determined as a combination of bad taste (Perez et al, 2008).

Table 2.9 : Chromatographic results (peak area: Ab*s) of meat spoilage compounds at different storage times obtained by HS-SPME sampling (Perez et al, 2008).

Compound	Day 0	Day 3	Day 6
Acetone	13.42±1.8	8.25±0.85	9.53±0.92
Ethanol	4.84±0.81	5.33±0.29	7.53±1.02
2,3-Butandione	6.54±1.73	51.06±6.49	42.41±3.67
Hexanal	70.7±6.26	32.92±8.10	-
1-Butanol	2.89±0.11	2.25±1.52	0.51±0.08
1-Penten-3-ol	3.01±0.25	2.81±0.35	0.62±1.47
3-Methyl 1-butanol	-	8.68±0.81	31.00±1.52
1-Pentanol	24.05±2.71	27.22±13.81	12.59±0.92
3-hydroxy-2-butanone	15.50±5.68	308.99±28.09	575.07±19.82
2-Methyl-3-octanone	3.68±0.96	5.67±1.02	0.43±0.093
1-Hexanol	3.63±0.19	53.39±4.05	39.28±3.90
3-Octen-1-ol	4.98±0.75	6.94±0.59	4.90±0.90
Acetic acid	5.56±1.46	3.86±0.37	7.18±1.41
2-Ethyl-1-hexanol	2.46±1.19	1.70±0.27	1.43±0.08
Benzaldehyde	3.14±1.51	2.02±1.44	1.69±0.62
Butyrolactone	1.11±0.26	0.95±0.04	1.11±0.21
Butanoic acid	1.18±0.10	3.17±0.41	1.75±0.50
3-Methylbutanoic acid	1.75±0.50	1.54±0.13	3.32±0.55
Hexanoic acid	4.42±0.24	5.48±0.74	1.75±0.50
Benzyl alcohol	3.05±1.36	3.10±1.96	2.08±0.42
Phenyl ethyl alcohol	1.69±0.07	1.09±0.11	2.89±0.43

Joshua et al. (2016) studied about volatile compound characterization of modified atmosphere packaged ground beef subjected to temperature abuse. The MAP (80% O_2, 20% CO_2) and CO-MAP (69.6% N_2, 0.4% CO, and 30% CO_2) were used as modified atmosphere conditions. The volatile compounds in the headspace and the effect of the packaging type were examined. SPME-GC-MS was preferred to characterize the quantitative and qualitative properties of volatile compounds. For SPME-GC-MS,

CAR/PDMS fiber was preferred according to the positive results in the literature. Twenty-one volatile compounds were observedand 10 of them were hydrocarbons, 7 of them were aldehydes, and 3 of them were ketones. When the volatile compounds obtained from the two packing types were compared, there was not much difference among them. Scenarios cannot be predicted because of the lack of sufficient work in this area (Joshua et al, 2016).

In another study, volatile compounds of Iberian dry-cured loins with different intramuscular fat contents were analysed by using SPME-DED. Dried steak samples having different fat ratios and maturation times were examined for VOC formation.

DED (direct extraction device) was used instead of headspace on the vial, which is the classic method in SPME, in the experiment of dried beef samples. SPME (carboxen-poly fiber coated) is placed in the DED. SPME-DED was immersed in dried meat. The SPME was then subjected to headspace into the DED to provide extraction. The purpose of the DED was to collect the air from the meat center into the device and extract it in the SPME for extraction (extraction temperature was chosen at room temperature and duration was 30 min).

According to the results obtained, the total chromatogram area in the sample with high fat ratio was higher than the low-fat samples. The gases formed due to the oxidation reaction of the fat were hexanol, octanal and etc. Dimethylsulfide, 3-methylbutanal increased depending on the decrease of amino acid. It has been found that the above-mentioned gas types had high chromatogram areas in the samples having high fat ratio. As a result, the fat ratio has been observed to affect the formation of VOCs and the proposed mechanism of movement of the gases from sample to the headspace (Ventanas et al, 2008).

Thermal desorber (TD) is another method that can be used for preparing samples for GC-MS. A fast and typical method is required for GC-MS sample preparation, which is an important step in laboratory work. Grab sampler was preferred in the study of Morris and Barden (2014) for capturing organic volatile components from headspace of packaging. The volatile compounds of meat were collected on a sorbent tube and then analyzed by thermal desorber and seperated with GC-MS. As a result, 46 organic volatile compounds were seperated at considerable levels.

In the GC-MS analysis, the moisture reactions cause problems such as decomposition, shifting in retention times, damage to the analytical system, and deterioration of the dedector sensitivity; the absence of intervention of water has been shown the most important feature. Itwas also emphasized that the selected sample preparation method was ideal for TD-GC-MS in terms of high sensitivity, quality and deterioration detection (Morris and Barden, 2014).

Another research about TD was an investigation of volatile compounds of raw beef from 5 local Spanish cattle breeds stored under modified atmosphere. All samples were stored at 2 ± 1°C and 90-95% relative humidity in the dark for a certain period of time (0, 5, 10, 15 min) and vacuum packed with the same machine and stored at -20°C until analysis. Headspace volatiles were thermally collected with Tenax GC trap at 300°C. In aliphatic hydrocarbons, the highest relative percent (RPA) belonged to pentane and 1,1'-oxybenzene. Hexanal from aliphatic aldehydes had the highest rate and gave off grassy smell. Aliphatic alcohols such as 1-penten-3-ol, ethanol and 1-octen-3-ol had ethereal butter, sugar, balsamic and fungal odor. One of the sulfur compounds which has important significance in the profile of the fried meat taste has been determined. The dimethyl sulphite compound is an amino acid-derived compound that is formed by physcrotroph bacteria. The methicyclohexane and propylcyclohexane compounds are aliphatic hydrocarbons presented in the sample (Insausti et al, 2002).

Lee et al. (1978) studied high-resolution gas chromatographic profiles of volatile organic compounds produced by microorganisms at refrigerated temperatures with TD. Three bacterial species isolated from deteriorated and uncooked chicken (*P. putida, P. fluorescens, Moraxella oxidative*) tyrptiaces were examined in GC-MS by concentrating in a porous polymer column at refrigerator temperatures. After 14 days of incubation of the cultures, 10 to 20 grams of the Trypticase soy yeast (TSY) agar containing specific culture was transferred to the headed sampling flask. The organic compounds were retained on 2.0 mg of porous polymer Tenax GC. After sampling, the compounds were identified on GC-MS. 20 different volatile compounds were obtained which are shown in Table 2.10.

Table 2.10 : The compounds identified after inoculation with different bacteria on agar (Lee et al, 1978).

Culture grown on TSY agar			Compounds
P. putida	***P. fluorescens***	***Moraxella oxidative***	
+	+	+	Acetone
+		+	2-Butanone
	+	+	Methyl propionate
+	+	+	Methyl butanal
+	+		Methyl thiolacetate
+	+		Methyl isothiocyanate
		+	Methyl isobutyrate
+	+	+	Dimethyl disulfide
+	+	+	Toluene
	+	+	Methyl-2-methyl butyrate
		+	n-Butyl acetate
+	+	+	Methyl pent-2-enoate
		+	Dimethyl benzene
+	+	+	Benzaldehyde
+	+	+	Dimethyl trisulfide
+			Methyl benzoate
+	+	+	2-Nonanone
+	+	+	1-Undecene

2.7.2 Selective ion flow tube-mass spectrometry

Different ionization techniques were developed for the direct analysis of volatile compounds in air. Selective ion flow tube-mass spectrometry (SIFT-MS) uses selected specific positive ions and investigate the chemical ionization of gas sample. SIFT-MS is a time-saving method. This technique can also run the analysis at high humidity medium and do not need any external calibration (Olivares et al, 2012).

In the study of Olivares et al. (2012), meat samples awaited at MAP conditions (80% O_2 and 20% CO_2) and at a temperature of 4°C. Definite storage days of meat were followed (0, 2, 5, 8 and 12 days). Other required sample preparations were done for both SIFT-MS SPME-GC-MS analysis and volatile compounds were detected. While 27 volatile compounds were detected via SIFT-MS, 21 volatile compounds were observed using SPME-GC-MS. The reasons for this difference were considered as low concentration in headspace and lack of proper binding to fiber. In SIFT-MS, extraction duration was 1 hour while analysis last about 3 minutes. However, with SPME-GC-MS, the sample was analysed for 5 hours in total. Also, there was no problem in overlapping the compounds with ions due to SIFT-MS having different precursor ions for each volatile compound.

Another important result of this study is that, while acetaldehyde, propanal, 2-pentanal, 2-heptanal, butyric acid, dimethyl sulphite and acetone were detected at 12th day of storage by SPME-MS, 8 days were enough for detection with SIFT-MS. SIFT-MS detected VOCs earlier than SPME-GC.

2.7.3 Proton transfer reaction-mass spectrometry

Proton transfer reaction-mass spectrometry (PTR-MS) was found by Werner Lindinger to correlate the VOCs and bacterial contamination. By PTR-MS, meat can be analyzed within minutes and multiple analysis of samples can be carried out rapidly. This method is dependent on concentration of H_3O^+ ions. Despite the fact that, dissolved protons do not react with compounds in air but they can only react with volatile compounds. Another advantage of this method is that, pre-concentration stage is not required (Mayr et al, 2003). Mayr et al. (2003) studied PTR-MS analyses on beef and pork meats. Headspace air was taken by vacuum pump with 114 mL/min. and 14 mL/min was sent to PTR/MS system by teflon transfer line.

2.7.4 Electronic nose

Electronic nose is a device that imitates the olfactometric characteristic of a human nose. It can be used separately or can be combined with other devices or methods. In electronic devices with sensors, metal oxides adsorb and desorb samples on the surface of the sensor. This method combines the data of collective interaction of headspace compounds with multiple sensors. Electronic noses are used to detect storage time, quality, processing time and their differences in meat and fish produced at industrial scale (Boothe and Arnold, 2002).

2.7.5 Determination of VOCs in meat by special apparatus

A new technique was presented by Bakhoum (2016), which is very sensitive to compounds and moreover reactivity with moisture is very low. The method is cheaper compared to other instrumental methods and easy to handle. Different barcodes are prepared for each compound using chemically sensitive dyes such as pH indicator dyes, metalloporphyrin dyes and solvatochromic dye. These barcodes are placed into meat package. While meat get spoiled, individual sticks on the barcode starts to change. Hand-held scanning device is used to scan the barcode which determines the spoilage through the color changes. Even though this method is more reliable

compared to electronic nose, it detects only very few number of volatile organic compounds.

In the study of Bakhoum (2016), low-cost, high-accuracy method and apparatus used to analyze spoilage of beef stored at 4°C. Concentration of VOCs for both scenario is shown in Table 2.11. Eleven volatile compounds were detected such as methanol, ethanol, methanethiol, 1-butanol, dimethyl sulfide etc. by this device. Their concentrations in room temperature substantially were higher than the ones stored at 4°C.

Table 2.11 : Concentrations of volatile compounds of beef sample stored for five days at room temperature and at 4°C (A: room temperature; B: 4°C).

Compound	Concentration (ppm)	
	A	B
Methanol	$5.2x10^2$	$1.9x10^2$
Ethanol	$2.5x10^2$	$1.1x10^2$
Methanethiol	$1.3x10^2$	$0.6x10^2$
1-Butanol	$1.1x10^3$	$0.2x10^3$
Dimethyl sulfide	$4.6x10^3$	$2.8x10^3$
Methyl acetate	$1.2x10^2$	$0.3x10^2$
Diethyl sulfide	$2.7x10^1$	$1.7x10^1$
Thioacetic acid methyl ester	$7.5x10^4$	$4.1x10^4$
2,3-Butanediol	$1.5x10^1$	$0.7x10^1$
Toluene	$2.1x10^3$	$0.5x10^3$
Dimethyl disulfide	$3.4x10^2$	$2.2x10^2$

This method can be selected for determination of spoilage based on concentrations of VOCs. It can detect VOCs in early stages of spoilage. Besides, it has no reaction with moisture. Reliability of this method is agreed to be better than electronic nose (Bakhoum, 2016).

2.7.6 Field asymmetric ion mobility spectrometry (FAIMS)

VOCs detection methods are generally expensive, technically complex and expensive. Besides, methods like mass spectrometry restrict usage without portability. Titration and colorimetry methods are simple. However, they are time-consuming and have low

efficiency. Therefore, ion mobility spectrometry has the advantage of being portable and consuming less power.

Principle of FAIMS is different than other spectrometric methods. Separation of different compounds is occurred due to ion mobility difference. This difference is obtained by changing the electrical field. The Owlstone Company in the U.K. developed micro dedector that is related to FAIMS technology. Their aim is to improve chemical sensors in the size of a coin. This sensor can be used in civil areas such as early disease diagnosis. The weight of multi-channel FAIMS chip structure with a built-in chargeable power supply is around 7-7.8 kg (Tang et al, 2011).

FAIMS technology was selected to monitor the out-gassed volatiles of pork samples kept at 25ºC over 24 h in a study. To show compatibility of FAIMS technology, adsorbent trap samples were taken for VOCs analysis. Besides, aerobic plate counts (APC) were detected. Peak height increased over time because of microbial growth. After 4h, FAIMS determined volatile organic compounds. At that time, bacterial count was log 2.96 (meat spoilage generally takes place at log 8.5). On the other hand, VOCs of spoiled meat was determined by TD-GC-MS. In both methods, signal of VOCs increased with the increase in bacterial growth. FAIMS helps to obtain results in a short time and usage of this method is very simple. With the use of this technique, quality control of meat is easy when during the delivery of meat to meat companies (Schrock, 2009).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Meat Samples

Beef striploin *"longissimus dorsi"* muscle was used as test sample. All beef samples were purchased from a local market in Istanbul. Purchased samples were transferred to Arçelik A.Ş. R&D laboratories in a cooler box, they were covered with polyethylene clinging film and stored at 4°C±0.6°C until they were used for analysis. The reason of choosing beef striploin as test sample is due to the fact that, more reproducible and repetable results could be obtained because of homogenous distribution of fat, connective tissue and free water ratio.

3.1.2 Chemicals

2,3-Butandione (Merck KGaA, Darmstadt, Germany), 3-hydroxy-2-butanone (Merck KGaA, Darmstadt, Germany), hexanal (Merck KGaA, Darmstadt, Germany), ethyl acetate (Merck KGaA, Darmstadt, Germany), 2-ethyl-1-hexanol (Sigma Aldrich Chemie GmbH, Steinheim, Germany) were standard chemicals that were used at recovery analyses of pre-concentration techniques. CASO Agar Base (Tryptic Soy Agar), CFC Agar Base (Cephalothin-Sodium Fusidate-Cetrimide Agar Base, OXOID), Modified CFC Selective Supplement (FD281), distilled water (Merck KGaA, Darmstadt, Germany), ethly alcohol (Merck KGaA, Darmstadt, Germany), NaCl (Merck KGaA, Darmstadt, Germany) were used for microbiological analysis and 7.5% trichloroacetic acid (TCA) (Merck KGaA, Darmstadt, Germany), thiobarbutyric acid (TBA) solution (Merck KGaA, Darmstadt, Germany), 1,1,3,3-tetramethoxypropane (Sigma Aldrich Chemie GmbH, Steinheim, Germany) were used for TBA analysis. All chemicals used for the analyses were of analytical or GC grade.

3.1.3 Sample preparation

First study was proceeded for investigating the effect of loading factors in two different pre-concentration techniques. For this study, two parallel group of samples were prepared, the first was used group for microbiological, chemical, physical and sensory

evaluations and the second group was used for volatile organic compound analyses. For all experiments, the samples were kept in cold storage at 4°C±0.6°C and sampling was performed at the day when the cold stored meat was spoiled. Sample weight were arranged according to loading factors. Loading factor has been calculated in terms of sample amount: total volume ratio that were 0.025 kg/L, 0.05 kg/L and 0.10 kg/L. Experiments were performed in glass vials for SPME and in a hermetic, stainless steel container for gas glushing on Tenax with Thermal Desorber (TD) sampling.

In the second study, in order to investigate the effects of disturbing temperature conditions for two different storage temperatures, beefs were sliced into pieces (each in 50 g) with 20 mm thickness and overwrapped with polyethylene film for storage. The samples were taken at day 0, 1, 3, 5 and 7 of storage for all cases. Three replicates were used for each analysis.

3.2 Methods

Experimental flow chart of first and second parts of the study is illustrated schematically in Figure 3.1 and Figure 3.2, respectively.

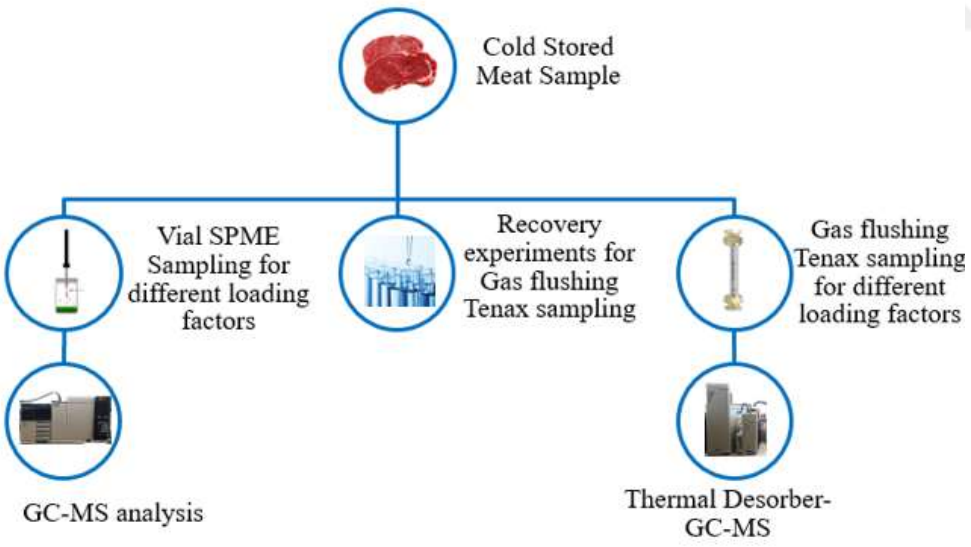


Figure 3.1 : Schematic of investigations on loading factor effect in spoiled meat.

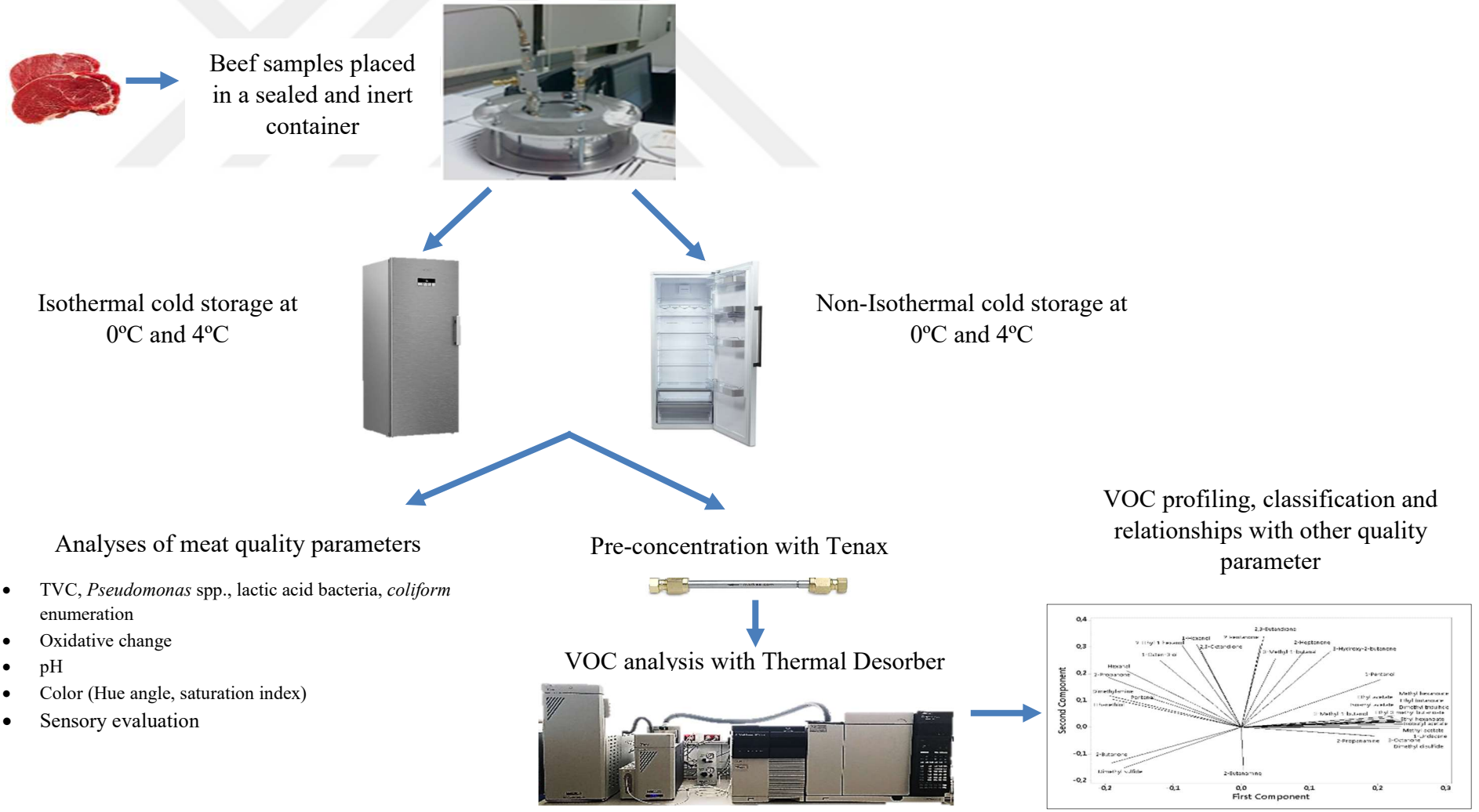


Figure 3.2 : Schematic of investigations on different storage conditions in meat.

3.2.1 Methods for investigations on loading factor effect

3.2.1.1 Volatile organic compounds analyses

SPME-GC-MS method

Volatile compfounds were determined with "headspace" (HS) Solid Phase Micro Extraction (SPME) sampling technique according to Perez at al (2008). The SPME fiber used for VOCs sorption was CARBOXEN/PDMS (Agilent, USA) with 75 μm film thickness, 1 cm needle length and 24 ga needle size. The procedures were as follows: 1) Meat samples were placed in 20 mL glass vials and sealed with PTFE/silicon septum (Agilent, USA), 2) Vials filled with samples were held in water bath on a heater plate (IKA C-MAG HS 7, Germany) at 40°C for 30 minutes to attain extraction equilibrium 3) After accumulation of volatiles in headspace, SPME fiber was inserted into vial via septum cape. Pre-equilibrium time was maintained for 60 minutes between the sample, headspace and fiber. The length of the needle in vial was kept constant during adsorption process. 4) Fiber was introduced into GC-MS injection port where desorbed for 10 minutes at 250°C.

GC-MS analyses were performed by using Agilent 7890A gas chromatography equipped with Agilent 5975C mass spectroscopy (Agilent, USA). Helium was used as carrier gas with a flow rate of 1 mL/min. Fiber was introduced into injection port using split mode at 1:10 split ratio. Analytical column used was DB-5MS (30 m x 0.25 mm and I.D 0.25 μm film thickness) (Agilent, USA). The temperature program for seperation were as follows: 40°C for 5 min., ramp to 150°C at a rate of 4°C/min, increased to 250°C with a rate of 30°C/min and held at this temperature for 5 min. Mass spectroscopy specifications were as follows: 70 eV electron energy at electron impact mode, 25-400 m/z mass range, MS "source" and "source quadrupole" temperatures were set as 230°C and 150°C, respectively. Detected volatiles were assessed regarding peak areas with arbitrary units. WILEY and NIST libraries were used for identification (Perez at al, 2008).

Gas flushing tenax sorbent tube- thermal desorption-GC-MS method

Meat volatiles were sampled from the headspace of a specially designed compartment where meats were stored in low temperature. High quality polished stainless steel container was produced with some critical features as follows: 1) No odor/volatiles

released from container material to eliminate interaction with volatiles formed from meat and no absorption/adsorption of volatiles released from meat, 2) Hermetically sealed and 3) Cylindrical shape to prevent contamination on edges. The container is shown in Figure 3.3.

Total volume of the container is 1 L. Food contact Polytetrafluoroethylene (PTFE) material was used as sealing gasket under flange cover. After meat samples were placed in the container, headspace was flushed with Helium gas at a flow rate of 50 mL/min for 10 min. Flow was controlled via needle vane measured with Ellutia 7000 GC Flowmeter (Ellutia Chromatography Solutions, UK).

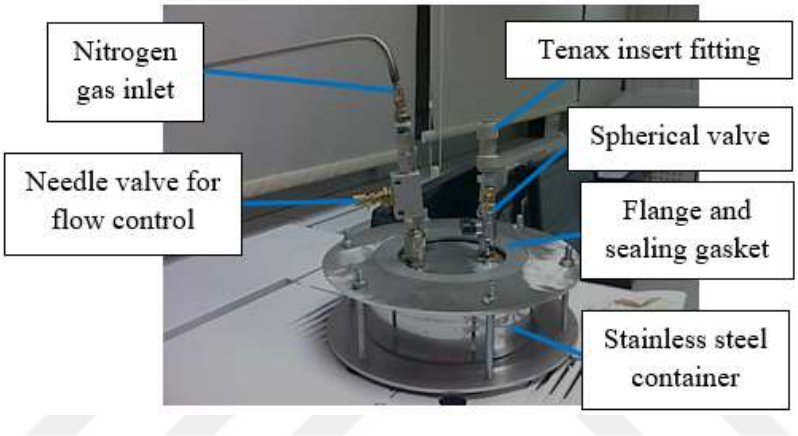


Figure 3.3 : Stainless steel sampling container for tenax studies.

Thermal desorber sampling unit (Markes, ULTRAA TD -100, USA) with Tenax tube (Markes, USA) autosampler was used. The Tenax tubes are shown in Figure 3.4. Sorbent Tenax Tube is a microporous polymer based on 2,6-diphenyl-p-phenylene oxide and its specific surface area is around 35 m^2/g (Table 3.1).

Figure 3.4 : Sorbent tenax tubes and caps (Markes, USA).

Table 3.1 : The properties of tenax tubes (Markes, USA).

Sorbent type	Porous polymer
Sorbent strenght	Weak
Specific surface area	~35 m^2/g
Approximate analyte volatility range	n-C7 to n-C30
Example analytes	Aromatics, apolar compounds b.p.>100°C, polar compounds b.p.>150°C, semi-volatile compounds
Recommended conditioning temperature	Up to 330°C
Recommended desorption temperature	280°C for trace levels, up to 320°C generally

Figure 3.5 : GC-MS system with thermal desorber unit

Desorption conditions were as follows; Pre-Purge time: 1-10 min, trap desorption time: 5 min., desorption temperature: 300-320°C, cold Trap Desorption Rate: 30 mL/min.

Tenax tubes were conditioned at 320°C desorption temperature for 4 h before each analysis. GC-MS analyses were performed with Agilent 7890B gas chromatography equipped with Agilent 5977A mass spectroscopy (Agilent, USA) as shown in Figure 3.5. Analytical column was DB-VRX (60mx0.25mmx1.4µm film thickness) (Agilent, USA). Column inlet temperature was 140°C and separation was performed with temperature program as follows: 40°C for 2 min, ramp to 250°C at a rate of 15°C/min and held at this temperature for 15 min. Helium was used as a carrier gas with a flow

rate of 1 mL/min. Mass spectroscopy specifications were similar to that of SPME technique.

Thermal desorption with Tenax in a special compartment is a unique technique. To ensure the accuracy of this technique and understand the matrix effect, the recoveries of the selected volatiles were calculated. 2,3-butandione, 3-hydroxy-2-butanone, hexanal and ethyl acetate were obtained from Merck (Merck KGaA, Darmstadt, Germany) and 2-ethyl-1-hexanol were purchased from Sigma Aldrich Chemie GmbH (Steinheim, Germany). They were used to spike the meat samples at different concentration levels in 20-200 ppb range. The work was conducted with three replicates.

Recoveries were calculated according to following equation (3.1)

$$\text{Recovery (\%)} = \frac{A_{spiked\ sample} - A_{unspiked\ sample}}{A_{spike}} x100 \quad (3.1)$$

Where,

$A_{spiked\ sample}$: The peak area of target analyte in spiked sample

$A_{unspiked\ sample}$: The peak area of target analyte in unspiked sample

A_{spike}: The peak area of spike added target analyte

The repeatibility and the reproducibility (with two different operators) for each spiked concentration levels of selected analytes were calculated to assess the variation of data and operator difference. The repeatibility and the reproducibility were expressed as relative standard deviations (%RSD) of results from repeated measurements by the following formula (3.2):

$$\%\ RSD\ = \frac{Standart\ Deviation\ (Data\ Range)}{Average\ (Data\ Range)} x100 \quad (3.2)$$

3.2.1.2 Meat quality assesment

Control meat samples were stored at 4°C for 7 days to determine the spoilage time of meat. Samples for volatile analyses were taken on the first day of spoilage (day 4 at 4°C). Decision was based on the results of microbiological analysis (Total viable counts, *Pseudomonas* spp.), lipid oxidation (Thiobarbutyric acid substances) and

sensory evaluations. Descriptive sensory analysis method was used. The details of methods are given under section 3.2. Meats were evaluated for different quality characteristic based on score scale. For microbiological analyses, three samples of meat (25 g each) were taken until the point of sensory rejection. Microbiological level limits were accepted as 6 log cfu/g for total viable counts and 5 log cfu/g for *Pseudomonas* spp.TBA limit is generally accepted as 1 mg malondialdehyde/kg meat. All analytical results were compared with sensory scores to predict shelf life.

3.2.1.3 Statistical analysis

Each volatile organic compound experiment was carried out in three replicates. A comparison between loading factor groups was performed by one-way ANOVA test. The differences were considered significant at $p < 0.05$. Loading factor levels were compared with Tukey's Pairwise Comparison test with 95% confidence. RSD% values were calculated according to means and standard deviations.

3.2.2 Methods for Investigations on the Effect of Isothermal and Non-Isothermal Storage Conditions

3.2.2.1 Isothermal and non-isothermal conditions

Isothermal and non-isothermal experiments were performed with a domestic cooler having thermocouples and PID (proportional–integral–derivative) controller. Door open-close cycles of non-isothermal conditions were specified based on a survey study (Thomas, 2007) that reflects the consumer habits on domestic refrigerators in Europe. In the study, it was found that door open time of refrigerator was commonly 15 seconds and total open time was between 1.5 min. – 19.3 min. per day. Door open time was selected as 15 seconds with 30 minutes' intervals between each door open-close cycles for non-isothermal condition 1. Non-isothermal condition 2 represents the same door open times with 15 minutes' intervals between door open-close cycles. Refrigerator door was opened with the angle of 90° at each cycle. Domestic refrigerators with 400 L of total internal volume were positioned at 25±3°C temperature. Time and temperature illustrations of mentioned conditions are shown for 4°C and 0°C in Figure 3.6 and Figure 3.7, respectively.

Storage temperatures and door open-close conditions in this study are shown in Table 3.2.

Table 3.2 : Storage parameters for isothermal and non-isothermal conditions.

Conditions	Storage temperature °C	t_{open}: total door open time (sec. per day)	n_{cycle}: door open-close cycle (times per day)
Isothermal	0 and 4	0	0
Non-isothermal 1	0 and 4	300	20
Non-isothermal 2	0 and 4	600	40

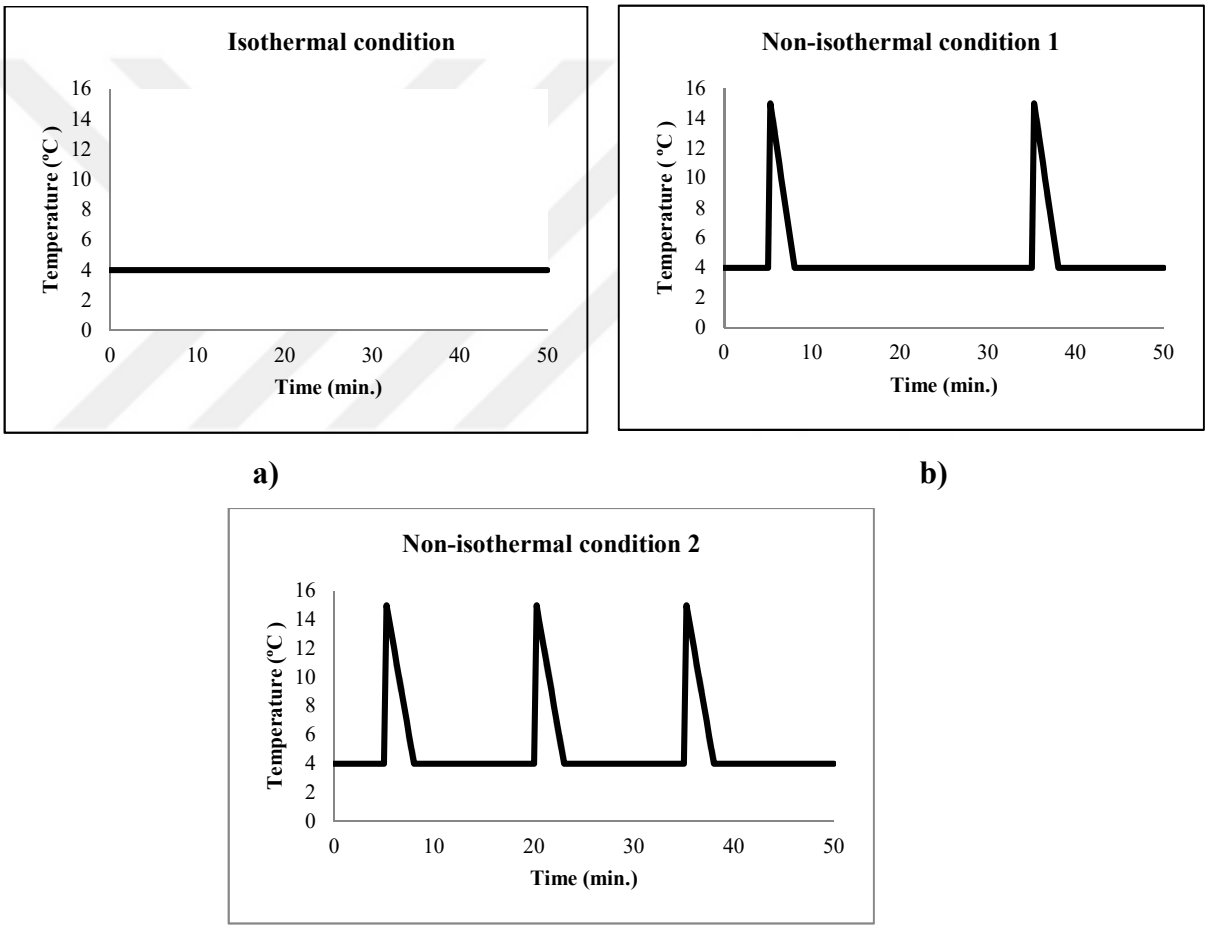


Figure 3.6 : Time-temperature illustrations of isothermal and non-isothermal conditions for 4°C. a) Isothermal condition, b) Non-isothermal condition 1, c) Non-isothermal condition 2.

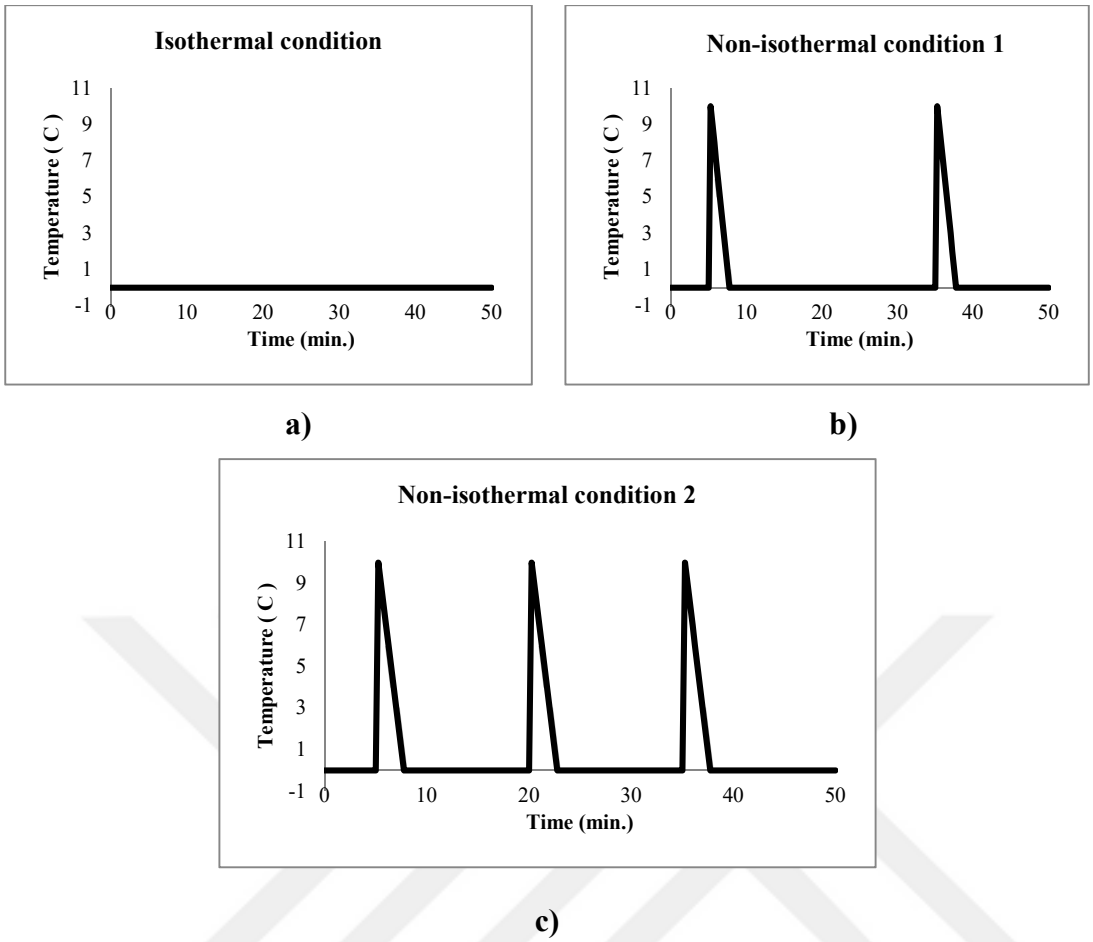


Figure 3.7 : Time-temperature illustrations of isothermal and non-isothermal conditions for 0°C. a) Isothermal condition, b) Non-isothermal condition 1, c) Non-isothermal condition 2

3.2.2.2 Microbiological analyses

Meat samples were stored at +4°C ± 2°C in the sterile box/petri dish/bag to prevent contamination from environment until the tests. Experiments were performed in the biosafety cabinet of a food microbiology laboratory at ambient temperature. 25 g of meat sample was added to 225 mL of sterile peptone salt water and homogenized in a stomacher (IUL, S.A., Barcelona, Spain) for 90 s at room temperature. Serial decimal dilutions in peptone salt water were prepared and 0.1 mL of samples at appropriate dilutions were spread on agar plates with drigalski spatula. Growth media were used as follows: total viable counts were determined on CASO Agar Base (Tryptic Soy Agar) and *Pseudomonas* spp. was determined on CFC Agar Base (Cephalothin-Sodium Fusidate-Cetrimide Agar Base) supplemented with Modified CFC Selective Supplement (FD281) incubated at 37°C for 48 h. The growth data is presented as log cfu g^{-1}. Colony growth at petri dish was enumerated on a colony counter visibly.

For total viable counts, CASO (Tryptic Soy Agar) powder was dissolved in distilled water (40 g/L as suggested by producer) and sterilized at 121°C for 15 minutes. Sterilized and melted agar was hold at 45°C in a water bath until it was used.

For *Pseudomonas* spp. counts, CFC Agar Base (Cephalothin-Sodium Fusidate-Cetrimide Agar Base) was dissolved in the distilled water (52.4 g/L as suggested by producer) and sterilized at 121°C for 15 minutes. Sterilized and melted agar was hold at 45°C in a water bath until it was used. Just before using of sterilized and melted agar base, Modified CFC Selective Supplement (FD281) was added (2 vial for 1 L as suggested by producer) and mixed homogenously.

For Supplement preparation, powder form of Modified CFC Selective Supplement was dissolved within the mix of 1 mL ethyl alcohol and 1 mL distilled water. This solution was poured on agar base and mixed homogenously. Supplemented agar solution was poured on petri dishes in the amount of 15-20 mL and dried under ventilated biosafety cabinet.

Preparation of peptone salt water for dilutions were 8.5 g of NaCl was dissolved in 1 L distilled pure water homogenously and portioned into glass tubes with 9 mL in each tube. Tubes were sterilized at 121°C for 15 minutes.

3.2.2.3 Thiobarbutyric acid reactive substances

Thiobarbutyric acid method defined by Pikul et al. (1989) was applied with minor modifications. Meat sample (15 g) was homogenized with 30 mL of cold extraction solution containing %7.5 trichloroacetic acid (TCA) at 5000 rpm for 1 min. The blended sample was filtered through the Whatman No.1 filter paper into tubes and centrifuged at 5000 rpm with a cooling centrifuge device (Nüve NF 800R, Turkey). 5 mL of supernatant was transferred to 10 mL test tube with screw cap and then, 5 mL of 20 mM thiobarbutyric acid (TBA) solution (0.72 g of TBA was dissolved in 250 mL of distilled water) was added and mixed vigorously. Test tubes were incubated at 85°C water bath for 45 minutes and then was cooled to room temperature. Absorbances were measured with a UV-Vis spectrophotometer (Lambda 35, Perkin Elmer Inc., USA) at 520 nm against a blank solution.

The TBA value was calculated by multiplying absorbance with a constant coefficient K, calculated from standard curves. Standard curves were prepared with 1,1,3,3-

tetramethoxypropane in 7.5% TCA solution. The TBA value was expressed as mg malondialdehyde (MDA) per kg of meat.

3.2.2.4 pH measurement

The pH value was recorded by a pH metre (WTW Inolab, Terminal 740, WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) with a glass electrode being immersed in the homogenate of meat (10 g) which was mixed with 100 mL of distilled water on a magnetic stirrer (IKA C-MAG HS 7, Germany) (Serdaroğlu, 2010).

3.2.2.5 Surface color measurement

Surface color of meat as L∗ (lightness), a∗ (redness), b∗ (yellowness), Hue angle and saturation index values were determined using a colorimeter (Konica Minolta CR 300, Osaka, Japan) calibrated against a standard seramic white plate. Duplicate measurements were taken at four different area of the same meat. Hue angle and saturation index were calculated according to the equations given below. One measurement includes the average of four different area of the same meat. Hue angle and chroma were calculated from a* and b* values according to the following equations (3.3 and 3.4) (Wrolstad and Smith, 2010).

$$\text{Hue angle} = \arctan [b^*/a^*] \quad (3.3)$$

$$\text{Saturation index} = \sqrt{a^{*2} + b^{*2}} \quad (3.4)$$

3.2.2.5 Sensory analysis

Descriptive sensory analysis was applied to the samples. Samples were evaluated for intensity of different quality characteristic based on score scale. Sensory evaluation was performed by trained panelists (minimum of 8 members) familiar with assesment of meat. Beef sensory quality were scaled based on the descriptive attributes of visual, off-odor and overall acceptability. All assesment scales are shown in Table 3.3. Samples that had overall acceptability below 4 were identified as rejected. All samples were hold at 20°C for 30 minutes prior to sensory analyses. Standard deviations between panelists were calculated among 0.5-1.0 for each of sensory panel. Sensory analyses were conducted as three replicates for each different case.

Table 3.3 : Sensory evaluation parameters and scores.

Visual Assesment		Off-Odor Assesment		Overall Acceptability	
No color change	7	No off-odor	7	Like extremely	7
Very slight color change	6	Very slight off-odor	6	Like very much	6
Sligth color change	5	Slight off-odor	5	Like moderately	5
Moderate color change	4	Moderate off-odor	4	Like slightly	4
High intensity color change	3	Mild-High intensity off-odor	3	Dislike moderately	3
Very high intensity color change	2	High intensity off-odor	2	Dislike very much	2
Completely changed color	1	Very high intensity off-odor	1	Dislike extremely	1

3.2.2.6 Statistical analyses

Means and standard deviations of all measured values were calculated for each condition. General Linear Model was applied to all set of data, followed by a multiple comparison, the Tukey's test. Significance value was $p<0.05$. Minitab 17 (Minitab Inc., USA) statistical program was used for data analyses. Sensory scores were represented as the mean of each panelist's scores. Means and standard deviations of the peak areas of volatile organic compounds were calculated for each condition. General Linear Model was applied to a set of VOC data, followed by a multiple comparison, the Tukey's test. Significance value was $p<0.05$. The data of the peak areas of VOC and sensory scores of overall acceptability were submitted to Principal Components Analysis (PCA) for qualitative classification. For the quantitative estimation of the biochemical changes and the microbial population of total viable counts, *Pseudomonas* spp., LAB and sensory quality, PLS regression (PLS-R) models were calculated using the volatile compounds as input variables (predictors) and the quality parameters as output variables (response). The leave-one-out cross validation (LOOCV) technique was applied to evaluate the performance of the models. The Minitab 17 (Minitab Inc., USA) statistical program was used for all mathematical data treatments and statistical analyses.

The PLS model is a bilinear regression model that extracts a small number of factors, which are a combination of the independent variables, and uses these factors as a regression generator for the dependent chemically and biologically measured variables

(Maleki et al, 2006). PLS regression is known for its simplicity, robustness, predictability, precision, and clearly quantitative explanations. Despite this, PLS regression does not present a quantitative relationship between predictor variables and response variables, and it does not support re-use of model algorithms between different instrumentations (Li et al, 2012). Some validation parameters of model are evaluated such as X Variance, Error, R-Sq, PRESS and R-Sq (pred.). Prediction errors (PE) or standardized residuals for each of individual prediction point were calculated with the equation (3.5) below and it can be served as the overall performance of the model (Oscar, 2009). The percentage of PE above 70% present that the prediction model can be used for prediction of test data in the acceptable range (Oscar, 2005).

$$\%PE = (PE_{in}/PE_{total}) \times 100 \quad (3.5)$$

Where,

PE_{in} : The number of PE between acceptable prediction zone ($1.5<PE<0.5$)

PE_{total} : The total number of PE

Discriminant analysis were applied to some selected volatile compounds according to outputs of PCA analysis to discriminate the freshness state of meat by using sensory evaluation results. Discriminant analysis classifies observations into two or more groups to investigate how the predictors contribute to the groupings.

4. RESULTS AND DISCUSSION

4.1 Comparison of Two Volatile Sampling Techniques Based on Different Loading Factors for Determination of Volatile Organic Compounds Released from Spoiled Raw Beef

Loading factor effect was investigated using two volatile sampling techniques such as: 1) Solid phase microextraction (SPME-GC/MS) and 2) Gas flushing on Tenax tube with Thermal Desorption (TD). These methods were used to investigate the interactions and relationship between sample amount, type of analyte and extraction method.

4.1.1 Effect of loading factor on volatile organic compound analysis with SPME-GC/MS

Three different loading factor such as 0.025 kg/L, 0.05 kg/L and 0.10 kg/L were studied. In the first part, solid phase microextraction (SPME-GC/MS) technique was used. Representative chromatography of the spoiled meat volatiles at 4th day measured with SPME technique is given in Figure 4.1.

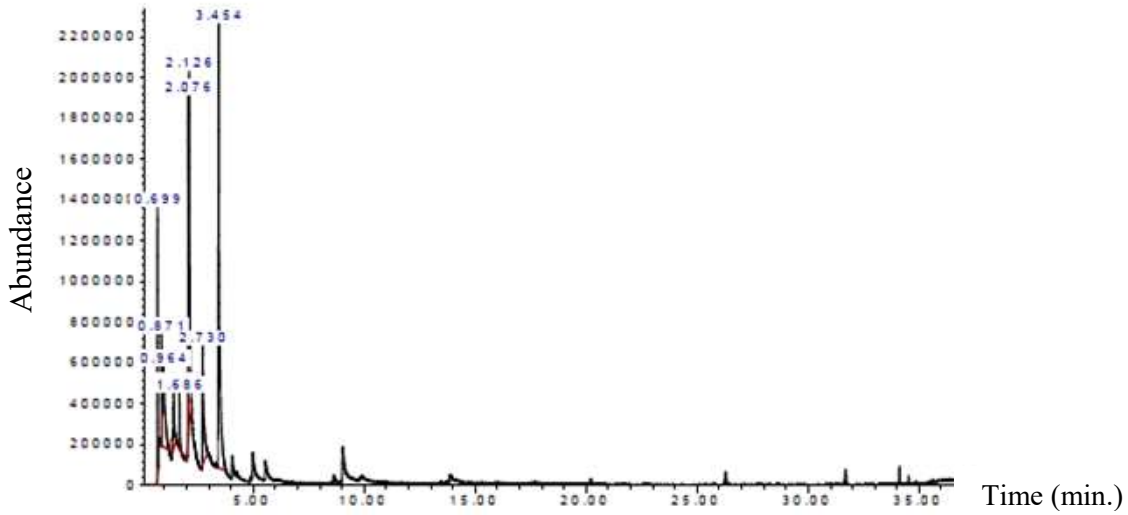


Figure 4.1 : Chromatography (abundance versus time) of the spoiled meat volatiles sampled with SPME fiber.

Effect of three loading factors on alcohol, aldehyde, ketone and ester compounds were investigated. Significance of differences were compared with statistical analysis and results are represented in Table 4.1.

Table 4.1 : Statistical analyses for comparison of loading factors with SPME-GC/MS method.

Volatile Organic Compounds	RSD% *			P-Value (Significance level α = 0.05)	Tukey Pairwise Comparisons** (95% Confidence)
	0.025 kg/L	0.05 kg/L	0.10 kg/L		
Ethanol	5.86	2.80	11.64	0.002	b, a, a
1-Butanol, 3-methyl-	21.60	24.16	5.28	0.009	b, a, b
1-Pentanol	N.D.	0.72	8.61	0.000	c, a, b
4-Amino-1-pentanol	2.56	15.64	11.79	0.018	a, b, a
1-Hexanol	N.D.	2.69	23.70	0.000	c, a, b
1-Octen-3-ol	7.59	4.07	8.97	0.000	b, a, b
2-Ethyl-1-hexanol	3.56	5.28	18.16	0.014	a, b, b
2,3-Butandione	27.11	16.99	16.19	0.018	b, a, ab
2-Butanone	5.67	3.42	12.92	0.030	b,a,a
2-Butanone, 3-hydroxy-	8.35	14.37	4.76	0.006	a,b,b
3-Octanone	33.89	2.01	21.73	0.071	a,a,a
2-Propanone	3.51	2.45	5.91	0.000	c,b,a
2-Heptanone	6.32	10.26	0.34	0.001	c,a,b
Pentanal	17.35	11.42	0.60	0.002	b,a,a
Hexanal	5.46	12.30	0.11	0.001	a,b,b
Butanal, 3-methyl-	1.33	22.20	20.34	0.012	ab, a,b
Ethyl acetate	4.20	9.72	10.26	0.025	a,b,b

*RSD%: Relative standard deviation

**Different letters within a row indicate significant differences among loading factors of 0.025 kg/L, 0.05 kg/L and 0.10 kg/L, respectively.

***N.D.: Not determined.

4.1.1.1 Alcohol compounds

3-Methyl-1-butanol, 2-ethyl-1-hexanol, 1-hexanol and 1-octen-3-ol can be listed as the most abundant alcohol compounds related with spoilage of meat. 3-Methyl-1-butanol, 2-ethyl-1-hexanol, 1-hexanol has etheric, fruity, 1-octen-3-ol shows musty aroma. 1-Octen-3-ol is defined as a product of oxidation in some food materials (Soncin et al, 2007).

A strain type of *Brochothrix thermosphacta* may produce 1-octen-3-ol and 3-methyl-1-butanol (Dainty et al, 1985; Casaburi et al, 2014). In recent studies, it is indicated that 2-ethyl-1-hexanol is formed in aerobic conditions by *Br. thermosphacta* and *P. fragi* (Casaburi et al, 2014; Ercolini et al, 2010). Similarly, 1-hexanol is also released with the activity of *P. fragi* and *C. maltaromaticum* (Ercolini et al, 2010; Casaburi et al, 2011). Effect of loading factor on alcohol compounds are shown in Figure 4.2. Detected alcohol compounds were found to be ethanol, 3-methyl-1-butanol, 1-pentanol, 4-amino-1-pentanol, 1-hexanol, 1-octene 3-ol and 2-ethyl-1-hexanol. It was observed that loading factor affected the peak levels of all alcohol compounds significantly (p<0.05). Highest peak areas were obtained when 0.05 kg/L loading factor was applied for most of the volatiles than results obtained with 0.025 kg/L and 0.10 kg/L. 0.05 kg/L loading factor was found to be significant with Tukey's pairwise comparison test. Lower peak areas were measured with the lowest loading factor of 0.025 kg/L except for 2-ethyl-1-hexanol. Especially, there was a considerable reduction on response for ethanol, 1-pentanol, 3-methyl-1-butanol, 1-hexanol, 1-octen-3-ol. Decreasing trend was observed only for 2-ethyl-1-hexanol with increasing loading factor. The reason of this may be due to the highest molecular weight of 2-ethyl-1-hexanol compared to other alcohol compounds. It means that 2-ethyl-1-hexanol may compete with the alcohols which has lower molecular weight at higher loading factors. Ethanol had great contribution to total peak area. It may be linked to the low retention index due to low molecular weight of ethanol.

Several reasons can be referred to explain this concentration effect according to theoretical and experimental studies. Analyte sorption on the vial wall or on the meat surface due to the super saturation of high partial pressure of related volatiles could be possible. It has been reported that there is a reality of lower degree of analyte absorption from humid headspaces (Chai and Pawliszyn, 1995). Fresh meat used in this study can cause high humid headspace due to high water content. High recoveries

of ethanol by CAR/PDMS fiber coating is characteristic for this coating (Marco et al, 2004).

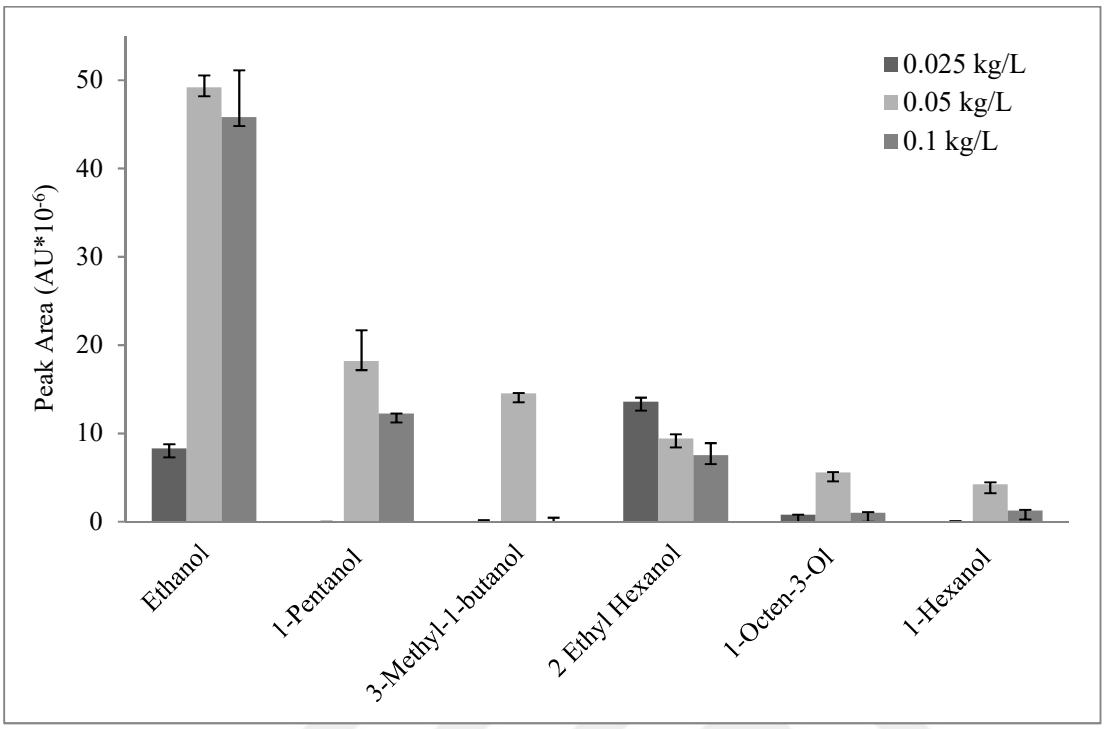


Figure 4.2 : Effect of loading factor on volatile alcohols extracted with SPME.

A study related with concentration effect has explained that higher concentrations could saturate small number of sites, especially fiber have high affinity for nitrogen compounds and alcohols. Additional concentration to the usual/normal absorption mechanism may cause to occupy these sites at high concentrations. The second possible reason could be the absorption of water vapour on sites if competition occurs between water vapour and polar analytes such as some alcohols (low molecular weight) for mixed ambient. In any event, the observed effects of concentration and solvent competition on K values (the amount of analyte in the fiber divided by the headspace concentration in the sampling bottle) were small relative to the effect of retention index. Higher K values had higher affinity of related compound on fiber (Bartelt, 1997). However; high K values do not always mean to be good. It is hard to eliminate losses of analytes with large K values due to the fact that such semi-volatile analytes tend to sorb at all surfaces when they are in contact with (Gorecki et al, 1998).

4.1.1.2 Aldehyde compounds

Aldehydes are responsible for fatty and grassy aroma (Pham et al, 2008). Aldehydes can be formed from hydrolysis of triglyceride, through fatty acid metabolism or lipid

autoxidation (Montel et al, 1998; Casaburi et al, 2015). 3-Methyl-1-butanal, hexanal and pentanal are commonly found off-flavor volatiles released during meat spoilage and can serve as reliable indicators for flavor deterioration (Wilkes et al, 2000). These compounds are related with the growth of *Br. thermosphacta, P. fragi* and *C. maltaromaticum* (Casaburi et al, 2015). 3-Methyl-1-butanal is derived from Leucine amino acid perceived as cheesy and pungent aroma (Casaburi et al, 2015; Smit et al, 2005). The peak areas of aldehyde compounds that were measured for three different loading factors are given in Figure 4.3. It was found that loading factor affected the peak levels of aldehydes significantly (p<0.05). Aldehydes showed low contribution to total peak level due to very low peak areas. Similar to behaviour of alcohol compounds, it was observed that the most effective extraction was obtained with 0.05 kg/L loading factor except for hexanal. However, it could be said that this difference of 0.05 kg/L loading factor is not clear due to the high relative standard deviations (RSD%) for aldehydes which have low peak areas relatively. Related with this, some studies have reported lower performances in measuring hexanal and pentanal in meat products with CAR/PDMS fiber in terms of reproducibility (Chiofalo and Lo Presti, 2012). Marco et al. (2004) showed that a competitive behaviour was existent between oxidation products (hexanal etc.) and ethyl acetate while using CAR/PDMS fiber due to its higher affinity to esters. Shorter extraction times may avoid this competitive effect due to the elimination of further lipid oxidation processes.

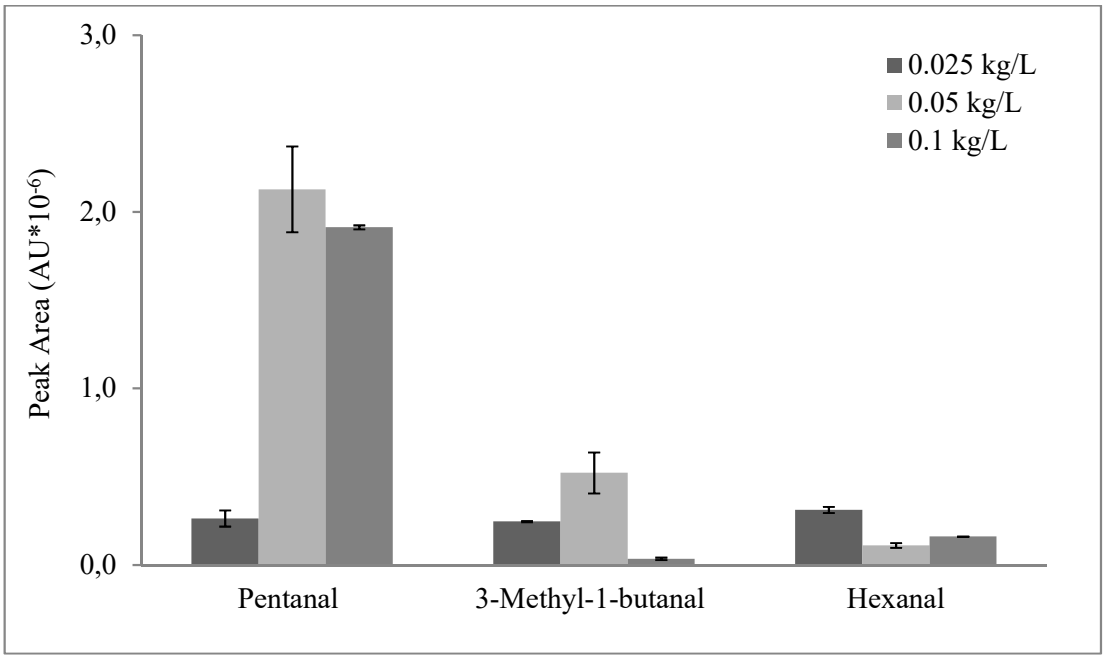


Figure 4.3 : Effect of loading factor on volatile aldehydes extracted with SPME.

4.1.1.3 Ketone compounds

Ketones are one of the most impact volatile compounds that are formed aggressively during meat spoilage. Major ketones commonly found in fresh meat are 2-propanone, 2-butanone, 2,3-butandione, 3-hydroxy- 2-butanone, 2-pentanone and 2-heptanone. Ketones are mostly produced due to the growth of *Pseudomonas* spp., *Carnobacterium* spp. and *Enterobacteriaceae* microorganisms (Casaburi et al, 2015). Moreover, 3-hydroxy-2-butanone is a product of glucose catabolism by *Br. thermosphacta, Carnobacterium spp.* and *Lactobacillus spp.* and/or aspartate degradation (Ardö, 2006). 3-Hydroxy- 2-butanone has cheesy aroma characteristic in fresh meat. In this study, the volatile ketones that were taken into account are 2-propanone, 2,3-butandione, 2-butanone, 3-hydroxy-2-butanone, 3-octanone and 2-heptanone which can be seen in Figure 4.4. Considerably high peak areas were obtained for ketones using SPME fiber. Total peak areas of ketones took higher percentage compared with other volatiles. Loading factor affected the peak levels of ketone compounds significantly ($p<0.05$). However, unlike most alcohol and aldehydes, ketones did not show a characteristic curvature trend with increasing loading factor. For each ketone compound, unique results were obtained. It can be explained that 0.05 kg/L and 0.10 kg/L loading factor may provide most considerable results for most of the aldehydes. Additionally, there was no clear difference between this two loading factors according to Tukey's test results. 2-Propanone was the dominant volatile compound with 0.05 kg/L and 0.10 kg/L loading factor resulted with nearly four times higher peak areas than the others. 3-hydroxy-2-butanone and 3-octanone followed 2-propanone.

Bartelt (1997) has stated that K values at different headspace concentrations of 3-hydroxy-2-butanone are affected by the presence of other compounds in the environment. Experiments have showed that the concentration of 3-hydroxy-2-butanone on fiber was depended to the headspace concentration in dichloromethane solvent unlike to methanol polar solvent (Bartelt, 1997). In our study, peak area of 3-hydroxy-2-butanone was the highest between other ketones with 0.025 kg/L loading while the peak area of 2-propanone was one of the lowest ones interestingly. In a study with goat meat, it was reported that the low-molecular-weight (MW) compounds like 2,3-butanedione were extracted efficiently by the CAR/PDMS fiber (Madruga et al, 2009). Both Tenax and SPME extraction involved a successful concentration process for extraction of compounds with low molecular weight (Madruga et al, 2009).

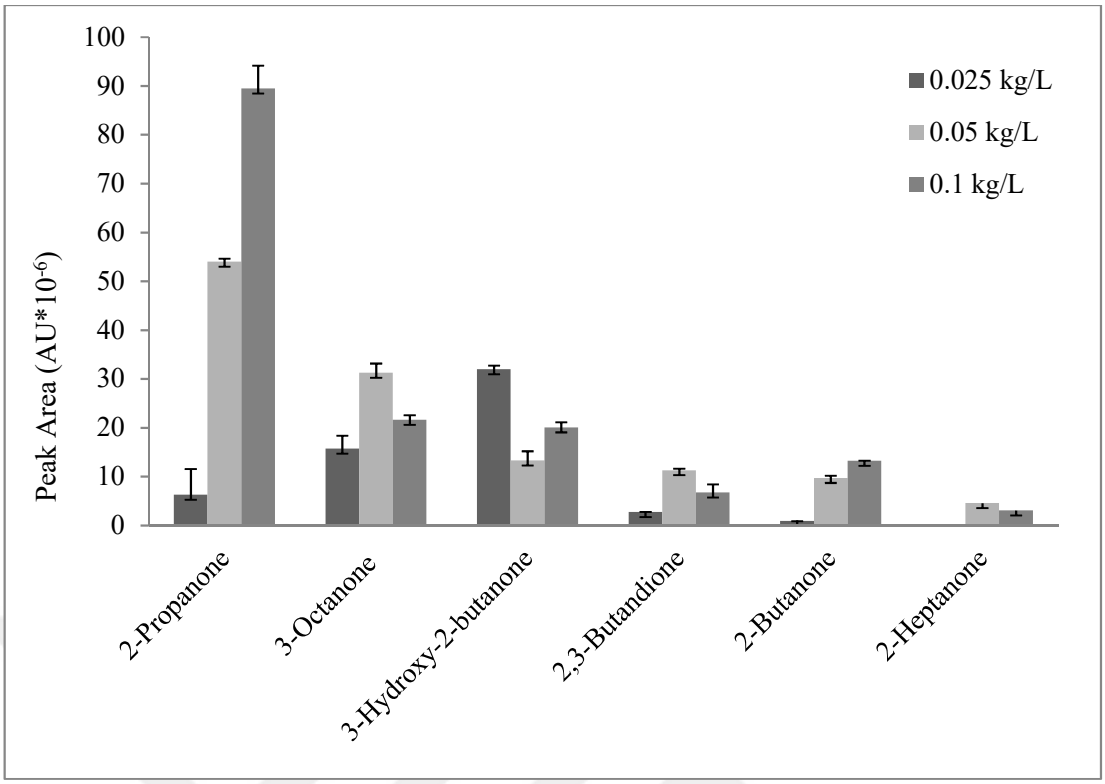


Figure 4.4 : Effect of loading factor on volatile ketones extracted with SPME.

4.1.1.4 Esters

Esters principally are formed in the presence of *P. fragi* at aerobic conditions (Ercolini et al, 2009). Some resources indicated that esters may occur with esterification of alcohol and carboxylic acid, and also with microbial esterase activity (Toldra, 1998). Most ester compound has sweet and flower odor (Curioni and Bosset, 2002). The only ester compound extracted with SPME was ethyl acetate. The peak areas of ethyl acetate are given in Figure 4.5.

Loading factor affected the peak levels of ethyl acetate significantly ($p<0.05$). Ethyl acetate resulted with having considerable peak area around $20x10^6$ AU for all loading factors while the highest extraction efficiency was obtained with 0.025 kg/L. As compatible with our results, it has been indicated that SPME fiber coated with CAR/PDMS had higher affinity to esters (Marco et al, 2004).

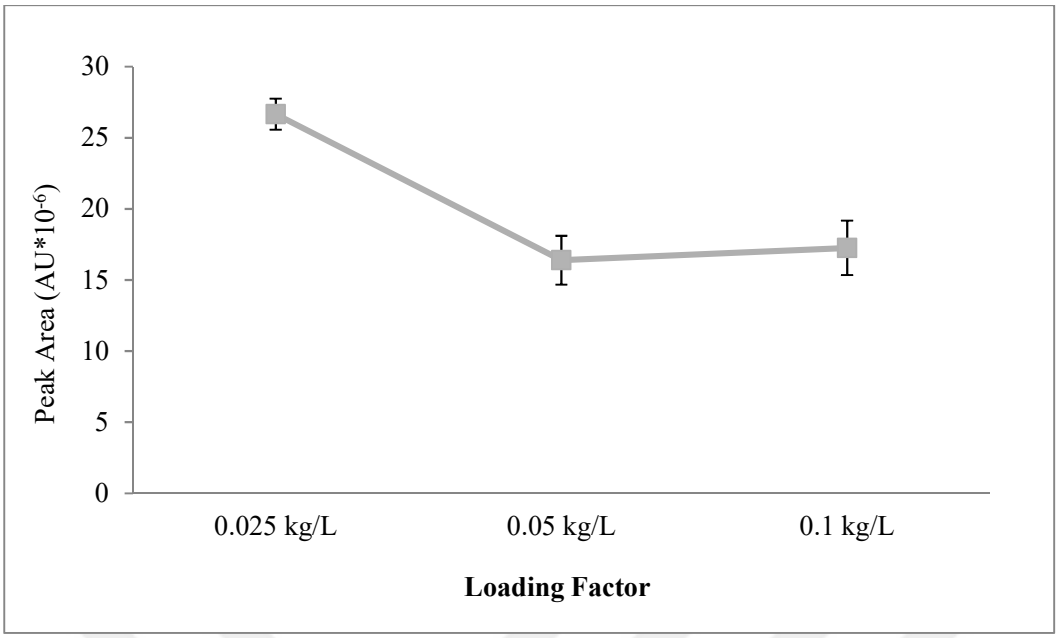


Figure 4.5 : Effect of loading factor on ethyl acetate extracted with SPME.

4.1.2 Effect of loading factor on volatile compound analysis with thermal desorber with gas flushing-tenax

Gas flushing on Tenax sampling with thermal desorber technique was the second technique to analyse spoiled meat volatiles. Chromatographic technique was more complicated compared with SPME technique due to flushing the whole air volume over Tenax. A representative chromatography of the spoiled meat volatiles measured with gas flushing on Tenax technique is given in Figure 4.6. Statistical comparison of loading factors is given in Table 4.2.

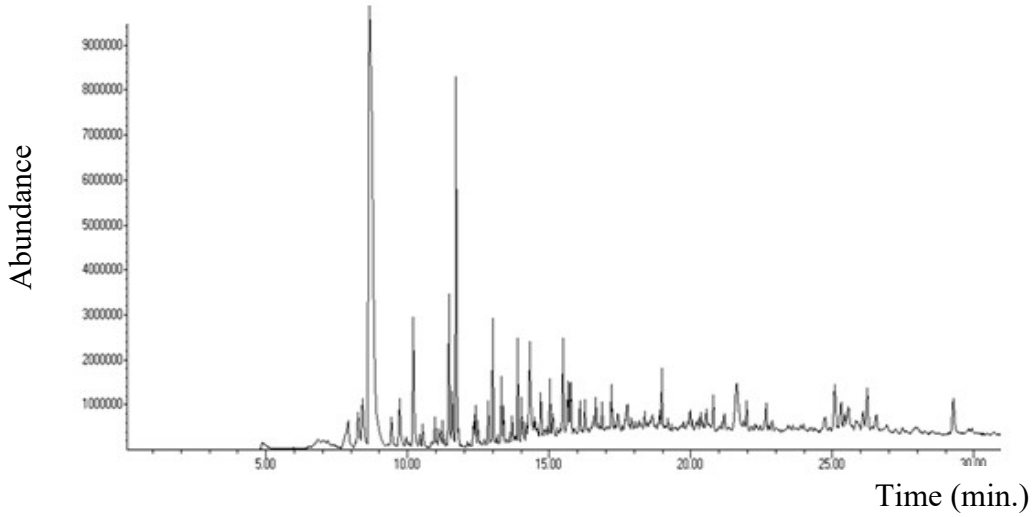


Figure 4.6 : Chromatography of the spoiled meat volatiles sampled with gas glushing on Tenax.

Table 4.2 : Statistical analyses for comparison of loading factors with gas-flushing on Tenax method.

Volatile Compounds	RSD% *			*p*-Value (Significance level α = 0.05)	Tukey Pairwise Comparisons** (95% Confidence)
	0.025 kg/L	0.05 kg/L	0.10 kg/L		
Ethanol	13.27	5.45	2.65	0.036	ab, a, b
3-Methyl-1-butanol	11.23	1.05	N.D.	0.000	b, a, c
1-Pentanol	7.66	16.32	3.20	0.020	b, a, ab
4-Amino-1-pentanol	9.34	7.83	13.99	0.347	a, a, a
1-Hexanol	3.98	4.91	6.07	0.000	b, a, b
1-Octen-3-ol	7.08	19.82	9.49	0.007	b, a, b
2-Ethyl-1-hexanol	1.20	7.42	6.01	0.011	b, b, a
2,3-Butandione	1.76	6.05	8.68	0.000	b, a, b
2-Butanone	5.36	4.92	1.07	0.001	b, a, c
3-Hydroxy-2-butanone	8.99	7.03	0.65	0.000	b, a, c
3-Octanone	2.83	2.71	12.60	0.000	b, a, b
2-Propanone	4.46	6.95	4.31	0.002	a, a, b
Pentanal	14.15	11.04	15.77	0.004	b, b, a
Hexanal	20.37	32.94	37.16	0.042	ab, a, b
3-Methyl-1-butanal	50.65	9.66	28.19	0.006	b, a, a
Butanal	79.59	68.12	55.08	0.163	a, a, a
2-Butanamine	19.76	5.53	3.76	0.007	a, b, b
Dimethylamine	57.14	14.62	7.09	0.003	c, a, b

*RSD%: Relative standard deviation

**Different letters within a row indicate significant differences among loading factors of 0.025 kg/L, 0.05 kg/L and 0.10 kg/L, respectively.

***N.D.: Not determined.

4.1.2.1 Recovery of target analytes

2,3-Butandione, 3-hydroxy-2-butanone, hexanal, ethyl acetate and 2-ethyl-1-hexanol were used to spike the meat samples at different concentration levels in 20-200 ppb range. The results of recovery calculations of selected analytes are given in Table 4.3. The average of recovery percentages with gas flushing on Tenax were between 70%-110% accepted as suitable for further analysis. At low concentrations of all analytes, recoveries were low that can be linked to the minimum detection limits. In addition, calibration curve equations and correlation coefficients for 20-200 ppb range are

shown in Table 4.4. Calculated calibration curves for each analyte were characterized by high correlation coefficients.

Table 4.3 : Recovery (%) of spiked selected analytes at different concentration ranges.

Concentration of Analytes	2,3-Butanedione	Ethyl acetate	2-Ethyl-1-hexanol	Hexanal	3-Hydroxy-2-butanone
20 ppb	77.7	69.6	76.4	70.2	84.5
200 ppb	82.4	90.9	92.7	79.9	97.9
2000 ppb	81.3	103.1	93.7	97.1	110.3

*Three replicate measurements were evaluated.

Precision of the method was assessed by repeatability for one operator and reproducibility for two operators (Table 4.5 and Table 4.6). The RSD (%) values of selected analytes at three different spiked concentration levels were between 1.13 and 7.29.

Table 4.4 : Calibration curve formula and coefficients for selected analytes.

Analytes	Calibration Curve Formula*	Correlation Coefficient (R^2)	Linear Range (ppb)
2,3-Butanedione	$y = 7.2 \times 10^3X - 4.6\times10^5$	0.9979	20-200
Ethyl acetate	$y = 2.6 \times 10^3X + 4.3\times10^5$	0.9988	20-200
2-Ethyl-1-hexanol	$y = 3.2 \times 10^3X - 5.5 \times 10^5$	0.9999	20-200
Hexanal	$y = 5.2 \times 10^4X$	0.9877	20-200
3-Hydroxy-2-butanone	$y = 1.4 \times 10^4X$	0.9893	20-200

*X is the quantity of each compound (ppb) solution in pure water. Y is the peak area.

Table 4.5 : Repeatability (%RSD) * results for selected analytes.

Concentration of Spiked Analytes	2,3-Butanedione	Ethyl Acetate	2-Ethyl-1-hexanol	Hexanal	3-Hydroxy-2-butanone
20 ppb	3.32	3.18	6.02	7.29	2.07
200 ppb	2.13	2.15	4.91	4.26	6.37
2000 ppb	2.82	1.13	2.97	1.29	2.16

*Three replicate measurements were evaluated for each spiked concentration level

Table 4.6 : Reproducibility (RSD%) * results for selected analytes.

Concentration of Spiked Analytes	2,3-Butanedione	Ethyl Acetate	2-Ethyl-1-hexanol	Hexanal	3-Hydroxy-2-butanone
20 ppb	5.61	3.17	6.71	5.59	2.38
200 ppb	2.02	2.40	4.26	2.97	5.32
2000 ppb	3.22	1.97	2.13	1.46	3.25

*Three replicate measurements of two operators were evaluated for each spiked concentration level.

4.1.2.2 Alcohol compounds

Volatile alcohol compounds sampled with gas flushing in a container are shown in Figure 4.7. Similar alcohol compound profile was observed with SPME technique regarding the type and number of compounds. Loading factor affected the peak levels of alcohol compounds significantly except 4-amino-1-pentanol (p<0.05). It was seen that optimum response was provided with 0.05 kg/L for most of the alcohol compounds. Especially, 3-methyl-1-butanol, 1-penthanol, 1-hexanol and 1-octen-3-ol showed great differences with 0.05 kg/L compared with the loading factors of 0.025 kg/L and 0.10 kg/L. This significant difference of 0.05 kg/L loading factor from the others was indicated with Tukey's comparing test. The behaviour of these compounds was consistent with the results found in SPME for vial samples while the peak area of compounds has showed different dynamics in both sampling methods. 2-Ethyl-1-hexanol had the highest peak area in alcohols for all conditions, while ethanol was dominant in SPME sampling. These results can be explained with the affinity of absorption materials used in SPME fiber and Tenax. It was shown that SPME fibers had high affinity to ethanol compound despite unaffected by loading factor generally. It was stated that high concentration of ethanol may interfere with other analytes due to high affinity (Kataoka et al, 2000). This results with higher peak areas of ethanol with SPME than Tenax. Moreover, the total alcohol peak area levels were higher compared to Tenax sampling.

Another possible theory is that the supersaturated volatiles may diffuse back to meat matrix. Therefore, concentration of some volatiles in the headspace can decrease at higher loading factors. The results were not in accordance with the comments stated by Ouyang and Pawliszyn (2006), as the amount of the analyte extracted from sample was measured as proportional to its initial concentration in sample. Bartelt (1997) also

found that, nitrogen- and hydroxy-containing compounds had concentration dependent results.

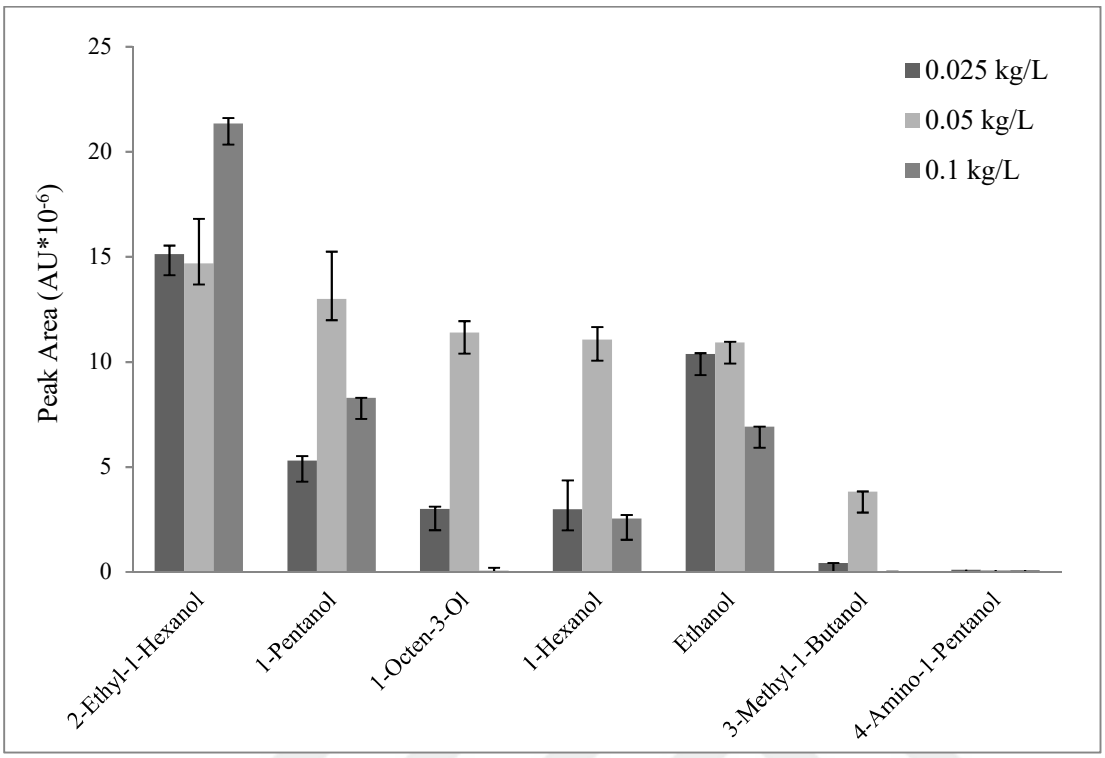


Figure 4.7 : Effect of loading factor on volatile alcohols extracted with gas flushing on Tenax.

4.1.2.3 Ketone Compounds

Although similar ketone compounds were found with HS-SPME fiber, their peak area levels were found to be different during gas flushing on Tenax sampling. Volatile ketones extracted with gas glushing on Tenax can be seen in Figure 4.8. Loading factor affected the peak area levels of all ketone compounds significantly (p<0.05).

Like alcohol compounds, most efficient extraction method for volatile ketones was measured with 0.05 kg/L followed by 0.025 kg/L and 0.10 kg/L, respectively for most of the compounds except 2-propanone. This difference was regarded as significant according to Tukey's test. 3-Hydroxy-2-butanone had the highest peak area between all ketone compounds. It was mentioned that 3-hydroxy-2-butanone could not compete with other ketones like acetone (lower molecular weight) at highest loading factor due to the higher molecular weight with Tenax sampling unlike with SPME fiber. Also, higher humid conditions may be the reason of decrease in all ketones at high product loads. There was a great decrease at the peak area of 2,3-butandione, 2-butanone, 2-propanone, 3-hydroxy-2-butanone and 3-octanone at a loading factor of 0.10 kg/L.

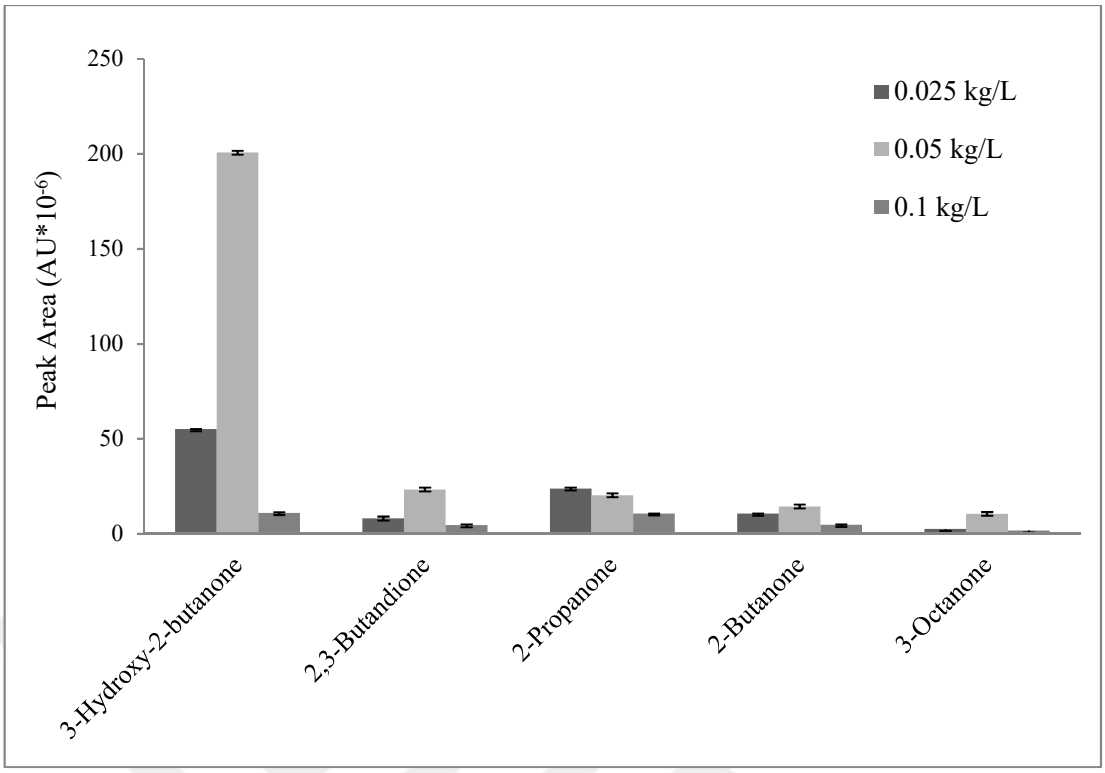


Figure 4.8 : Effect of loading factor on volatile ketones extracted with gas flushing on Tenax.

It was clearly observed that 0.05 kg/L loading factor resulted with optimal results for Tenax sampling techniques contrary to mixing trend of SPME results. It may be deduced that; competitive effect was dominant in passive sampling like SPME. Peak areas of total ketones extracted with Tenax were slightly higher than SPME sampling technique. Stationary conditions may offer more competitious conditions. In goat meat study, 2,3-butanedione ketone compounds were extracted only by headspace extraction on Tenax (Madruga et al, 2009). In consistence with the result of Madruga et al. (2009), the peak area level of 2,3-butanedione was higher in Tenax sampling than SPME in this study.

4.1.2.4 Aldehyde compounds

The peak area of aldehyde compounds that are measured at three different loading factors are given in Figure 4.9. Peak area levels were found to be very low when compared with other compounds. With gas flushing-tenax, the types of aldehyde compounds and low-level characteristic were similar to SPME fiber except butanal. Butanal could only be extracted with Tenax sampling.

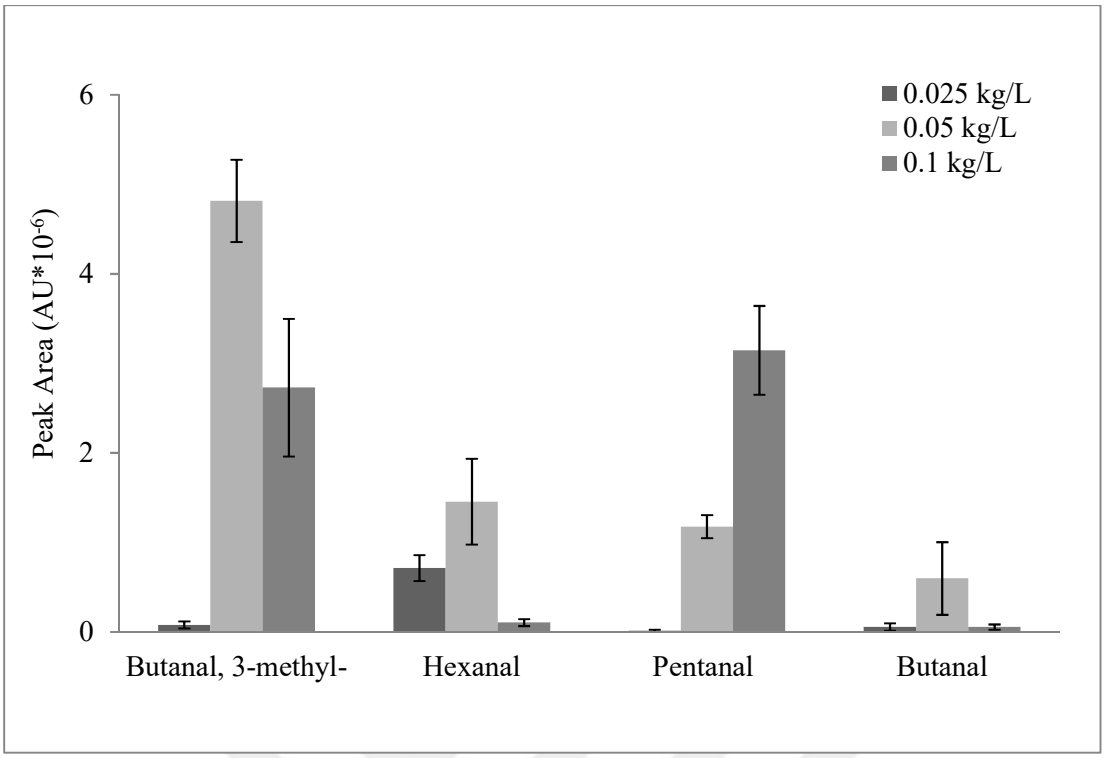


Figure 4.9 : Effect of loading factor on volatile aldehydes extracted with gas flushing on Tenax.

The most efficient extraction was obtained with 0.05 kg/L except for pentanal. Due to the comparable low abundance and high relative standard deviations (%RSD) of aldehydes, this effect cannot be considered distinguishable as similar to SPME results according to Tukey's comparison test. However, only clear results for 0.025 kg/L were obtained as inefficient for extraction.

4.1.2.5 Amines

Malodorous amines are the metabolites produced from proteolysis or can be formed by the action of *Pseudomonads* using nitrogenous compounds (Nychas et al, 1998). While amines were not detected by SPME sampling, 2-butanamine and dimethylamine were the only volatile amines extracted with Thermal desorption of Tenax tube sampling without a characteristic effect of the loading factor (Figure 4.10). Regarding total peak areas, contribution of amines was minor like aldehydes. Wzorek et al, (2010) studied about the detection of dimethylamine and trimethylamine in gaseous samples with SPME and Tenax sampling. They reported that SPME method was not satisfactory for dimethylamine while Thermal desorption with Tenax allowed reliable measurements both for dimethylamine and trimethylamine similar to our study. Difficulties in measurement of dimethylamine (DMA) with SPME method were linked

to its properties since it is highly basic and polar which have high tendency of adsorption on the surface of the equipment (Wzorek et al, 2010; Namiesnik et al, 2000). It was also noted that Thermal desorption with Tenax is a good preconcentration technique for amines without derivatization (Wzorek et al, 2010). Due to high humid conditions in meat headspace, it may be difficult step to preconcentrate amines in headspace because of their high polarity properties interacting with water in air. Therefore, gas flushing on Tenax sampling may be more suitable than passive sampling (SPME) due to the forced accumulation on sorbent Tenax material.

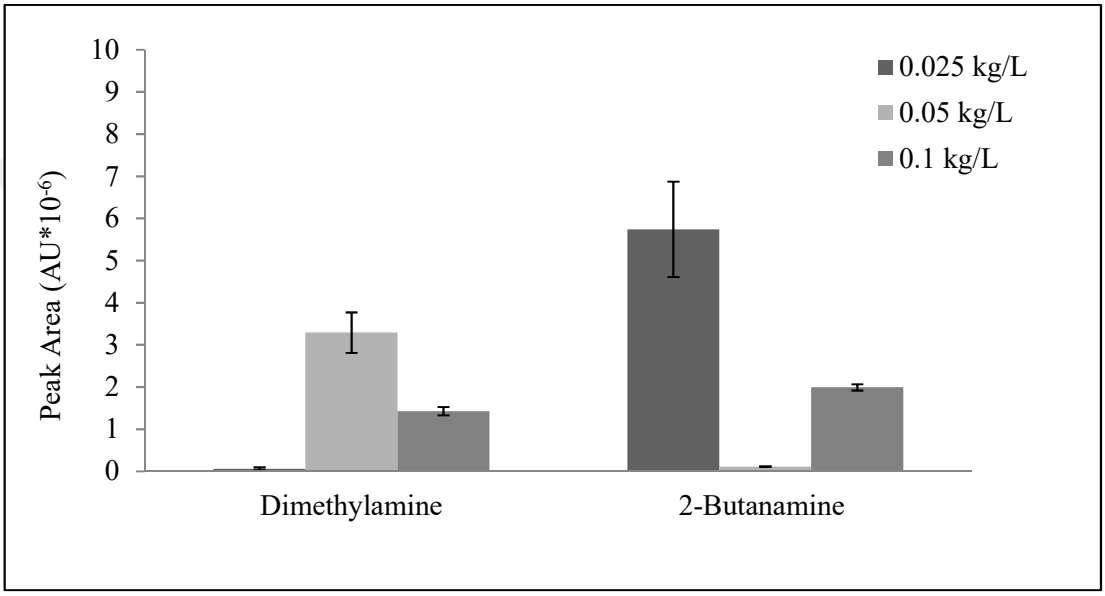


Figure 4.10 : Effect of loading factor on volatile amines extracted with gas flushing on Tenax.

4.1.3 Comparison of SPME and thermal desorber with gas flushing-Tenax sampling methods according to peak area of total volatile organic compounds

Total peak areas of volatiles measured with SPME and Tenax sampling are shown in Figure 4.11. As the volatile profiles did not vary significantly with the preconcentration technique according to previous results, the peak area levels were affected with preconcentration method and loading factor. Amines were the only chemical group measured with gas flushing on Tenax method. The total peak area levels of volatiles with Tenax (~390x10^6 AU at 0.05 kg/L loading factor) were higher than the results obtained by SPME sampling (~290x10^6 AU at 0.05 kg/L loading factor) for each loading factor. Loading factor of 0.05 kg/L were found to be the most efficient among the preconcentration techniques regarding the total peak area of

volatiles. Normally, Tenax is a very hydrophobic material and is unsuitable for highly volatiles (carbon numbers lower than four) due to its low specific surface area (Yang and Lo, 1997; Dettmer and Engewald, 2002).

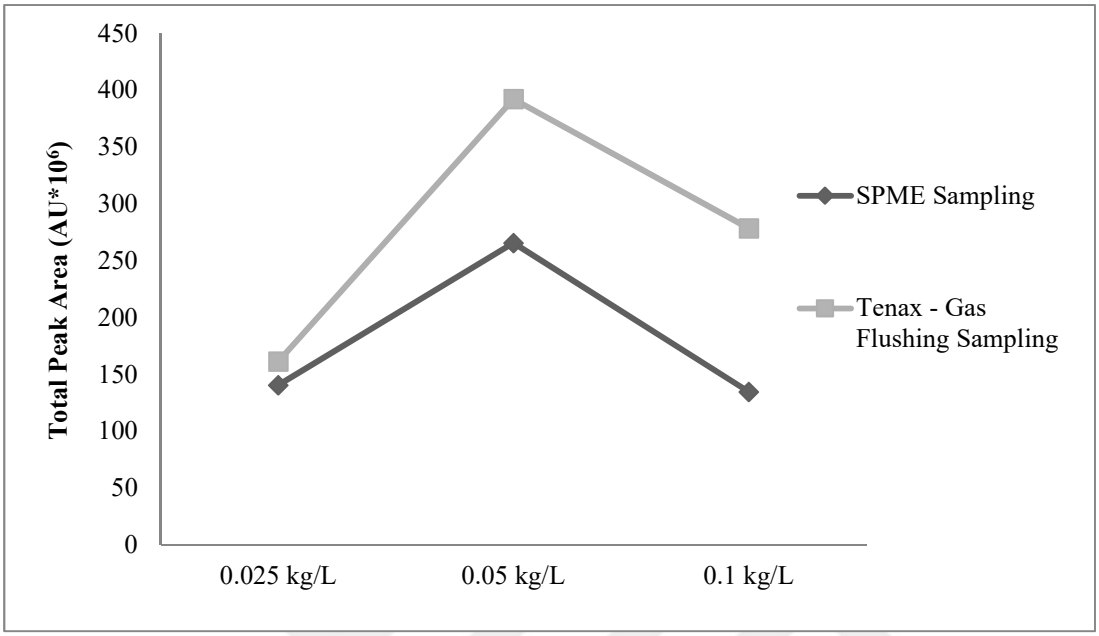


Figure 4.11 : Effect of loading factor on total peak area extracted with SPME and gas flushing on Tenax.

It can be seen that gas flushing process increased the adsorption capability of Tenax with forced circulation than static headspace sampling. SPME fiber coated with the CAR (Carboxen) layer allows extracting the low-MW compounds (Machiels and Istasse, 2003). In this study, low MW compounds were extracted well with CAR/PMDS coated SPME fiber. However, it seems that the molecular weight is not the only criteria; partial pressure, polarity, volatility, and pH of analyte are also important parameters during extraction. However, the effects of these parameters are not fully understood yet. Consistent with our study, Elmore et al. (2001) reported that levels for headspace concentration on Tenax were five times higher compared to SPME in the extracts of cooked beef for major compounds. It has been stated that the primary parameter affecting preconcentration was the partial pressure of the analytes. Higher the partial pressure, quicker the passage into the gaseous form (Elmore et al, 2001). Water condensation on the fiber and inside the walls of vial or container could be the possible loss of volatiles limiting the sorption (Stevenson et al, 1996). Also, analyte sorption on the container walls may cause losses of less volatile compounds for large surface-to-volume ratios (Gorecki et al, 1998). These could be the possible explanations of loss in analyte at higher sample: volume (0.10 kg/L in this study).

Beside the condensation on the wall of container, stripped water vapour may condense on interior walls of the connections and plug the traps and cause losses of peaks when higher amount of loadings was used (Valero et al, 2000). High volumes of humid air may also result shifts in retention times (Peng and Batterman, 2000). Heidari et al (2008) studied different volumes for SPME extraction of toluene and showed that increasing volume to 5 mL, increased the extraction efficiency, but at higher volumes, there was no significant difference. Another main problem could be the dissolving of volatile analytes back in the wet matrix even at small sample size.

According to the results of loading effect studies for two different pre-concentration methods, 0.05 kg/L loading factor with Tenax sampling and TD-GC-MS system were selected for the second part of the study.

4.2 Investigation on the Volatile Organic Compounds of Raw Beef Preserved at Isothermal and Non-Isothermal Cold Storage Conditions

In this part of the study, the volatile organic compound profile released from beef meat under the effect of isothermal and non-isothermal cold storage conditions of two temperatures, simulating the domestic conditions were investigated. Non-isothermal conditions such as 15 min. and 30 min. intervals between cooler door open-close cycles at two different temperatures (0°C and 4°C) were studied. Additionally, the relationship between volatile organic compounds and other quality parameters such as sensory, color, microbiological and oxidation quality was established by using different mathematical tools.

4.2.1 Effects of isothermal and non-isothermal conditions on volatile organic compounds

Results of volatile organic compounds that were obtained for three different conditions (Isothermal condition, non-isothermal condition 1: door open time is 15 seconds with 30 minutes' intervals between each door open-close cycles, non-isothermal condition 2: door open time is 15 seconds with 15 minutes' intervals between door open-close cycles) for 4°C storage temperature are shown in Table 4.7. TD-GC/MS method presented representative 32 types of volatile organic compounds. Alcohols, aldehydes, ketones, esters and sulphur compounds were the main volatile groups formed during spoilage of meat in all three conditions. Most of the identified volatile compounds

found in meat were mentioned in various publications (Insausti et al, 2002; Perez et al, 2008; Ercolini et al, 2009; Ercolini et al, 2010; Ercolini et al, 2011; Arygri et al, 2015; Casaburi et al, 2015).

Based on the results, it was found that storage conditions influenced the intensity of volatile compounds. Characteristic trends of volatiles showed differences between early and late period of storage. The significant increasing trend was obtained for 2-propanone, 2,3-butandione, ethyl acetate, 3-hydroxy-2-butanone and 1-pentanol for each condition during 5 days of storage time (p<0.05). The peak areas of these compounds significantly were higher at dynamic fluctuating conditions than isothermal condition. Detected volatile organic compounds for three different conditions at 0°C are shown in Table 4.8. Total number of detected volatiles were 26, which were less than the detected compounds at 4°C conditions due to the retarded spoilage at low temperatures. At isothermal conditions, for most of the volatiles, there was a slight increase until day 7. At the day 7, statistically significant increase was observed especially for 2,3-butandione, 3-hydroxy-2-butanone, 1-pentanol, 1-octen 3-ol, 2-ethyl-1-hexanol, 3-methyl-1-butanol, 1-hexanol and 2,3-octandione (p<0.05). Similar increase was observed with the beginning of day 5 for dynamic conditions. This observed increase in VOC intensity were in consistency with sensory evaluation in which off-odor perception was clear after day 7 and 5 for isothermal and dynamic conditions, respectively. The VOC intensity when meat was unacceptable for 0°C was lower than the intensity of all 4°C conditions when meat was unacceptable. Additionally, no ester compounds were measured at all conditions of 0°C.

Ketones had a major contribution in all cases with mostly increasing ratios, only started to decrease at late period of 4°C. Sulphur compounds decreased during cold storage especially due to the dimethyl sulfide and carbon disulfide dominantly found at initial period without a significant difference in all cases. Similarly, amines also showed decreasing trend and were found dominantly at 4°C storage temperatures. There was no major difference in contribution of volatile compound classes between isothermal and non-isothermal conditions, hovewer storage temperature affected their contribution.

Table 4.7 : Volatile organic compounds identified in beef for the effects of isothermal and non-isothermal conditions at 4°C (means expressed as AUx10^6).

Volatile Organic Compounds	Isothermal condition*				Non-isothermal condition 1**				Non-isothermal condition 2***			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
1-Pentanol	0.00_{c}	1.05_{c}^{ab}	3.10_{b}^{c}	12.44_{a}^{c}	0.00_{c}	0.89_{c}^{b}	9.86_{b}^{c}	21.81_{a}^{b}	0.00_{c}	1.33_{c}^{a}	12.98_{b}^{a}	27.57_{a}^{a}
1-Octen-3-ol	0.00_{c}	4.55_{b}^{a}	6.52_{a}^{ab}	0.36_{c}^{a}	0.00_{c}	1.07_{b}^{b}	5.60_{a}^{b}	0.00_{c}^{b}	0.00_{c}	3.09_{b}^{a}	6.60_{a}^{a}	0.00_{c}^{b}
2 Ethyl-1-hexanol	0.00_{b}	0.33_{a}^{b}	0.02_{b}^{c}	0.00_{b}	0.00_{c}	0.47_{b}^{ab}	2.09_{a}^{b}	0.00_{c}	0.00_{c}	1.06_{b}^{a}	4.95_{a}^{a}	0.00_{c}
3-Metil-1-butanol	0.00_{c}	0.00_{c}	6.92_{b}^{a}	21.77_{a}^{a}	0.00_{c}	0.00_{c}	3.48_{a}^{b}	2.10_{b}^{b}	0.00_{b}	0.00_{b}	2.78_{a}^{b}	3.10_{a}^{b}
1-Hexanol	0.00_{b}	0.00_{b}	0.31_{a}^{c}	0.00_{b}	0.00_{b}	0.00_{b}	3.74_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	6.75_{a}^{a}	0.00_{b}
Hexanal	1.16_{c}^{c}	4.38_{a}^{ab}	3.62_{b}^{b}	0.48_{d}^{a}	2.82_{b}^{a}	2.00_{b}^{b}	4.73_{a}^{b}	0.00_{c}^{c}	1.75_{b}^{b}	5.63_{a}^{a}	6.83_{a}^{a}	0.13_{b}^{b}
3-Methyl-1-butanal	0.00_{b}	4.36_{a}^{b}	4.50_{a}^{b}	0.06_{b}^{b}	0.00_{c}	4.91_{b}^{a}	4.87_{b}^{b}	6.31_{a}^{a}	0.00_{d}	5.06_{c}^{a}	5.84_{b}^{a}	6.64_{a}^{a}
Pentanal	0.00_{c}^{a}	3.21_{b}^{b}	4.61_{a}^{a}	0.00_{c}	0.18_{bc}^{a}	2.46_{a}^{b}	1.25_{ab}^{b}	0.00_{c}	0.22_{c}^{a}	6.94_{a}^{a}	3.46_{b}^{ab}	0.00_{c}
2-Butanamine	14.70_{b}^{a}	12.78_{b}^{a}	16.59_{b}^{a}	31.77_{a}^{a}	5.84_{a}^{b}	2.56_{b}^{c}	0.02_{b}^{c}	0.00_{b}^{b}	10.66_{a}^{a}	10.29_{a}^{b}	3.95_{b}^{b}	0.00_{c}^{b}
Dimetilamine	2.32_{c}^{b}	3.00_{b}^{c}	3.69_{a}^{b}	0.00_{d}	1.36_{b}^{c}	4.39_{a}^{b}	4.07_{a}^{b}	0.00_{b}	4.23_{c}^{a}	6.55_{a}^{a}	5.86_{b}^{a}	0.00_{d}
2-Propanamine	0.00_{b}	0.00_{b}	0.00_{b}	48.08_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	12.93_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	22.37_{a}^{b}
2-Propanone	29.21_{c}^{a}	39.23_{a}^{c}	34.95_{b}^{c}	40.53_{a}^{d}	24.55_{c}^{b}	54.39_{b}^{a}	61.12_{a}^{b}	0.00_{d}	22.48_{c}^{b}	48.50_{b}^{b}	71.29_{a}^{a}	0.00_{d}
2-Butanone	33.91_{a}^{a}	28.68_{b}^{a}	28.05_{b}^{a}	13.25_{c}^{a}	36.16_{a}^{a}	24.88_{b}^{ab}	20.97_{b}^{c}	14.71_{c}^{a}	33.36_{a}^{a}	24.88_{b}^{b}	25.39_{b}^{b}	12.54_{c}^{a}
2.3-Butandione	4.72_{c}^{a}	8.50_{b}^{ab}	14.83_{a}^{b}	13.43_{a}^{a}	2.98_{d}^{c}	6.85_{c}^{b}	19.54_{a}^{a}	12.33_{b}^{a}	3.84_{d}^{b}	9.88_{c}^{a}	23.69_{a}^{a}	13.08_{b}^{a}
3-Hydroxy-2-butanone	11.88_{c}^{a}	55.96_{b}^{a}	120.74_{a}^{c}	119.08_{a}^{c}	1.90_{c}^{b}	37.94_{b}^{c}	172.00_{a}^{b}	169.28_{a}^{b}	1.18_{d}^{b}	48.10_{c}^{b}	184.06_{b}^{a}	193.04_{a}^{a}
2-Pentanone	1.00_{a}^{a}	0.00_{b}	0.00_{b}^{b}	0.00_{b}^{c}	0.00_{c}^{b}	0.00_{c}	4.03_{a}^{a}	2.41_{b}^{a}	0.00_{c}^{b}	0.00_{c}	4.20_{a}^{a}	1.59_{b}^{b}
2.3-Octandione	0.00	0.00	0.00^{b}	0.00	0.00_{b}	0.00_{b}	3.67_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	2.24_{a}^{a}	0.00_{b}
2-Heptanone	0.00_{b}	0.00_{b}	0.00_{b}^{b}	1.07_{a}^{b}	0.00_{b}	0.00_{b}	1.18_{a}^{b}	1.04_{a}^{b}	0.00_{b}	0.00_{b}	2.54_{a}^{a}	1.93_{a}^{a}

Table 4.7 (continued) : Volatile organic compounds identified in beef for the effects of isothermal and non-isothermal conditions at 4°C (means expressed as AUx10^6).

Volatile Organic Compounds	Isothermal condition*				Non-isothermal condition 1**				Non-isothermal condition 2***			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
3-Octanone	0.00_{b}	0.00_{b}	0.00_{b}	17.16_{a}^{ab}	0.00_{b}	0.00_{b}	0.00_{b}	13.82_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	17.07_{a}^{a}
Dimetyl sulphide	50.84_{a}^{a}	28.08_{b}^{a}	14.82_{c}^{b}	0.00_{d}	23.01_{a}^{c}	19.24_{a}^{a}	11.43_{b}^{c}	0.00_{c}	33.01_{a}^{b}	23.28_{b}^{a}	18.73_{b}^{a}	0.00_{c}
Etanethiol	2.16_{c}^{c}	27.21_{a}^{a}	19.41_{b}^{c}	2.90_{c}^{a}	23.60_{b}^{b}	24.20_{ab}^{b}	29.30_{a}^{b}	2.28_{c}^{a}	33.85_{a}^{a}	28.18_{b}^{ab}	32.89_{a}^{a}	1.98_{c}^{a}
Dimetyl disulphide	0.00_{b}	0.00_{b}	0.00_{b}	7.68_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	2.88_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	2.01_{a}^{b}
Dimetyl trisulphide	0.00_{b}	0.00_{b}	0.00_{b}	0.45_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	0.65_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	0.68_{a}^{a}
Ethyl acetate	2.83_{b}^{a}	4.15_{b}^{b}	5.56_{b}^{b}	61.77_{a}^{c}	2.93_{c}^{a}	4.89_{bc}^{ab}	6.15_{b}^{b}	149.64_{a}^{b}	2.64_{c}^{b}	5.79_{bc}^{a}	9.02_{b}^{a}	163.05_{a}^{a}
Methyl acetate	0.00_{b}	0.00_{b}	0.00_{b}	11.36_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	12.48_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	15.33_{a}^{a}
Ethyl butanoate	0.00_{b}	0.00_{b}	0.00_{b}	12.54_{a}^{c}	0.00_{b}	0.00_{b}	0.00_{b}	22.91_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	27.17_{a}^{a}
Ethyl 3-methyl butanoate	0.00_{b}	0.00_{b}	0.00_{b}	4.60_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	8.38_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	8.55_{a}^{a}
Ethyl hexanoate	0.00_{b}	0.00_{b}	0.00_{b}	26.26_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	38.06_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	42.09_{a}^{a}
Methyl hexanoate	0.00_{b}	0.00_{b}	0.00_{b}	1.84_{a}^{c}	0.00_{b}	0.00_{b}	0.00_{b}	5.64_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	6.35_{a}^{a}
Ethyl octanoate	0.00_{b}	0.00_{b}	0.00_{b}	3.02_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	5.77_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	6.01_{a}^{a}
Isoamyl acetate	0.00_{b}	0.00_{b}	0.00_{b}	2.96_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	7.30_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	7.15_{a}^{a}
Isobutyl acetate	0.00_{b}	0.00_{b}	0.00_{b}	13.09_{a}^{c}	0.00_{b}	0.00_{b}	0.00_{b}	22.36_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	20.36_{a}^{b}
1-Undecene	0.00_{b}	0.00_{b}	0.00_{b}	64.65_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	53.45_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	51.95_{a}^{b}

- *Different subscript letters in the same row for each condition indicates significant difference between storage days ($p<0.05$).*
- *Different superscript letters in the same column for each day indicates significant difference between storage conditions ($p<0.05$).*

*Isothermal condition: 4ºC constant temperature condition, **non-isothermal condition 1: door open time is 15 seconds with 30 minutes' intervals between each door open-close cycles at 4ºC mean temperature, ***non-isothermal condition 2: door open time is 15 seconds with 15 minutes' intervals between door open-close cycles at 4ºC mean temperature.

Table 4.8 : Volatile organic compounds identified in beef for the effects of isothermal and non-isothermal conditions at 0°C (means expressed as AUx10^6).

Volatile Organic Compounds	Isothermal condition*				Non-isothermal condition 1**				Non-isothermal condition 2***			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
1-Pentanol	3.31_{c}^{a}	5.36_{b}^{b}	5.34_{b}^{c}	13.33_{a}^{b}	3.54_{c}^{a}	8.21_{b}^{a}	12.55_{a}^{b}	14.40_{a}^{ab}	3.74_{c}^{a}	8.45_{b}^{a}	15.20_{a}^{a}	15.77_{a}^{a}
1-Octen-3-ol	2.38_{c}^{a}	2.71_{c}^{c}	4.37_{b}^{c}	8.29_{a}^{b}	1.42_{c}^{b}	12.64_{b}^{a}	12.64_{b}^{b}	18.30_{a}^{a}	2.34_{d}^{ab}	9.63_{c}^{b}	14.67_{b}^{a}	17.53_{a}^{a}
2 Ethyl-1-hexanol	2.23_{c}^{a}	2.58_{c}^{a}	7.32_{b}^{b}	12.59_{a}^{c}	3.65_{c}^{b}	2.21_{d}^{a}	8.15_{b}^{a}	52.75_{a}^{b}	2.50_{c}^{b}	2.42_{c}^{a}	8.23_{b}^{a}	55.88_{a}^{a}
3-Metil-1-butanol	0.00_{b}^{c}	0.12_{b}^{b}	0.26_{b}^{b}	6.34_{a}^{a}	0.07_{b}^{a}	0.17_{b}^{a}	0.71_{b}^{a}	3.34_{a}^{b}	0.03_{c}^{b}	0.17_{bc}^{a}	0.72_{b}^{a}	4.60_{a}^{b}
1-Hexanol	0.00_{b}^{c}	0.26_{b}^{a}	0.57_{b}^{b}	10.63_{a}^{b}	0.54_{c}^{a}	0.35_{c}^{a}	3.64_{b}^{a}	13.53_{a}^{a}	0.12_{c}^{b}	0.27_{c}^{a}	3.54_{b}^{a}	14.36_{a}^{a}
1-Heptanol	0.00	0.00	0.00	0.00^{b}	0.00_{b}	0.00_{b}	0.00_{b}	$0.22a^{a}$	0.00_{b}	0.00_{b}	0.00_{b}	0.22_{a}^{a}
Hexanal	3.46_{b}^{b}	7.29_{a}^{c}	1.41_{c}^{b}	0.94_{c}^{a}	5.25_{c}^{a}	18.48_{a}^{a}	7.71_{b}^{a}	1.48_{d}^{a}	3.45_{c}^{b}	14.33_{a}^{b}	8.51_{b}^{a}	1.08_{d}^{a}
3-Methyl-1-butanal	1.28_{c}^{a}	1.75_{c}^{a}	2.55_{b}^{b}	5.26_{a}^{a}	0.67_{d}^{a}	1.65_{c}^{a}	7.82_{a}^{a}	5.13_{b}^{a}	1.33_{c}^{a}	1.73_{c}^{a}	2.65_{b}^{b}	5.41_{a}^{a}
Pentanal	0.00	0.00	0.00	0.00_{b}	0.00_{b}	0.00_{b}	0.00_{b}	1.24_{a}^{a}	0.00_{a}	0.00_{a}	0.00_{a}	0.05_{a}^{b}
Heptanal	0.09_{a}^{b}	0.04_{b}^{a}	0.02_{b}^{c}	0.00_{c}^{b}	0.15_{a}^{ab}	0.00_{b}^{b}	0.00_{b}^{b}	0.00_{b}^{a}	0.24_{a}^{a}	0.00_{b}^{b}	0.00_{b}	0.00_{b}^{a}
Nonanal	0.00_{c}^{b}	0.50_{b}^{a}	0.96_{a}^{a}	0.00_{c}	0.24_{a}^{a}	0.00_{b}^{a}	0.00_{b}^{b}	0.00_{b}	0.24_{a}^{a}	0.00_{b}^{a}	0.00_{b}^{b}	0.00_{b}
2-Butanamine	0.00_{b}	2.29_{a}^{b}	0.00_{b}^{c}	0.00_{b}	0.00_{b}	3.69_{a}^{a}	0.79_{b}^{b}	0.00_{b}	0.00_{c}	3.49_{a}^{ab}	0.81_{b}^{a}	0.00_{c}
Dimetilamine	0.00	0.00	0.00	0.00^{c}	0.00_{b}	0.00_{b}	0.00_{b}	3.79_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	3.19_{a}^{b}
2-Propanone	24.30_{d}^{a}	26.69_{c}^{c}	47.46_{a}^{c}	39.40_{b}^{c}	23.54_{d}^{a}	40.9_{c}^{a}	64.56_{a}^{a}	42.22_{b}^{b}	24.05_{d}^{a}	29.30_{c}^{b}	58.38_{a}^{b}	46.04_{b}^{a}
2-Butanone	6.34_{a}^{c}	6.15_{b}^{b}	3.76_{c}^{b}	3.22_{d}^{b}	9.13_{a}^{a}	7.18_{b}^{a}	4.51_{c}^{a}	4.52_{c}^{a}	7.29_{a}^{b}	7.26_{a}^{a}	3.89_{b}^{ab}	3.35_{b}^{b}
2.3-Butandione	1.85_{c}^{a}	2.94_{b}^{a}	2.19_{c}^{a}	11.09_{a}^{c}	2.46_{b}^{a}	2.72_{b}^{a}	2.30_{b}^{a}	24.72_{a}^{b}	2.13_{b}^{a}	2.54_{b}^{a}	2.43_{b}^{a}	27.27_{a}^{a}
3-Hydroxy-2-butanone	5.45_{c}^{a}	6.26_{bc}^{b}	7.18_{b}^{b}	87.51_{a}^{c}	3.78_{c}^{b}	7.31_{bc}^{a}	10.70_{b}^{a}	184.09_{a}^{a}	4.44_{b}^{b}	7.22_{b}^{a}	10.48_{b}^{a}	166.79_{a}^{b}

Table 4.8 (continued) : Volatile organic compounds identified in beef for the effects of isothermal and non-isothermal conditions at 0°C (means expressed as AUx10^6).

Volatile Organic Compounds	Isothermal condition*				Non-isothermal condition 1**				Non-isothermal condition 2***			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
2-Pentanone	1.24_{c}^{a}	1.48_{c}^{c}	2.14_{b}^{c}	2.80_{a}^{b}	0.79_{d}^{b}	3.18_{c}^{a}	6.60_{a}^{a}	4.39_{b}^{a}	0.91_{c}^{b}	2.20_{b}^{b}	3.58_{a}^{b}	4.35_{a}^{a}
2.3-Octandione	2.07_{b}^{a}	1.33_{c}^{b}	2.42_{b}^{b}	8.70_{a}^{a}	2.24_{b}^{a}	2.36_{b}^{a}	4.45_{a}^{a}	0.00_{c}^{b}	2.84_{b}^{a}	2.36_{b}^{a}	4.34_{a}^{a}	0.14_{c}^{b}
2-Heptanone	0.00_{b}	0.00_{b}	0.00_{b}	2.12_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	1.56_{a}^{b}	0.00_{b}	0.00_{b}	0.00_{b}	1.92_{a}^{ab}
3-Octanone	0.00	0.00	0.00	0.00^{b}	0.00_{b}	0.00_{b}	0.00_{b}	4.99_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	4.48_{a}^{a}
Dimetyl sulphide	7.44_{a}^{a}	8.02_{a}^{b}	4.76_{b}^{a}	4.66_{b}^{a}	8.20_{b}^{a}	12.39_{a}^{a}	4.72_{c}^{a}	4.21_{c}^{a}	7.50_{b}^{a}	8.76_{a}^{b}	4.35_{c}^{a}	3.76_{c}^{a}
Etanethiol	5.23_{c}^{a}	18.70_{a}^{c}	9.68_{b}^{b}	3.10_{d}^{a}	5.50_{c}^{a}	22.55_{a}^{a}	15.34_{b}^{a}	2.81_{d}^{b}	5.54_{c}^{a}	20.96_{a}^{b}	16.01_{b}^{a}	2.75_{d}^{b}
Carbon disulphide	10.58_{a}^{a}	5.90_{b}^{a}	6.52_{b}^{a}	0.00_{c}^{b}	9.26_{a}^{a}	6.34_{b}^{a}	4.23_{c}^{b}	2.20_{d}^{a}	10.32_{a}^{a}	6.12_{b}^{a}	3.58_{c}^{b}	2.20_{c}^{a}
Tiourea	0.00	0.00	0.00	0.00^{c}	0.00_{b}	0.00_{b}	0.00_{b}	2.69_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	1.45_{a}^{b}
1-Undecene	0.00	0.00	0.00	0.00^{c}	0.00_{b}	0.00_{b}	0.00_{b}	2.39_{a}^{a}	0.00_{b}	0.00_{b}	0.00_{b}	0.63_{a}^{b}

- *Different subscript letters in the same row for each condition indicates significant difference between storage days ($p<0.05$).*
- *Different superscript letters in the same column for each day indicates significant difference between storage conditions ($p<0.05$).*

*Isothermal condition: 0ºC constant temperature condition, **non-isothermal condition 1: door open time is 15 seconds with 30 minutes' intervals between each door open-close cycles at 0ºC mean temperature, ***non-isothermal condition 2: door open time is 15 seconds with 15 minutes' intervals between door open-close cycles at 0ºC mean temperature.

4.2.1.1 Alcohol compounds

Detected alcohol compounds were found to be 1-pentanol, 3-methyl-1-butanol, 1-hexanol, 1-octen-3-ol and 2-ethyl-1-hexanol both at 4°C and 0°C storage temperatures. 3-Methyl-1-butanol, 2-ethyl-1-hexanol, 1-hexanol has etheric, fruity aroma, whereas 1-octen-3-ol has musty aroma. Alcohol formation occurs mostly as a result of microbial growth. *Brochothrix thermosphacta* has capability to produce 1-octen-3-ol and 3-methyl-1-butanol compounds (Dainty et al, 1985; Casaburi et al, 2014). 1-Octen-3-ol is known as an oxidation product in some food materials (Soncin et al, 2007). 2-Ethyl-1-hexanol is also a well-known volatile compound in meats and is formed in aerobic conditions by *Br. thermosphacta* and *P. fragi* (Ercolini et al, 2010; Casaburi et al, 2014) and 1-hexanol is mostly formed with the activity of *P. fragi, C. maltaromaticum* (Ercolini et al, 2010; Casaburi et al, 2011). Fluctuating temperature conditions affected 1-pentanol concentration significantly ($p<0.05$), which increased with storage time. Highest peak areas for 1-pentanol were obtained when non-isothermal 2 (door open time is 15 seconds with 15 minutes' intervals between door open-close cycles) condition was applied. The peak area of 1-pentanol at the time of spoilage was higher at 0°C than 4°C. Due to the low abundance of other alcohols, there were no considerable differences between all conditions of 4°C. 3-Methyl-1-butanol was lower at non-isothermal conditions compared to isothermal conditions. There was a dynamic trend for 1-octen-3-ol and 2-ethyl-1-hexanol which increased until the day 3 and decreased after the day 5 with very low peak area levels at 4°C. Similar behaviours was observed in the study of Argyri et al. (2015) for 1-octen-3-ol both at 0°C and 5°C temperatures. However, these chemicals were in an increasing trend during 7 days due to the extended spoilage time point at 0°C. Especially, 2-ethyl-1-hexanol had very high peak areas with the spoilage of meat at day 7 for non-isotermal conditions compared with isothermal conditions at 0°C. 2-Ethyl-1-hexanol may be served as a good indicator for lower storage temperatures near to 0°C. Contribution of 3-methly-1-butanol and 1-hexanol was very low compared with other alcohols for all conditions of two temperatures.

4.2.1.2 Aldehyde Compounds

Aldehydes are generally produced by the hydrolysis of triglycerides through fatty acid metabolism or lipid autoxidation (Montel et al, 1998; Casaburi et al, 2015) and they

are responsible for oily and grassy flavour and odor (Pham et al, 2008). 3-Methyl-1-butanal, hexanal and pentanal are commonly observed off-flavor volatiles released during meat spoilage and can serve as reliable indicators for flavor deterioration (Wilkes et al, 2000). These compounds are related with the growth of *Br. thermosphacta, P. fragi* and *C. maltaromaticum* (Casaburi et al, 2015). 3-Methyl-1-butanal is derived from leucine amino acid and perceived as cheesy and pungent aroma (Smit et al, 2005; Casaburi et al, 2015). Observed aldehydes were hexanal, 3-methyl-1-butanal and pentanal both for 0°C and 4°C conditions. Aldehydes had lower peak areas compared to other volatiles. No clear affect was observed for non-isothermal conditions on the peak area of aldehydes significantly both for 0°C and 4°C conditions. Hexanal generally showed curved trends for all conditions of two temperatures similar to the study of Argyri et al. (2015). Hexanal, the product of oxidation tended to increase at the early period of storage and then decreased after the day 3 for isothermal condition and after the day 5 for dynamic conditions at 4°C. For 0°C, hexanal started to decrease from the day 3 for all conditions with higher intensity at non-isothermal conditions siginificantly (p<0.05). Pentanal was only recorded at non-isothermal conditions for 0°C. It may be concluded that aldehydes are not good indicators to predict the spoilage of beef and they were not differentiable regarding storage conditions. Heptanal and nonanal were presented only at 0°C without a distinguishable result.

4.2.1.3 Ketone compounds

Ketones are one of the most impact volatile compounds that are formed aggressively during meat spoilage. Major ketones commonly found in fresh meat are 2-propanone, 2-butanone, 2,3-butandione, 3-hydroxy-2-butanone, 2-pentanone and 2-heptanone. Ketones are the spoilage metabolites due to the growth of *Pseudomonas* spp., *Carnobacterium* spp. and *Enterobacteriaceae* microorganisms (Casaburi et al, 2015). Moreover, 3-hydroxy-2-butanone is a product of glucose catabolism by *Br. thermosphacta, Carnobacterium spp.* and *Lactobacillus spp.* and/or aspartate degradation (Ardö, 2006) and it has cheesy/buttery aroma in fresh meat. In this study, ketones were: 2-propanone, 2,3-butandione, 2-butanone, 3-hydroxy-2-butanone, 2,3-octandione, 3-octanone, 2-pentanone and 2-heptanone. All ketone compounds showed increasing trend until the point of spoilage except 2-butanone. 2-Butanone decreased clearly during storage for all conditions of two storage temperatures with higher peak

areas at 4°C. Total peak areas of ketones had a higher percentage compared to other volatiles in total volatile compounds. Dynamic conditions generally affected the intensities significantly for most of the aldehydes (p<0.05). 3-Hydroxy-2-butanone and 2-propanone were the dominant volatile compounds regarding peak areas and showed similar behaviour both for two temperature conditions. Beside this, the peak areas of 3-hydroxy-2-butanone were higher at 4°C. Effect of dynamic conditions on 3-hydroxy-2-butanone was observed after day 3 with at least 30% increase at 4°C and after day 5 with at least 100% increase in peak areas at 0°C significantly with late spoilage. 2,3-Butandione increased in the first stage of spoilage, then decreased in late spoilage stage at 4°C. Additionally, 2-propanone disappeared in the last day of dynamic conditions at 4°C. 2,3-Octandione, 3-octanone, 2-pentanone and 2-heptanone were just measured after day 5 when meats spoiled completely at 4°C. Despite this, 3-octanone and 2-heptanone were present and they were increased during storage period of 0°C. Although, the temperature effect was clear during all storage period, it can be noted that the effect of open-close cycle was dominant at late storage period.

4.2.1.4 Esters

Esters are principally formed in the presence of *P. fragi* at aerobic conditions (Ercolini et al, 2009) with esterification of alcohol and carboxylic acid, as well as with microbial esterase activity (Toldra, 1998; Insausti et al, 2002). Most ester compounds have sweet odor (Curioni and Bosset, 2002). In this study, esters were detected only at 4°C conditions. The only ester compound measured at early storage period was ethyl acetate. The peak areas of ethyl acetate under non-istohermal conditions were higher two times than isothermal conditions. Ethyl acetate increased with increasing storage time with clear effect of dynamic conditions. Methyl acetate, ethyl butanoate, 3-methyl ethyl butanoate, ethyl hexanoate, methyl hexanoate and ethyl octanoate were produced at late storage period in all conditions. These ester compounds may serve as good indicators for completely spoiled meats in which higher amounts were observed at conditions that have open-close cycles.

4.2.2 Sensory analysis

Effects of isothermal and non-isothermal conditions on discoloration, off-odor formation and overall acceptability are given in Table 4.9 for 4˚C and Table 4.10 for 0˚C. Overall acceptability trend was fitted into second order polynomial for all cases.

All sensory scores decreased during storage time significantly (p<0.05) and off-odor score was more effective on overall acceptability. Kotsumanies et al. (2006) indicated that negative correlation is present between low glucose concentration and the first signs of off-odor formation. Shelf life (the time when overall acceptability < 4.0) values of 0˚C conditions were longer than that of 4˚C. Overall acceptability of beef samples severely decreased at the fluctuating conditions compared with isothermal temperature of 4˚C and 0˚C conditions after 24 h and 72 h, respectively. However, there was no considerable difference in quality perception between two non-isothermal conditions for 4˚C. This may be related with the difficulty of differentiation due to short shelf life. When the minimum acceptible shelf life was checked from the trendline equations, 101 h, 54 h and 53 h were calculated for isothermal, non-isothermal condition 1 and non-isothermal condition 2 at 4˚C, respectively. Shelf life of isothermal, non-isothermal 1 and non-isothermal 2 at 0˚C was found to be 157 h, 119 h and 102 h, respectively. Off-odor intensity of meat stored at isothermal condition started to increase significantly with the day 7 while beefs stored at dynamic conditions were unacceptable after day 5 parallel to overall acceptability. It is obvious that effect of increasing open-close cycle was more differentiable at 0˚C conditions due to the slower degradation and longer shelf lifes. Kotsumanies et al. (2006) studied the ability of bacteria growth model for ground meat under dynamic conditions and found that the conditions of “24 h at 0°C followed by 24 h at 10°C” and “12 h at 0°C followed by 6 h at 10°C, and 6 h at 15°C” resulted with 85.3 and 98 h shelf life. Long time exposure to high temperatures resulted with shorter shelf life. Ammor et al. (2009) indicated that shelf-life at 0°C was longer nearly two times than 5°C and four times longer than 10°C, according to hedonic scores. Argyri et al. (2015) showed that the shelf life of aerobically stored meat decreased with increasing storage temperature for approximately 270 h at 0°C and 150 h at 5°C. The spoilage potential of different bacteria and differences on the species of the same bacterial group are affected by different storage temperatures (Doulgeraki et al, 2012).

Table 4.9 : Sensory evaluation results of beef samples for the effects of isothermal and non-isothermal conditions at 4°C

Time (h)	Visual Assesment		
	Isothermal Condition*	**Non-Isothermal Condition 1****	**Non-Isothermal Condition 2*****
0	$7.0\pm0.0_{a}^{a}$	$7.0\pm0.0_{a}^{a}$	$7.0\pm0.0_{a}^{a}$
24	$6.7\pm0.1_{a}^{a}$	$6.3\pm0.2_{b}^{a}$	$6.5\pm0.3_{a}^{a}$
72	$6.2\pm0.2_{a}^{a}$	$5.7\pm0.1_{b}^{a}$	$5.6\pm0.2_{b}^{a}$
120	$5.1\pm0.1_{b}^{a}$	$4.2\pm0.3_{c}^{ab}$	$4.2\pm0.2_{c}^{b}$
168	$2.7\pm0.5_{c}^{a}$	$2.0\pm0.0_{d}^{a}$	$2.0\pm0.0_{d}^{a}$
Time (h)	**Off-Odor Assesment**		
	Isothermal Condition	**Non-Isothermal Condition 1**	**Non-Isothermal Condition 2**
0	$7.0\pm0.0_{a}^{a}$	$7.0\pm0.0_{a}^{a}$	$7.0\pm0.0_{a}^{a}$
24	$6.6\pm0.6_{a}^{a}$	$6.9\pm0.1_{a}^{a}$	$7.0\pm0.1_{a}^{a}$
72	$5.9\pm0.1_{a}^{a}$	$4.3\pm0.3_{b}^{b}$	$4.8\pm0.2_{b}^{b}$
120	$3.6\pm0.2_{b}^{a}$	$3.0\pm0.0_{c}^{a}$	$3.0\pm0.2_{c}^{a}$
168	$2.4\pm0.5_{b}^{a}$	$2.0\pm0.0_{d}^{a}$	$2.0\pm0.0_{d}^{a}$
Time (h)	**Overall Acceptability**		
	Isothermal Condition	**Non-Isothermal Condition 1**	**Non-Isothermal Condition 2**
0	$7.0\pm0.0_{a}^{a}$	$7.0\pm0.0_{a}^{a}$	$7.0\pm0.0_{a}^{a}$
24	$6.7\pm0.3_{a}^{a}$	$6.5\pm0.2_{a}^{a}$	$6.7\pm0.2_{a}^{a}$
72	$5.1\pm0.4_{b}^{a}$	$3.6\pm0.0_{b}^{b}$	$3.6\pm0.3_{b}^{b}$
120	$3.2\pm0.3_{c}^{a}$	$2.2\pm0.3_{c}^{b}$	$2.1\pm0.1_{c}^{b}$
168	$1.8\pm0.4_{d}^{a}$	$1.0\pm0.0_{d}^{a}$	$1.2\pm0.2_{d}^{a}$
Mathematical Formula of Overall Acceptability Values	$y = -3E^{-05}x^2 - 0.0281x + 7.1656$	$y = 0.0001x^2 - 0.0548x + 7.253$	$y = 0.0001x^2 - 0.0582x + 7.3681$
Minimum Acceptible Storage Time (h)	101 h (4.2 days)	54 h (2.3 days)	53 h (2.2 days)

*Isothermal condition: 4ºC constant temperature condition, **non-isothermal condition 1: door open time is 15 seconds with 30 minutes' intervals between each door open-close cycles at 4ºC mean temperature, ***non-isothermal condition 2: door open time is 15 seconds with 15 minutes' intervals between door open-close cycles at 4ºC mean temperature.

Table 4.10 : Sensory evaluation results of beef samples for the effects of isothermal and non-isothermal conditions at 0°C.

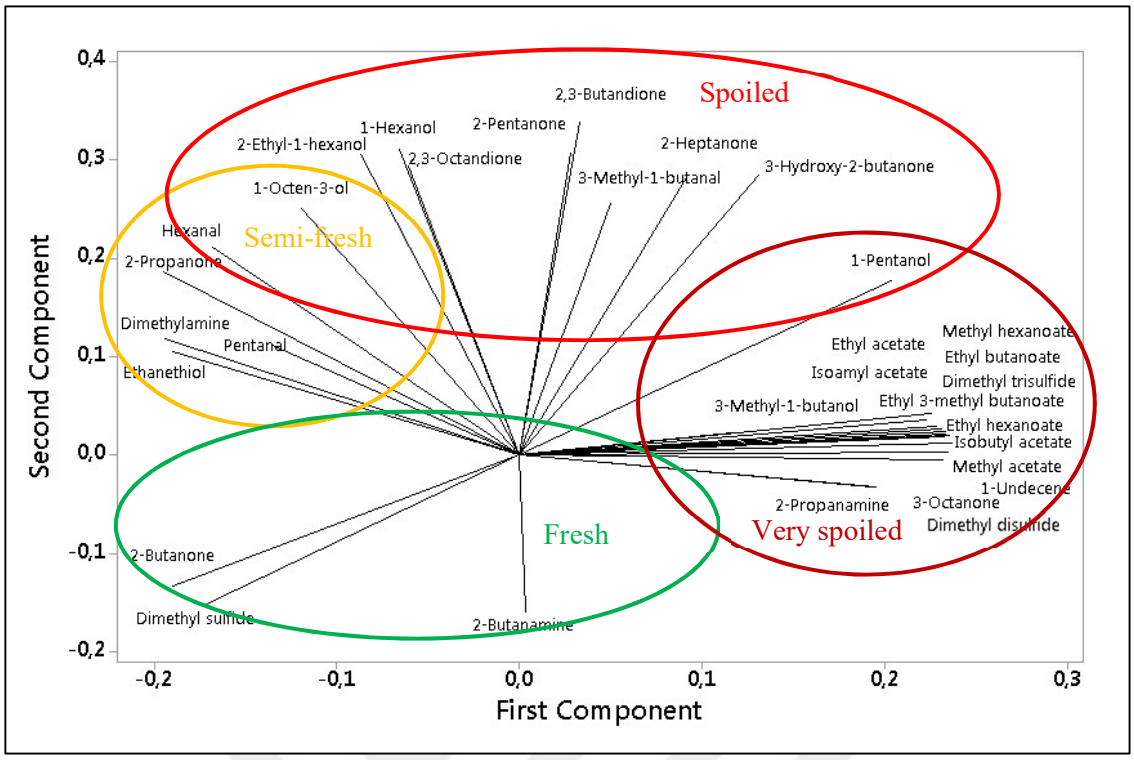
Time (h)	Visual Assesment		
	Isothermal Condition	**Non-Isothermal Condition 1**	**Non-Isothermal Condition 2**
0	$7.0_{a}{}^{a}$	$7.0_{a}{}^{a}$	$7.0_{a}{}^{a}$
24	$6.8_{a}{}^{a}$	$6.7_{ab}{}^{a}$	$6.6_{ab}{}^{a}$
72	$6.2_{ab}{}^{a}$	$5.9_{b}{}^{a}$	$6.0_{b}{}^{a}$
120	$5.3_{bc}{}^{a}$	$4.4_{c}{}^{a}$	$4.2_{c}{}^{a}$
168	$5.1_{c}{}^{a}$	$4.1_{c}{}^{b}$	$3.9_{c}{}^{b}$
Time (h)	**Off-Odor Assesment**		
	Isothermal Condition	**Non-Isothermal Condition 1**	**Non-Isothermal Condition 2**
0	$7.0_{a}{}^{a}$	$7.0_{a}{}^{a}$	$7.0_{a}{}^{a}$
24	$6.8_{ab}{}^{a}$	$6.7_{a}{}^{a}$	$6.8_{a}{}^{a}$
72	$5.9_{bc}{}^{a}$	$5.0_{b}{}^{a}$	$5.0_{b}{}^{a}$
120	$5.2_{c}{}^{a}$	$4.2_{b}{}^{b}$	$4.0_{b}{}^{c}$
168	$4.0_{d}{}^{a}$	$2.6_{c}{}^{b}$	$2.8_{d}{}^{b}$
Time (h)	**Overall Acceptability**		
	Isothermal Condition	**Non-Isothermal Condition 1**	**Non-Isothermal Condition 2**
0	$7.0_{a}{}^{a}$	$7.0_{a}{}^{a}$	$7.0_{a}{}^{a}$
24	$6.8_{a}{}^{a}$	$6.8_{a}{}^{a}$	$6.8_{a}{}^{a}$
72	$5.9_{ba}{}^{a}$	$5.1_{b}{}^{a}$	$4.8_{b}{}^{a}$
120	$5.0_{b}{}^{a}$	$4.0_{c}{}^{b}$	$3.4_{c}{}^{b}$
168	$3.9_{c}{}^{a}$	$2.5_{d}{}^{b}$	$2.2_{d}{}^{b}$
Mathematical Formula of Overall Acceptability Values	$y = -4E^{-05}x^2 - 0.0131x + 7.045$	$y = -1E^{-05}x^2 - 0.025x + 7.1199$	$y = 2E^{-05}x^2 - 0.0334x + 7.2063$
Minimum Acceptable Storage Time (h)	157 h (6.5 days)	119 h (5.0 days)	102 h (4.3 days)

*Isothermal condition: 0ºC constant temperature condition, **non-isothermal condition 1: door open time is 15 seconds with 30 minutes' intervals between each door open-close cycles at 0ºC mean temperature, ***non-isothermal condition 2: door open time is 15 seconds with 15 minutes' intervals between door open-close cycles at 0ºC mean temperature.

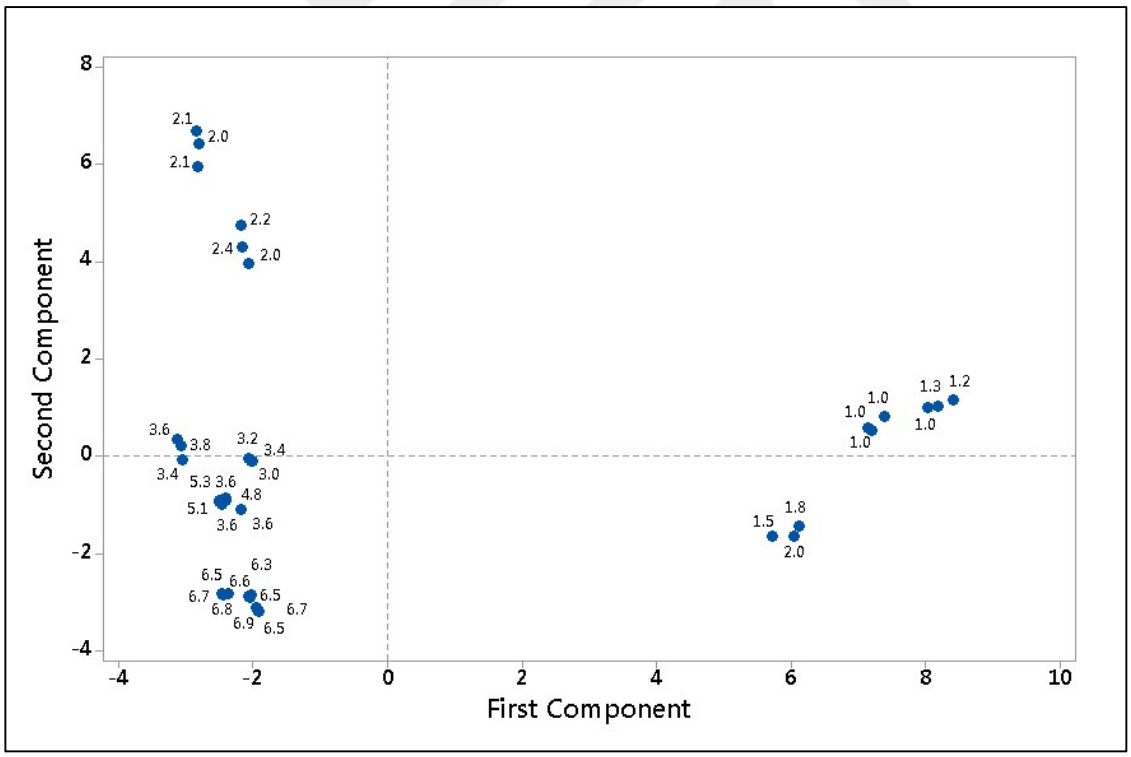
4.2.3 Principal Component Analyses of Volatile Organic Compounds

Principal component analysis (PCA) was used to find out the relation between obtained results of measured variables. All volatile compounds and overall acceptability scores were included to PCA classification. PCA of two storage temperatures were studied separately. Score plot and loading plot of 4°C are given in Figure 4.12. On the loading plot of PCA, it was seen that mainly four different regions were observed (Figure 4.12a). Regions were shown as different colors. Volatiles were dedicated to fresh, semi-fresh, spoiled and very spoiled regions by PCA. 2-Butanone and dimethyl sulphide may be related with fresh and acceptable meat, whereas hexanal, 2-propanone, dimethylamine pentanal, 1-octen-3-ol, 2-ethly-1-hexanol, 1-hexanol and 2,3-octandione may be correlated with first signs of spoilage. In the second spoilage period of beef, 2,3-butandione, 3-hydroxy-2-butanone, 2-pentanone, 3-methyl-1-butanal and 1-pentanol were associated with spoilage. All ester compounds (ethly acetate, methyl acetate, ethyl butanoate, ethyl hexanoate, methyl hexanoate, etc.) and dimethyl disulfide, dimethyl trisulfide were encountered in completely spoiled beef.

Score plot and loading plot of 0°C are given in Figure 4.13. Due to the retarded spoilage process at 0°C, minimum sensory score was 2. Similar with the results obtained at 4°C, 2-butanone and dimethyl sulphide may be related with fresh and acceptable meat. Beside this, heptanal, nonanal and carbon disulphide also appeared at fresh period. Hexanal, ethanethiol, 2-butanamine and 2,3-octandione were related with semi-fresh beefs at acceptable limits. In the early period of spoilage, 2-propanone, 2-pentanone, 1-octen-3-ol, 1-pentanol and 3-methyl-1-butanal. 2,3-butandione, 3-hydroxy-2-butanone, 2-pentanone, 3-methyl-1-butanal were recored as good indicators of spoilage like volatiles mentioned at 4°C. 3-Octanone was the common volatile which was observed at very spoiled state of meat both at 4°C and 0°C storage temperatures.

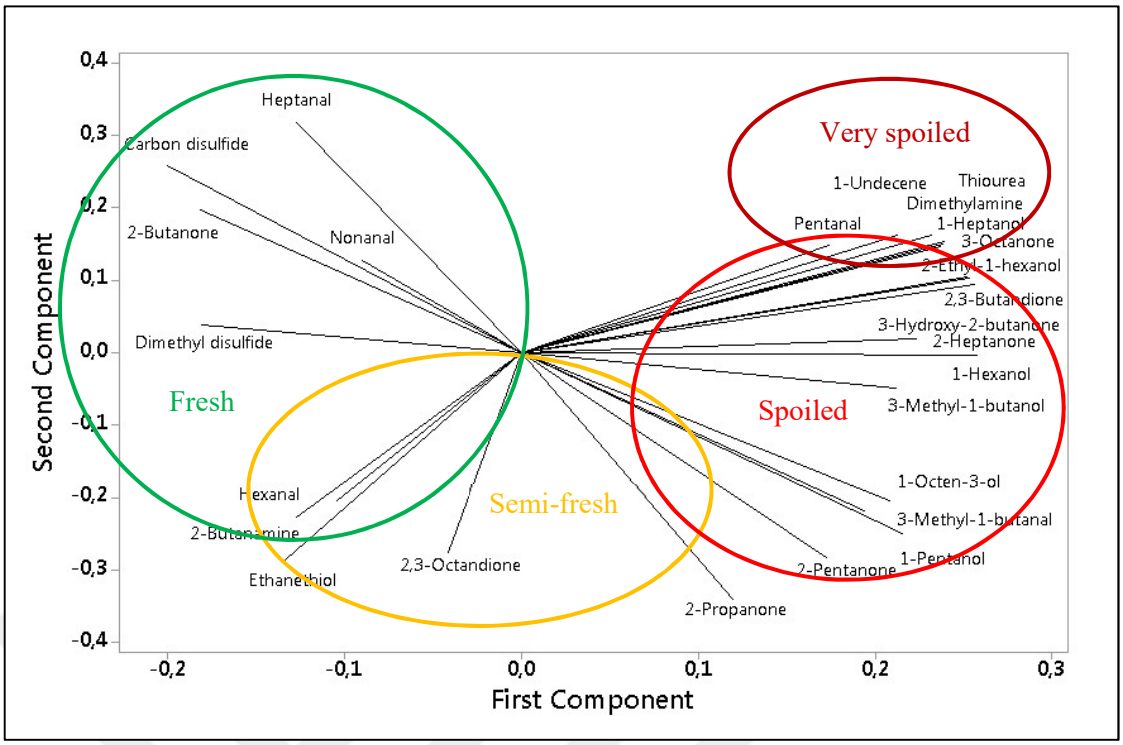


a)

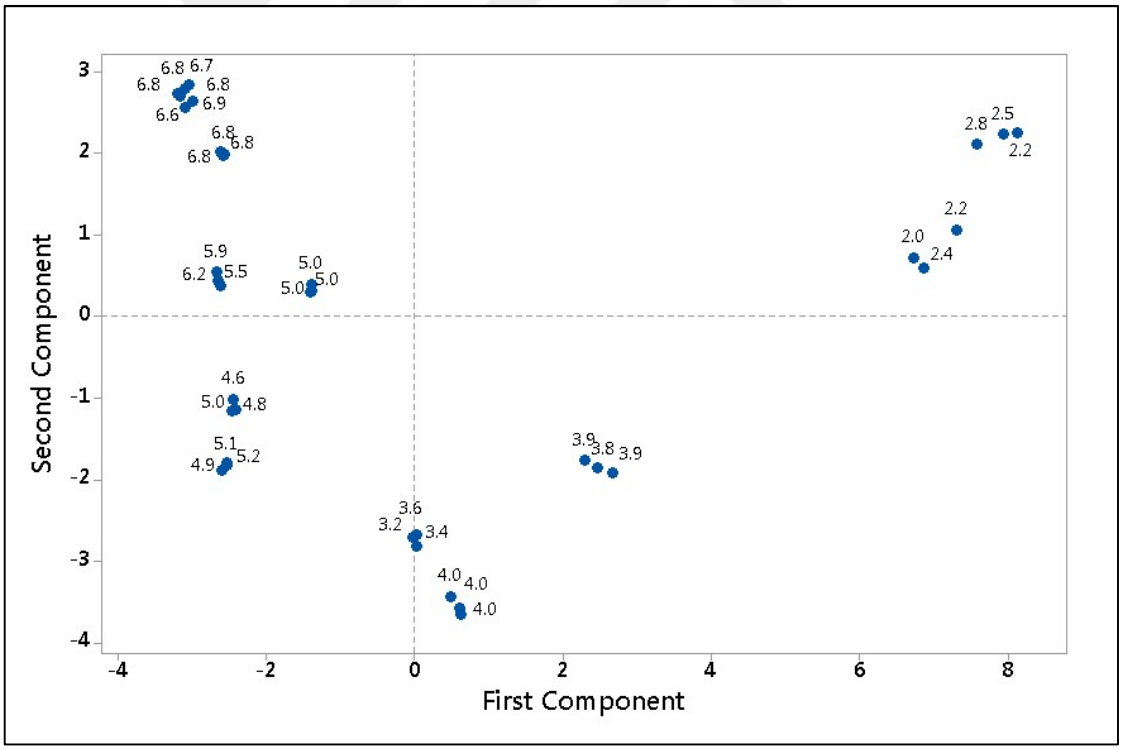


b)

Figure 4.12 : (a) Loading plot, (b) Score plot of volatile compounds labelled with sensory overall acceptability scores for 4°C.



a)



b)

Figure 4.13 : (a) Loading plot, (b) Score plot of volatile compounds labelled with sensory overall acceptability scores for 0°C.

4.2.4 Discriminant analysis of volatile organic compounds

Discriminant analysis was used for classification performance of GC-MS data on shelf life prediction. For representing different state of meat for 4°C storage temperature, 2-butanone for fresh state, hexanal for semi-fresh state and 3-hydroxy-2-butanone for spoiled state were selected for discriminant analysis from PCA results. Results are presented in Table 4.11, Table 4.12 and Table 4.13. 100% classification of fresh and semi-fresh state of meat can be classified by using 2-butanone. 2-Butanone could not be characterizable for spoiled meat. However, hexanal can be used for indicative compound to classify semi-fresh meat with 100% percentage. For spoiled meat, 3-hydroxy-2-butanone has been regarded as a good predicter for shelf life with 75% ratio of total sample. 3-hydroxy-2-butanone was also a good predicter for fresh states but not selective one.

Table 4.11 : Summary of classification with cross-validation by using 2-butanone.

Put into group	True Group		
	Fresh	Semi-Fresh	Spoiled
Fresh	9	0	0
Semi-fresh	0	3	10
Spoiled	0	0	14
Total N	9	3	24
N correct	9	3	14
Proportion	1.000	1.000	0.583

N=36, N correct=26, Proportion correct: 0.722

Table 4.12 : Summary of classification with cross-validation by using hexanal.

Put into group	True Group		
	Fresh	Semi-Fresh	Spoiled
Fresh	6	0	10
Semi-fresh	0	3	10
Spoiled	3	0	4
Total N	9	3	24
N correct	6	3	4
Proportion	0.667	1.000	0.167

N=36, N correct=13, Proportion correct: 0.361

Table 4.13 : Summary of classification with cross-validation by using 3-hydroxy-2-butanone.

Put into group	True Group		
	Fresh	Semi-Fresh	Spoiled
Fresh	9	0	0
Semi-fresh	0	3	6
Spoiled	3	0	18
Total N	9	3	24
N correct	9	3	18
Proportion	1.000	1.000	0.750

N=36, N correct=30, Proportion correct: 0.833

For representing different states of meat for 0°C storage temperature, heptanal for fresh state, hexanal for semi-fresh state and 2,3-butandione for spoiled state were selected for classifications. Results are presented in Table 4.14, Table 4.15 and Table 4.16. 80% classification of fresh and semi-fresh state of meat can be classified by using heptanal. 2-Butanone also appears for spoiled meat; however, it can not be used to predict spoiled time since it is not a selective compound for spoiled state. Hexanal can be used as an indicative compound to classify semi-fresh meat from other states with 71.4% percentage. For spoiled meat, 2,3-butandione is a good predicter for shelf life with 75% ratio of total sample. This compound is not indicative for fresh states. Therefore, the spoiled time can be predicted specifically when 2,3-butandione level changes characteristically. It is obvious that storage temperature can affect the type of indicative compound for different state of meat during storage.

Table 4.14 : Summary of classification with cross-validation by using heptanal.

Put into group	True Group		
	Fresh	Semi-Fresh	Spoiled
Fresh	8	0	0
Semi-fresh	2	5	0
Spoiled	0	9	12
Total N	10	14	12
N correct	8	5	12
Proportion	0.800	0.357	1.000

N=36, N correct=25 Proportion correct: 0.694

Table 4.15 : Summary of classification with cross-validation by using hexanal.

Put into group	True Group		
	Fresh	Semi-Fresh	Spoiled
Fresh	3	1	0
Semi-fresh	1	10	6
Spoiled	6	3	6
Total N	10	14	12
N correct	3	10	6
Proportion	0.300	0.714	0.500

N=36, N correct=22 Proportion correct: 0.510

Table 4.16 : Summary of classification with cross-validation by using 2,3-butandione.

Put into group	True Group		
	Fresh	Semi-Fresh	Spoiled
Fresh	6	7	0
Semi-fresh	4	7	3
Spoiled	0	0	9
Total N	10	14	12
N correct	6	7	9
Proportion	0.600	0.500	0.750

N=36, N correct=22 Proportion correct: 0.611

4.2.5 Effects of isothermal and non-isothermal cold storage conditions on oxidation of meat

Oxidative rancidity may contribute to undesired taste and odor formation after a specific TBARS levels. TBARS formation is the secondary event that contributes to the spoilage at refrigeration temperatures (Limbo et al, 2010). Oxidation degree accelerates with increasing temperature.

Thiobarbutyric acid reactive substances (TBARS) results which give information about the lipid oxidation degree of meats stored at 4°C conditions are given in Table 4.17. As can be seen from the Table, TBARS values increased over time significantly for all preservation conditions. Ferioli et al. (2010) indicated similar increasing trend in their study. The initial mean TBA values were around 0.45 mg MDA/kg meat. This value was in consistent with the studies of Chounou et al (2012). On the same days of storage period, all three conditions differed from each other statistically after day 1 (p<0.05). Highest values were measured by condition 3 for all storage period. Isothermal conditions affected the oxidation degree directly. The highest frequency of open-close cycles resulted with highest oxidation level. Although there is no existing

legal threshold, 1 mg malonaldehyde/kg meat is suggested as limit value in terms of perceived rancidity (Limbo et al, 2010). When the limit values of malondialdehyde levels exceeded after three days at 4°C constant temperature, the limit TBARS levels were measured after day 1 for both non-isothermal conditions. Opposite to this threshold limit, Zakrys et al. (2008) accepted the perceived limit by consumer as 2 mg MDA/kg meat. By the end of storage period, TBARS values were recorded as 1.67, 2.27 and 2.74 mg MDA/kg meat for condition 1, 2 and 3, respectively.

Table 4.17 : TBA changes of fresh meat during storage at isotermal and dynamic conditions at 4°C storage temperature (mg MDA / kg meat).

Days	4°C Constant Temperature	4°C / t_{open}= 300 sn, n_{cycle}=20	4°C / t_{open}= 600 sn, n_{cycle}=40
0	$0.44\pm0.12_{e}^{a}$	$0.44\pm0.12_{e}^{a}$	$0.44\pm0.12_{e}^{a}$
1	$0.68\pm0.02_{d}^{b}$	$0.77\pm0.03_{d}^{a}$	$0.79\pm0.02_{d}^{a}$
3	$0.86\pm0.02_{c}^{c}$	$1.07\pm0.10_{c}^{b}$	$1.23\pm0.11_{c}^{a}$
5	$1.21\pm0.33_{b}^{c}$	$1.66\pm0.10_{b}^{b}$	$1.79\pm0.05_{b}^{a}$
7	$1.67\pm0.07_{a}^{c}$	$2.27\pm0.00_{a}^{b}$	$2.74\pm0.10_{a}^{a}$

- *The different letters with subscript in the same column means the difference is significant between days for a condition ($p<0.05$).*
- *The different letters with superscript in the same line means the difference is significant between conditions for a day ($p<0.05$).*

PLS-R prediction models were applied for estimation of different TBARS value by using GC-MS volatile compounds data. PLS-R is generally used in the applications that have many variables such as spectral measurements which mostly correlated with each other to model some physicochemical changes. GC-MS data have also similar type of variables like spectral measurements and suitable for PLS-R analysis. PLS-R technique limits the many predictor variable (GC-MS data in this study) to a small number of uncorrelated components and apply least squares regression on selected components. These limited components are selected according to their explanations on variance between predictor data and response data. PLS model has ability to make a single model with multiple response variables in a multivariate way.

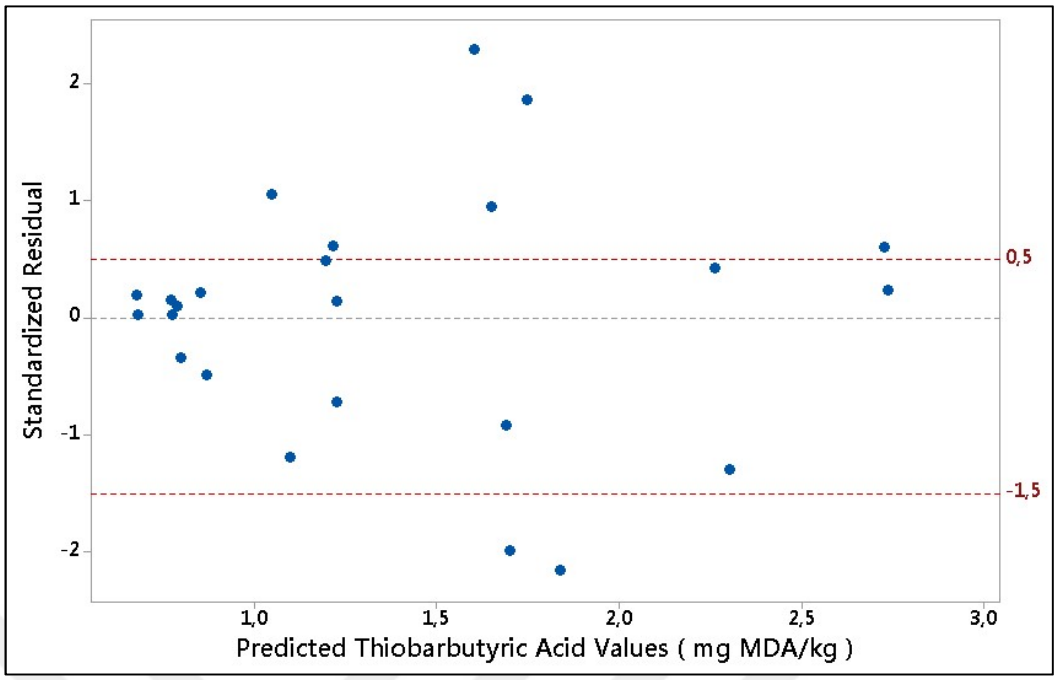
According to Table 4.18, 24 unit of VOC variable was reduced to 10 components by one-leave out validation method. 10-component model explains 99.45% of the variance in the predictors according to the X-variance that indicates the amount of variance in the predictors. Prediction Error Sum of Squares (PRESS) values also

smaller with 10-components model means high prediction ability of the model. PRESS is found out by excluding the i^{th} observation from the data and estimating the regression from n - 1 observations. Then, the fitted regression function is used to obtain the predicted value for the i^{th} observation.

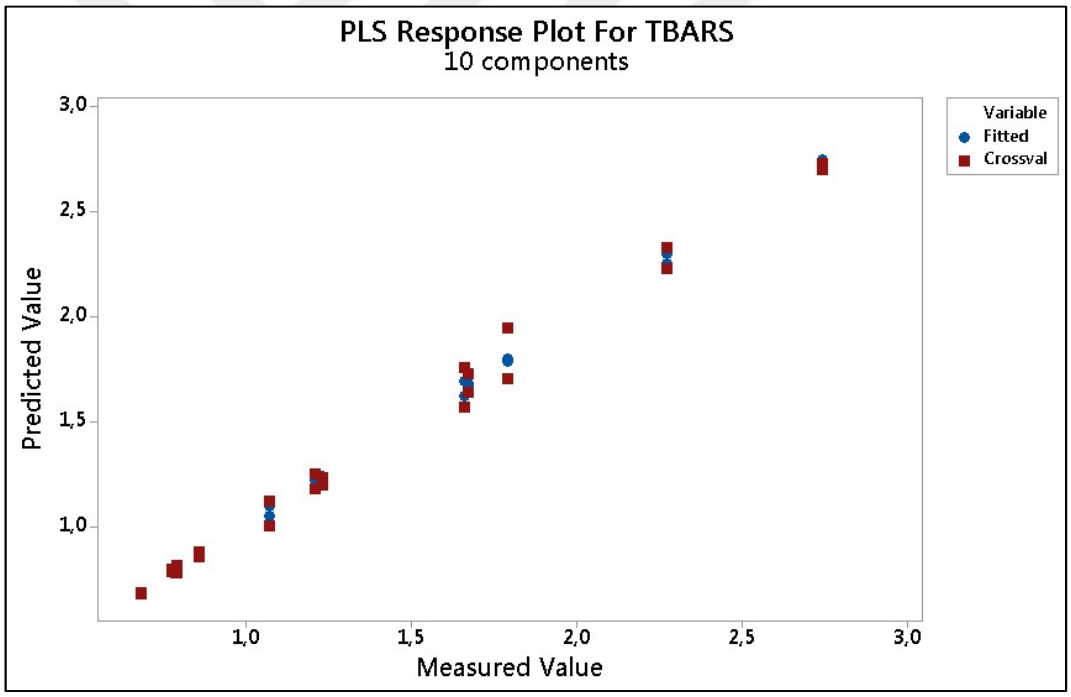
Table 4.18 : Model selection and validation parameters for TBA at 4°C.

Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred.)
1	0.509004	1.22838	0.866327	1.49584	0.837223
2	0.768574	0.38837	0.957738	0.55995	0.939066
3	0.835933	0.36395	0.960394	0.61517	0.933058
4	0.926625	0.17536	0.980917	0.27811	0.969736
5	0.944024	0.16019	0.982569	0.2823	0.969281
6	0.965372	0.15611	0.983012	0.29928	0.967432
7	0.981345	0.15593	0.983032	0.33125	0.963953
8	0.985423	0.02719	0.997041	0.14924	0.983759
9	0.990645	0.01401	0.998476	0.09512	0.989649
10	0.994581	0.01332	0.998551	0.08196	0.991081

Standardized residual values above or below 0 show that there is an under- or overestimates of the used PLS-R models (Figure 4.14a). For predicted TBA values, predicted errors was presented mostly between 0.5 and 1.5 log with 66.7% for predicted TBA values. The minimum portion should be 70% at least. Therefore, GC-MS data is not a sufficient tool to predict the oxidation level clearly in average. Low TBA ranges distributed very near to 0 line means that good prediction of low TBARS values (lower than 1.0 mg MDA/kg meat) were by GC-MS data predictors.



(a)



(b)

Figure 4.14 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of TBA value as estimated from the volatile organic compounds PLS-R model for 4°C.

Cross-validation PLS model explain how the predicted data fit with measured data set, individually. According to the plot shown in Figure 4.14b, the predicted values of cross-validation model showed a good distribution around the fitted line for 4°C storage temperature. However, there was over and underestimed differences between

measured and predicted values for some measured data range, especially between 1 and 2 mg MDA/kg meat.

Thiobarbutyric acid reactive substances (TBARS) values of meat stored at 0°C conditions are given in Table 4.19. Generally, measured values were not lower enough than the results obtained at 4°C conditions as expected. Similar to results obtained for 4°C, TBARS values increased significantly for all preservation conditions over storage period. Isothermal and non-isothermal conditions differed from each other statistically (p<0.05) after day 3, a little bit later than conditions at 4°C. Despite this, no clear differences were recorded between two non-isothermal conditions. The limit values of malondialdehyde levels were exceeded after 3 days for all conditions of 0°C. Isothermal conditions affected the oxidation degree directly without no significant differences between dynamic conditions. By the end of storage period, TBARS values were measured as 1.38, 2.08 and 2.23 mg MDA/kg meat for condition 1, 2 and 3, respectively. It seems that temperature fluctuations affected the TBARS levels, but its frequency did not alter the MDA concentration when the storage temperature was lowered.

Table 4.19 : TBA changes of fresh meat during storage at isotermal and dynamic conditions at 0°C storage temperature (mg MDA/kg meat).

Days	0°C Constant Temperature	0°C / t_{open}= 300 sn, n_{cycle}=20	0°C / t_{open}= 600 sn, n_{cycle}=40
0	0.28 ±0.03 $_{d}^{a}$	0.28 ±0.03 $_{d}^{a}$	0.28 ±0.03 $_{e}^{a}$
1	0.44 ±0.03 $_{c}^{b}$	0.77 ±0.02 $_{c}^{a}$	0.43 ±0.01 $_{d}^{b}$
3	1.05 ±0.00 $_{b}^{a}$	1.16 ±0.01 $_{b}^{a}$	1.12 ±0.01 $_{c}^{a}$
5	1.22 ±0.04 $_{b}^{b}$	1.95 ±0.02 $_{a}^{a}$	2.01 ±0.03 $_{b}^{a}$
7	1.38 ±0.01 $_{a}^{b}$	2.08 ±0.06 $_{a}^{a}$	2.23 ±0.04 $_{a}^{a}$

- *The different letters with subscript in the same column means the difference is significant between days for a condition (p<0.05).*
- *The different letters with superscript in the same line means the difference is significant between conditions for a day (p<0.05)*

Similar to previous results, 24 unit of VOC variable was reduced to 10 components by one-leave out validation method for TBA results of 0°C storage temperature (Table 4.20). 9 and 10-components model explains 99.13% and 99.29% of the variance in the predictors respectively according to X variance that indicates the amount of variance in the predictors. Prediction Error Sum of Squares (PRESS) values were also smaller

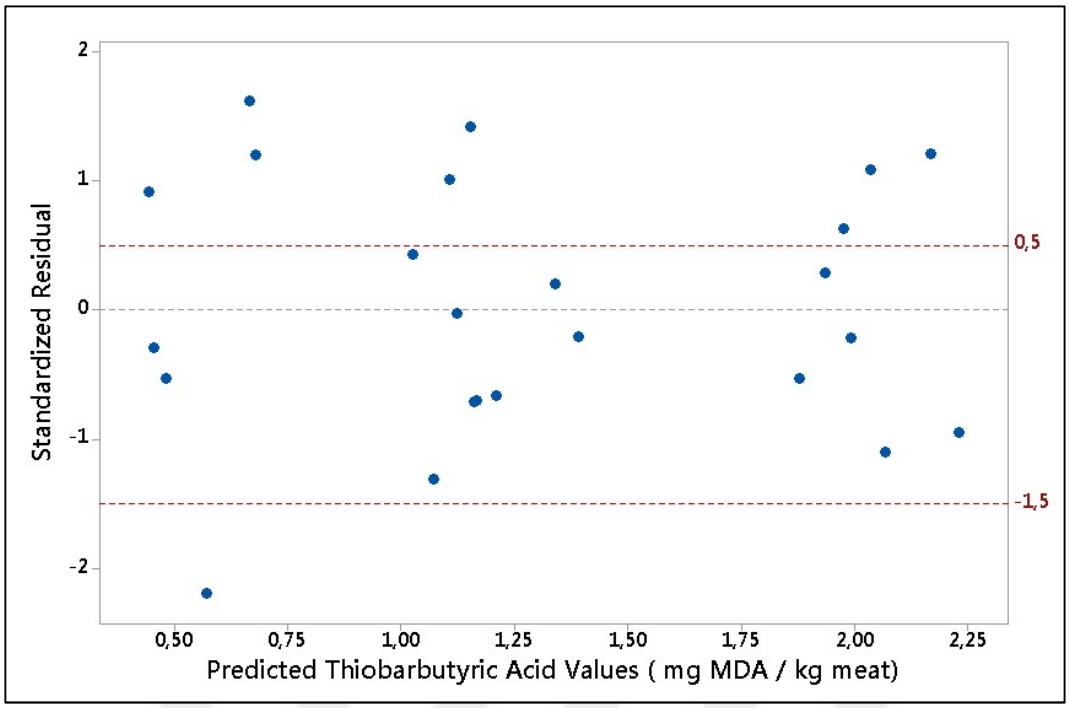
with 9 and 10-components model means high predictive ability of model with 0.36 and 0.37 values.

Table 4.20 : Model selection and validation parameters for TBA at 0°C.

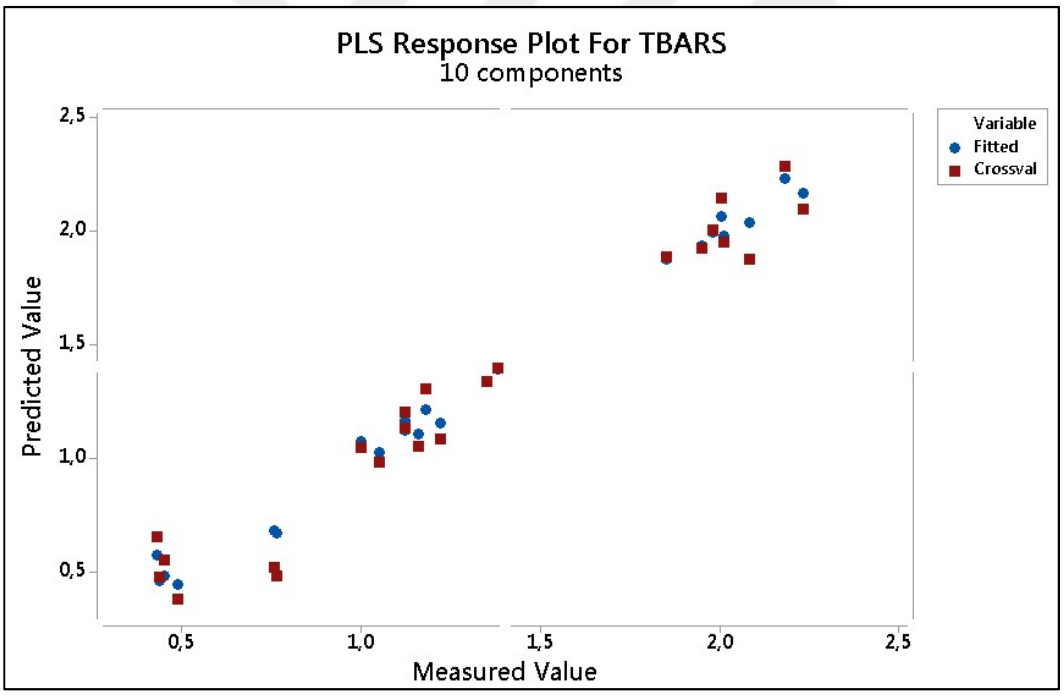
Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred)
1	0.534784	2.03175	0.752154	2.42343	0.704375
2	0.711755	0.76299	0.906926	1.08127	0.868100
3	0.829310	0.62449	0.923821	1.13737	0.861257
4	0.866662	0.4826	0.941129	1.16089	0.858388
5	0.923443	0.33967	0.958565	0.7168	0.912560
6	0.936023	0.25871	0.968441	0.59274	0.927693
7	0.973807	0.25711	0.968636	0.56638	0.930909
8	0.986436	0.23780	0.970992	0.56636	0.930912
9	0.991300	0.07344	0.991041	0.36290	0.955731
10	0.992996	0.07210	0.991205	0.37657	0.954064

Standardized residual values above or below 0 shows that there is an under-or overestimates of the developed models (Figure 4.15a). For predicted TBA values, predicted errors were presented mostly between 0.5 and 1.5 log with 62.5 % for predicted TBA values of 0°C conditions. It has slightly lower prediction quality than TBA values of 4°C conditions. The predicted TBA values generally distributed equally under and over-estimates zone with homogenous distribution. TBA ranges of 1.0-1.5 distributed near to 0 line with higher pecentage means that there was a good prediction of mid TBARS values by GC-MS predictors. It can be said that TBA values were predicted well when it reached to 1.0 mg/kg in which it was rancidity perception limit for meats. The results showed that prediction of oxidation level by using GC-MS data is more accurate at slightly higher temperatures.

The correlation of observed versus predicted TBARS values also presented good agreement for 0°C (Figure 4.15b). However, predicted values of cross validation model have under-prediction values for low TBARS concentrations, generally for under 1 mg/kg. Model is more predictive at high concentrations of TBARS.



(a)



(b)

Figure 4.15 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of TBA value as estimated from the volatile organic compounds PLS-R model for 0°C.

4.2.6 Effects of isothermal and non-isothermal cold storage conditions on pH value of meat

pH values of meat during cold storage at 4°C conditions are given in Table 4.21. pH values showed increasing trend for all storage conditions at 4°C. However, the most drastic increase was observed with condition 3. pH values in condition 2 and condition 3 increased significantly after day 3 when pH values changed after day 5 significanlty for isothermal conditions. Similar results about constant temperatures were obtained with different researches. Alomirah et al. (1998) presented that pH changes were more considerable after day 4 for fresh red meat. Despite this study, Ercolini et al. (2011) recorded no change in pH values of meat over time during storage at 5°C. Differences between two non-isothermal conditions were statistically significant however this difference was not important at all. Non-isotermal condition 2 had slightly higher pH values. It is clear that temperature fluctuations during cooler door open-close cycles affected pH changes similar to TBA values. pH value changes were generally related with microbial activity because of accumulation of biogenic amines after degradation in proteins and degradation products of proteins such as ammonia, hydrogen sulphite and free amino acids, etc. (Jay et al, 2003). Shelef (1977) related the pH increase with glucose consumption by microorganisms in their studies.

At the end of the storage period, pH values reached to 6.70, 6.87 and 6.93 for condition 1, 2 and 3 respectively. According to Turkish Standardization Regulation (TS 11566), meat and meat products should not be consumed when they exceeded pH value of 6.2. This means that all conditions were suitable for consumption until the day 5.

Table 4.21 : pH changes of fresh meat during storage at isotermal and dynamic conditions of 4°C storage temperature.

Days	4°C Constant Temperature	4°C / t_{open}= 300 sn, n_{cycle}=20	4°C / t_{open}= 600 sn, n_{cycle}=40
0	$5.66\pm0.01_{b}^{a}$	$5.66\pm0.01_{c}^{a}$	$5.66\pm0.01_{c}^{a}$
1	$5.48\pm0.01_{c}^{b}$	$5.60\pm0.03_{d}^{a}$	$5.57\pm0.01_{d}^{a}$
3	$5.67\pm0.04_{b}^{a}$	$5.58\pm0.01_{d}^{b}$	$5.69\pm0.03_{c}^{a}$
5	$5.66\pm0.03_{b}^{c}$	$5.90\pm0.02_{b}^{b}$	$6.02\pm0.03_{b}^{a}$
7	$6.70\pm0.03_{a}^{c}$	$6.87\pm0.05_{a}^{b}$	$6.93\pm0.02_{a}^{a}$

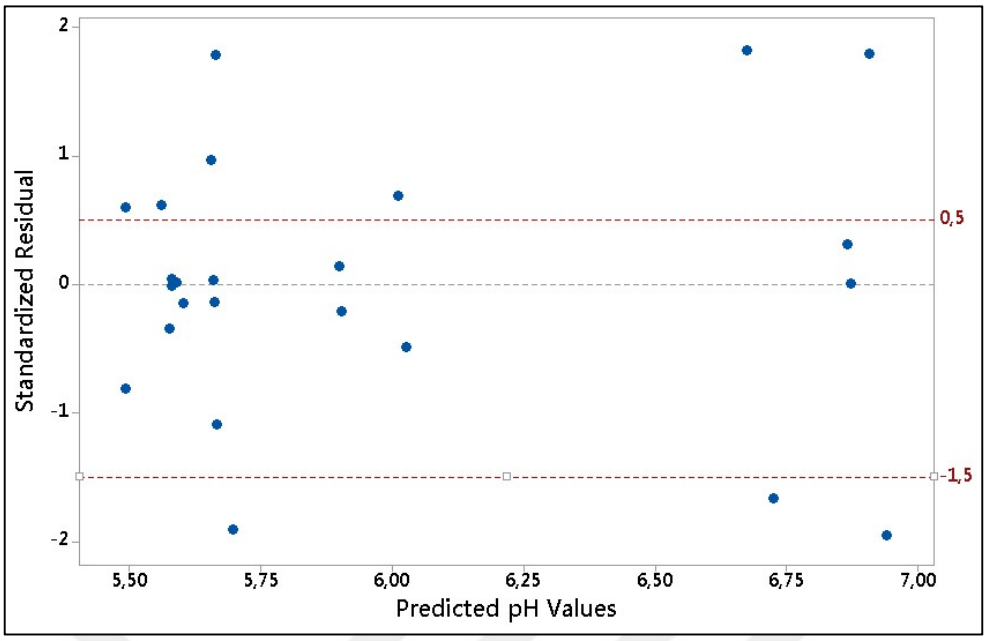
- *The different letters with subscript in the same column means the difference is significant between days for a condition ($p<0.05$).*
- *The different letters with superscript in the same line means the difference is significant between conditions for a day ($p<0.05$).*

According to Table 4.22, 24 unit of VOC variable was reduced to 10 components by one-leave out validation method for pH outputs of 4°C storage temperature. 9-component model explains 99.1% of the variance in the predictors according to the X variance. Prediction Error Sum of Squares (PRESS) values were also smaller with 9-components model means high prediction ability of the model.

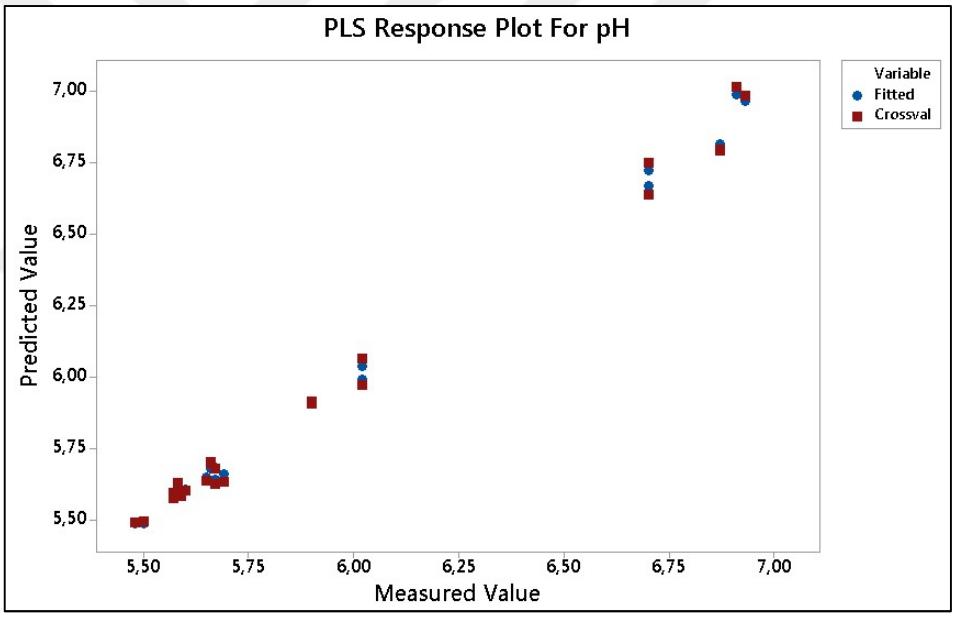
Table 4.22 : Model selection and validation parameters for pH at 4°C.

Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred.)
1	0.509004	0.0890639	0.986139	0.112045	0.982562
2	0.768574	0.0864291	0.986549	0.112742	0.982453
3	0.835933	0.0835079	0.987003	0.125609	0.980451
4	0.926625	0.067268	0.989531	0.106566	0.983415
5	0.944024	0.056630	0.991186	0.095688	0.985108
6	0.965372	0.0278558	0.995665	0.074555	0.988397
7	0.981345	0.0234747	0.996347	0.064818	0.989912
8	0.985423	0.0145161	0.997741	0.052521	0.991826
9	0.990645	0.0052345	0.999185	0.01936	0.996987
10	0.994581	0.0050822	0.999209	0.01978	0.996921

According to standardized residual values for predicted pH values, predicted errors are presented mostly between 0.5 and 1.5 log with %58.3 at 4°C conditions (Figure 4.16a). The prediction quality of pH by GC-MS data is very low. The predicted pH values have uniform distribution values both around over estimate and under estimates zone near to 0. pH values lower than 6.0 distributed near to 0 line with higher by GC-MS predictors. pH values at higher values near to 6.75 and higher is relatively hard to predict well because of low number of data and the low percentage between limit residuals. The predicted values against observed pH values plot also showed linearity well up to pH 5.8. Above pH 5.8 value, linearity is disturbed with under estimated datas (Figure 4.16b).



(a)



(b)

Figure 4.16 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of pH value as estimated from the volatile organic compounds PLS-R model for 4°C.

The result of pH values of meat stored at 0°C conditions are presented in Table 4.23. As described in previous results for 4°C temperature, drastic changes were observed only for condition 3 after day 1. pH levels were reasonably lower than the pH values of meats stored at 4°C. No significant increase was observed in constant temperature. While the frequency of open-close cycle affected the changes of pH, changes in pH

values were much slower in condition 2 than condition 3. Despite the effect of temperature fluctuation on pH was reasonably clear, values did not exceed the TS limits by the end of storage. At the end of the storage period, pH values reached to 5.53, 5.67 and 5.98 for condition 1, 2 and 3 respectively. Statistically significant difference was observed between non-isothermal conditions after day 3.

Table 4.23 : pH changes of fresh meat during storage at isotermal and dynamic conditions of 0°C storage temperature.

Days	0°C Constant Temperature	0°C / t_{open}= 300 sn, n_{cycle}=20	0°C / t_{open}= 600 sn, n_{cycle}=40
0	$5.48 \pm 0.01_{d}^{a}$	$5.48 \pm 0.01_{c}^{a}$	$5.48 \pm 0.01_{d}^{a}$
1	$5.42 \pm 0.06_{c}^{b}$	$5.52 \pm 0.03_{c}^{a}$	$5.50 \pm 0.02_{d}^{a}$
3	$5.50 \pm 0.00_{bc}^{b}$	$5.62 \pm 0.02_{b}^{a}$	$5.61 \pm 0.01_{c}^{a}$
5	$5.52 \pm 0.01_{ab}^{c}$	$5.60 \pm 0.00_{b}^{b}$	$5.83 \pm 0.03_{b}^{a}$
7	$5.53 \pm 0.04_{a}^{c}$	$5.67 \pm 0.02_{a}^{b}$	$5.98 \pm 0.05_{a}^{a}$

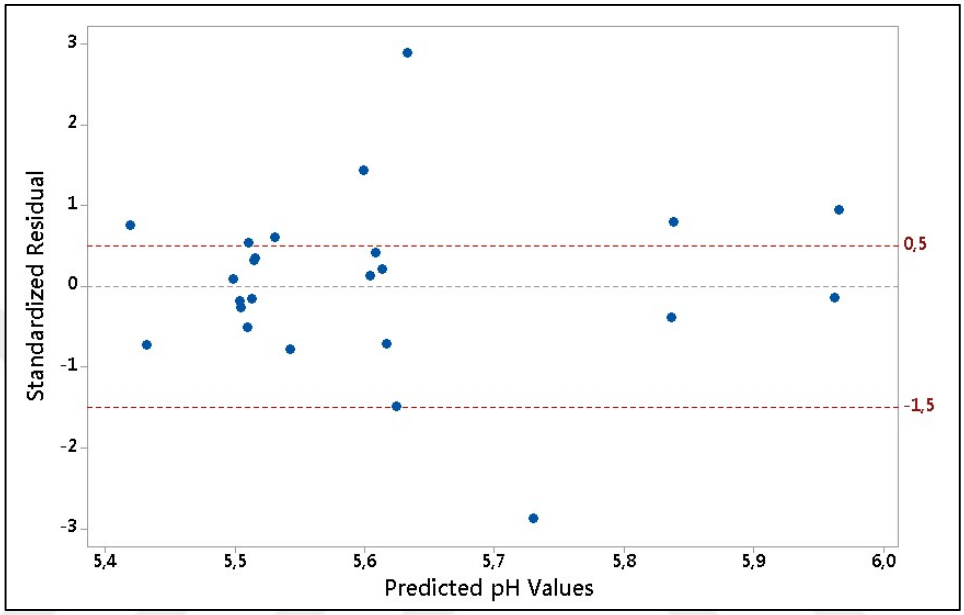
- *The different letters with subscript in the same column means the difference is significant between days for a condition ($p<0.05$).*
- *The different letters with superscript in the same line means the difference is significant between conditions for a day ($p<0.05$).*

Model selection and validation parameters for pH at 0°C are given in Table 4.24. Variance in model can be explained with 99.2% percentage for 10-component model when it is assessed together with lowest error and PRESS value. The results were very similar with the results obtained for 4°C.

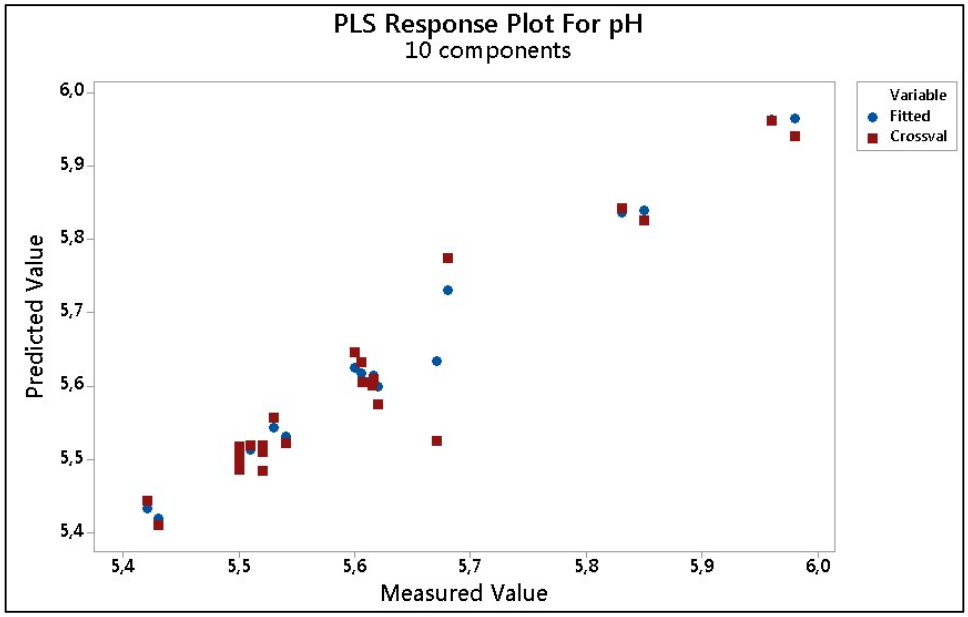
Table 4.24 : Model selection and validation parameters for pH at 0°C.

Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred)
1	0.534784	0.279566	0.479091	0.363684	0.322356
2	0.711755	0.24948	0.535149	0.360673	0.327966
3	0.829310	0.17229	0.678976	0.307538	0.426970
4	0.866662	0.046619	0.913135	0.132321	0.753450
5	0.923443	0.040578	0.924392	0.131634	0.754729
6	0.936023	0.016731	0.968826	0.090169	0.831989
7	0.973807	0.010615	0.980222	0.058968	0.890126
8	0.986436	0.007539	0.985953	0.04188	0.921967
9	0.991300	0.006426	0.988026	0.040626	0.924302
10	0.992996	0.006365	0.988139	0.041627	0.922437

Since most of the pH data for 0°C were around 5.5-5.6, it can be concluded that predictive quality was high for these ranges (Figure 4.17a). pH values can be predictable by GC-MS inputs with high percentage around 75% which means that residuals were between accepted limit values. Residuals were distributed homogenously around 0. According to the plot of predicted and measured response, there was some over and underestimated regions.



(a)



(b)

Figure 4.17 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of pH value as estimated from the volatile organic compounds PLS-R model for 0°C.

More data is needed to explain the estimation direction for all ranges because there is no sufficient data for the pH values higher than 5.6. pH values under 5.6 seems to have a lineer equality.

PLS response plot for pH of meat stored at 0°C is given in Figure 4.17b, since most of the clustered data is lower than pH 5.7, no clear comparison is possible with the results of 4°C. One similar point is that the predictive quality is slightly higher at the lower pH ranges for both of two temperature conditions.

4.2.7 Effects of isothermal and non-isothermal cold storage conditions on microbiological quality of meat

Together with chemical parameters, microbiological growth at 4°C storage temperature is shown in Table 4.25.

For 4°C storage temperature, total viable counts and *Pseudomonas* spp. had similar growth characteristics. Considerable time-depended growth rates were observed for all conditions. It is noticable that total viable counts and *Pseudomonas* spp. growth is sensitive to changing temperatures. Difference between isothermal and dynamic conditions started to begin immediately after day 1 for TVC and *Pseudomonas* spp.. However, no significant difference was observed between two different dynamic conditions until day 5 for TVC. After day 5, difference began to increase significantly between two dynamic conditions. For *Pseudomonas* spp. level, no difference was recorded under dynamic conditions during all the storage period. *Pseudomonas* spp. level at 4°C conditions was similar with the results obtained by Ercolini et al. (2006) and Esmer et al. (2012).

According to recommendations of Turkish Standardization Institute for total viable counts, threshold levels (6 log cfu/g) were reached between day 3 and day 5 in accordance with sensorial evaluations for isothermal conditions of 4°C. This limit value was recorded at day 3 for dynamic conditions. Total viable counts at the end of the storage period were 6.88, 7.05 and 6.95 log cfu/g for condition 1, 2 and 3, respectively.

Table 4.25 : Total viable, *Pseudomonas spp*. and lactic acid bacteria counts of fresh meat during storage at isotermal and dynamic conditions of 4°C storage temperature.

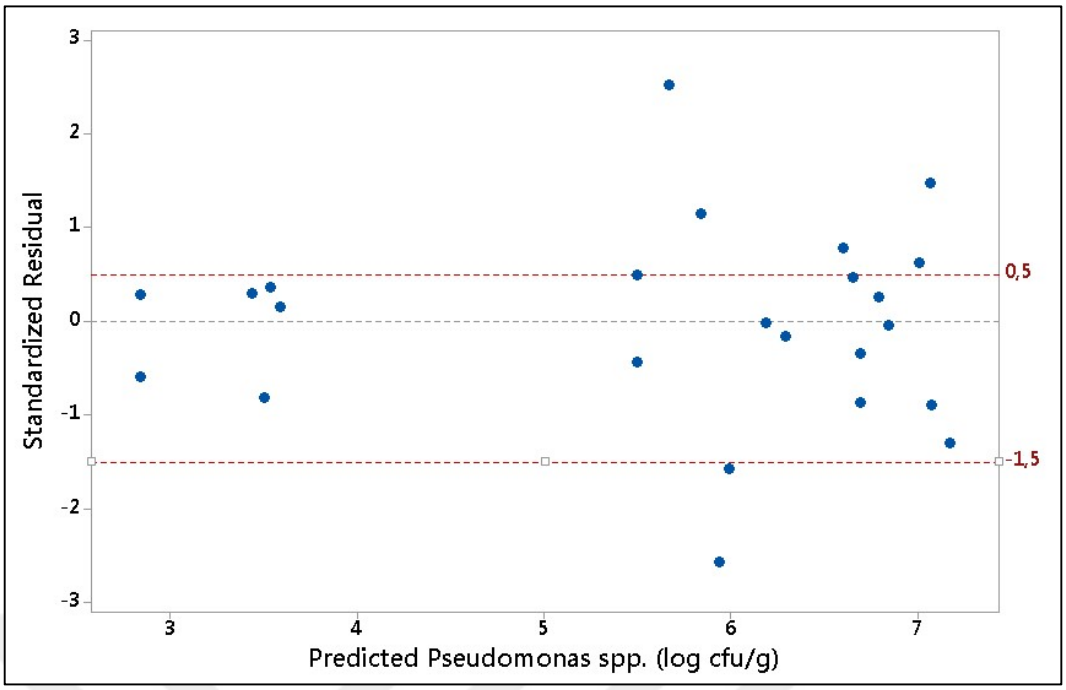
Total Viable Counts			
Days	**4°C Isothermal Condition**	**4°C / t_{open}= 300 sn, n_{cycle}=20**	**4°C / t_{open}= 600 sn, n_{cycle}=40**
0	$4.75\pm0.06_{e}^{a}$	$4.75\pm0.06_{e}^{a}$	$4.75\pm0.06_{e}^{a}$
1	$4.83\pm0.05_{d}^{b}$	$4.93\pm0.09_{d}^{a}$	$4.91\pm0.06_{d}^{a}$
3	$5.71\pm0.33_{c}^{b}$	$6.14\pm0.13_{c}^{a}$	$6.13\pm0.05_{c}^{a}$
5	$6.32\pm1.48_{b}^{c}$	$6.83\pm0.18_{b}^{a}$	$6.76\pm0.11_{b}^{b}$
7	$6.88\pm0.17_{a}^{c}$	$7.05\pm0.11_{a}^{a}$	$6.95\pm0.13_{a}^{b}$
Pseudomonas spp.			
Days	**4°C Isothermal Condition**	**4°C / t_{open}= 300 sn, n_{cycle}=20**	**4°C / t_{open}= 600 sn, n_{cycle}=40**
0	$2.69\pm0.12_{d}^{a}$	$2.69\pm0.12_{e}^{a}$	$2.69\pm0.12_{e}^{a}$
1	$2.85\pm0.53_{d}^{c}$	$3.60\pm0.28_{d}^{a}$	$3.45\pm0.18_{d}^{b}$
3	$5.52\pm0.31_{c}^{b}$	$5.82\pm0.17_{c}^{a}$	$5.90\pm0.07_{c}^{a}$
5	$6.28\pm0.25_{b}^{b}$	$6.64\pm0.09_{b}^{a}$	$6.68\pm0.13_{b}^{a}$
7	$6.84\pm0.33_{a}^{b}$	$7.15\pm0.21_{a}^{a}$	$7.04\pm0.20_{a}^{a}$
Lactic Acid Bacteria			
Days	**4°C Isothermal Condition**	**4°C / t_{open}= 300 sn, n_{cycle}=20**	**4°C / t_{open}= 600 sn, n_{cycle}=40**
0	3.02±0.23 d	3.02±0.23 e	3.02±0.23 e
1	3.02±0.18 d	3.43±0.03 d	3.33±0.08 d
3	3.74±0.23 c	3.81±0.14 c	3.86±0.11 c
5	3.98±1.34 b	4.92±0.02 b	4.85±0.09 b
7	5.34±0.06 a	5.76±0.03 a	5.84±0.03 a

- *The different letters with subscript in the same column means the difference is significant between days for a condition ($p<0.05$).*
- *The different letters with superscript in the same line means the difference is significant between conditions for a day ($p<0.05$).*

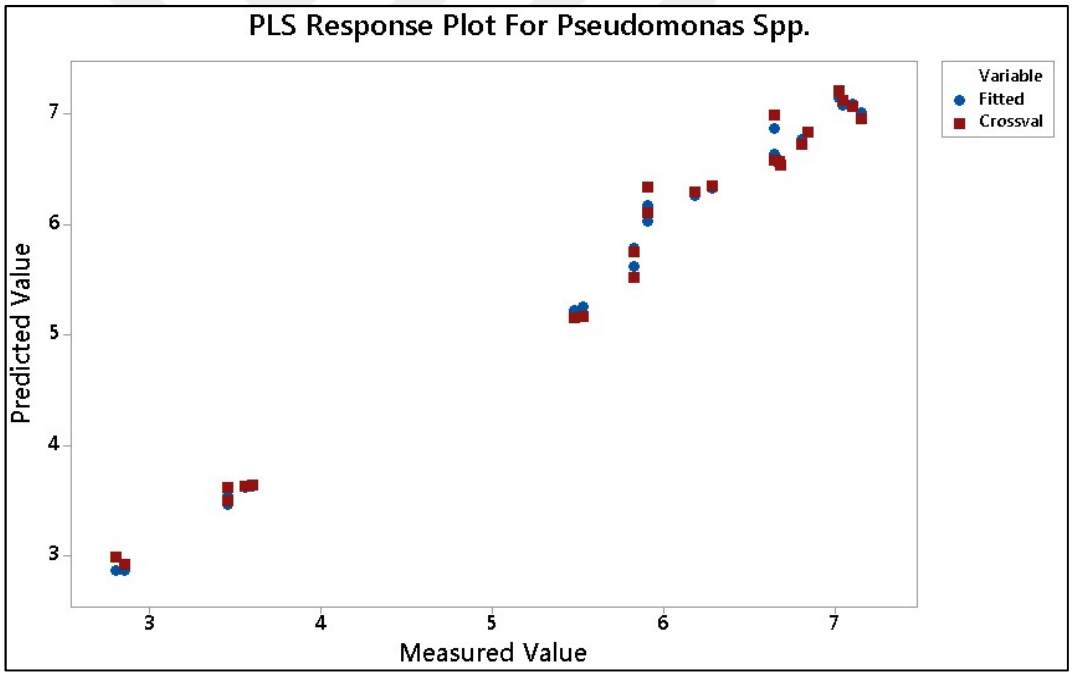
There are no clear spoilage threshold limits for bacterial counts. Several scientists indicated different threshold levels for meat that have off-odors (Irkın et al, 2011). Berruga et al. (2005) stated that critical level for meat spoilage is around 6-7 log cfu/g. Additionally, some researchers claimed that 7-8 log cfu/g of *Pseudomonas* level results unpleasant odor and slime surface on fresh meat (Gill and Newton,1977; Olaoye and Ntuen, 2011). Koutsoumanis et al. (2006) explained the threshold level as around 7 log cfu/g for total viable counts. Limbo et al. (2010) indicated that proteolytic activity did not begin until total viable count reached to $3.2x10^9$ cfu/g level.

Number of LAB is lower than *Pseudomonas* spp.. Since LAB is a microaerophilic microorganism group, aerobic and cold conditions of this study did not allow the growth of LAB as much as *Pseudomonas* spp. during storage. The change in LAB level in each condition by the time was found to be statistically significant for all conditions of 4°C. Despite this significance, changes were not major due to the low standard deviation and remained at lower levels in accordance with the study of Koutsoumanis et al. (2006) for isothermal storage temperatures. Additionally, no significant differentiation was observed between three different conditions based on time. By some studies, it was indicated that average spoilage treshold for LAB was around 10^7 cfu/g (Koutsoumanis et al, 2008 and Limbo et al, 2010). After day 7, this threshold level was not reached for all conditions of 4°C. According to standardized residual values (log) for predicted *Pseudomonas* spp counts in Figure 4.18a, predicted errors (log) mostly distributed between 0.5 and 1.5 log. With the highest predictive quality compared to other microorganism enumerations, %70.83 of data were between desired ranges for 4°C conditions. This model provided acceptable prediction quality (%70) of *Pseudomonas* spp. by using GC-MS data. Data in limit range distributed well around 0. There was also underestimate and overestimate zones. Residuals were higher between 5 and 6 log levels. Lower *Pseudomonas* levels are more predictable compared to higher levels. As can be seen on the response plot of measured values versus predicted values (Figure 4.18b), underestimated data exist between 5 and 6 log levels. Remaining predicted values that generated with GC-MS data by PLS-R model are very well fitted with measured values.

According to standardized residual values (log) for predicted total viable counts (Figure 4.19a), significant rate of predicted errors (log) are out of desired limits (0.5 and 1.5 log). 62.50% percentage of data were between desired ranges for 4°C conditions. Thus, PLS-R model was not sufficent to predict the total viable count by GC-MS data. The prediction variance was higher at high counts of microorganism near to spoilage of meat. Underestimate and overestimate zones were equally distributed around 0. As can be seen on the response plot of measured values versus predicted values (Figure 4.19b), overestimated data existed between 6 and 6.5 log levels. Despite this, lower predicted values of PLS-R model were well fitted with measured values.

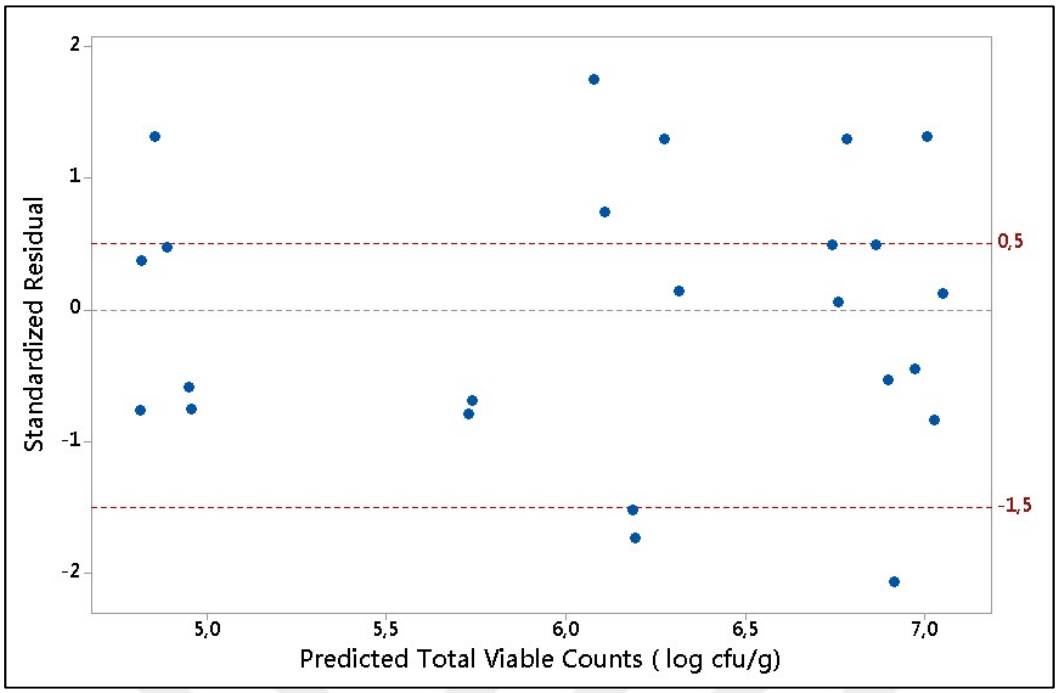


(a)

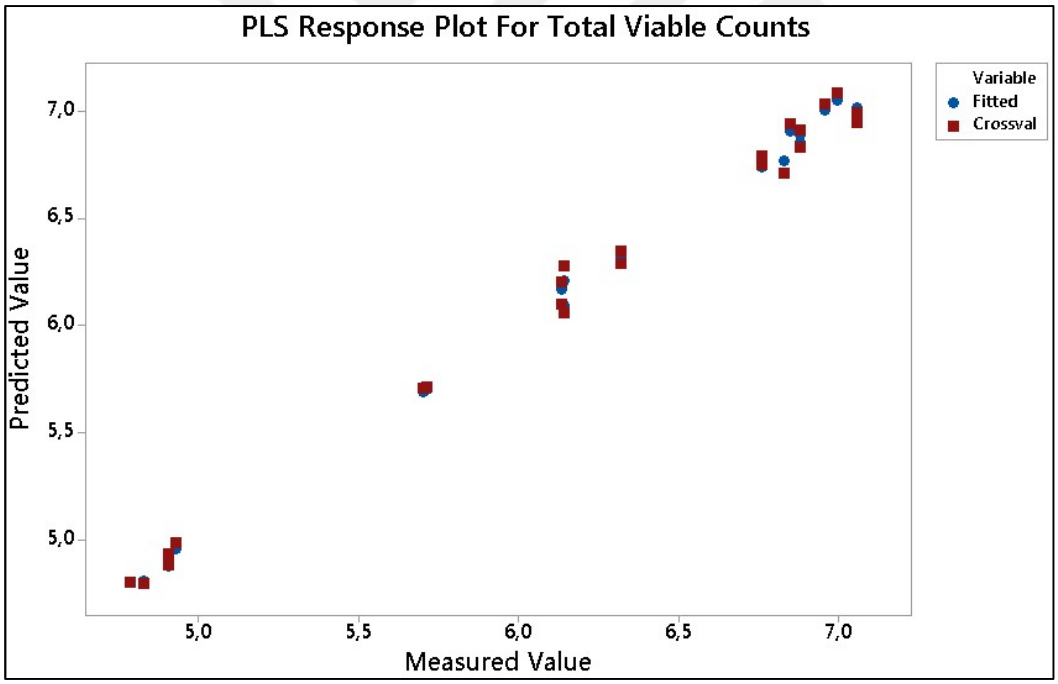


(b)

Figure 4.18 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of *Pseudomonas* spp. as estimated from the volatile organic compounds by PLS-R model for 4°C.



(a)

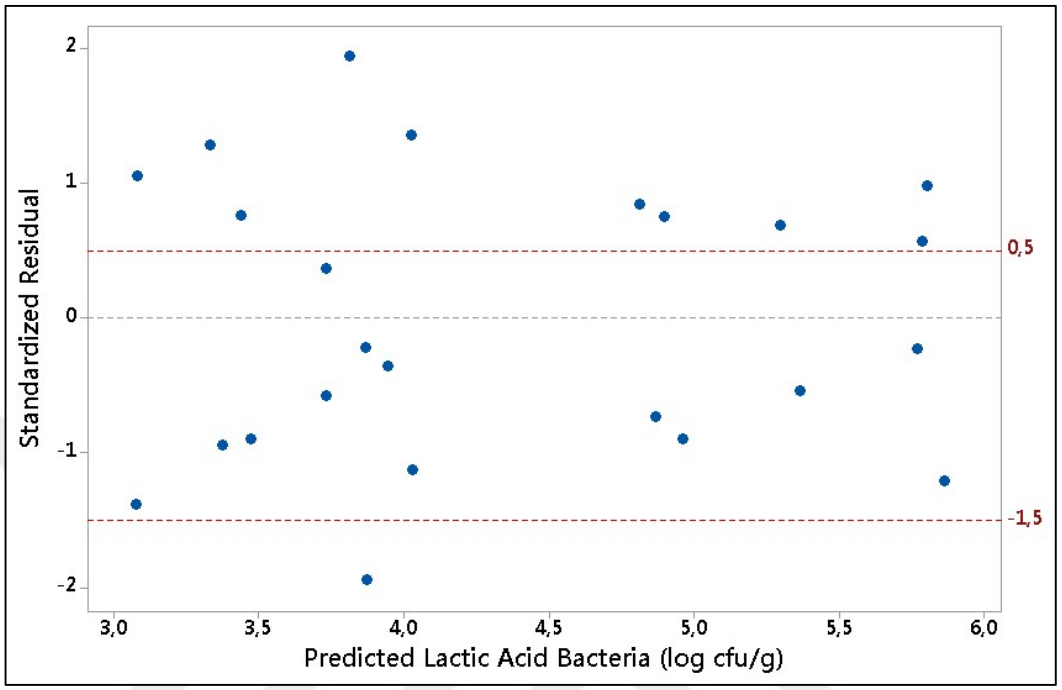


(b)

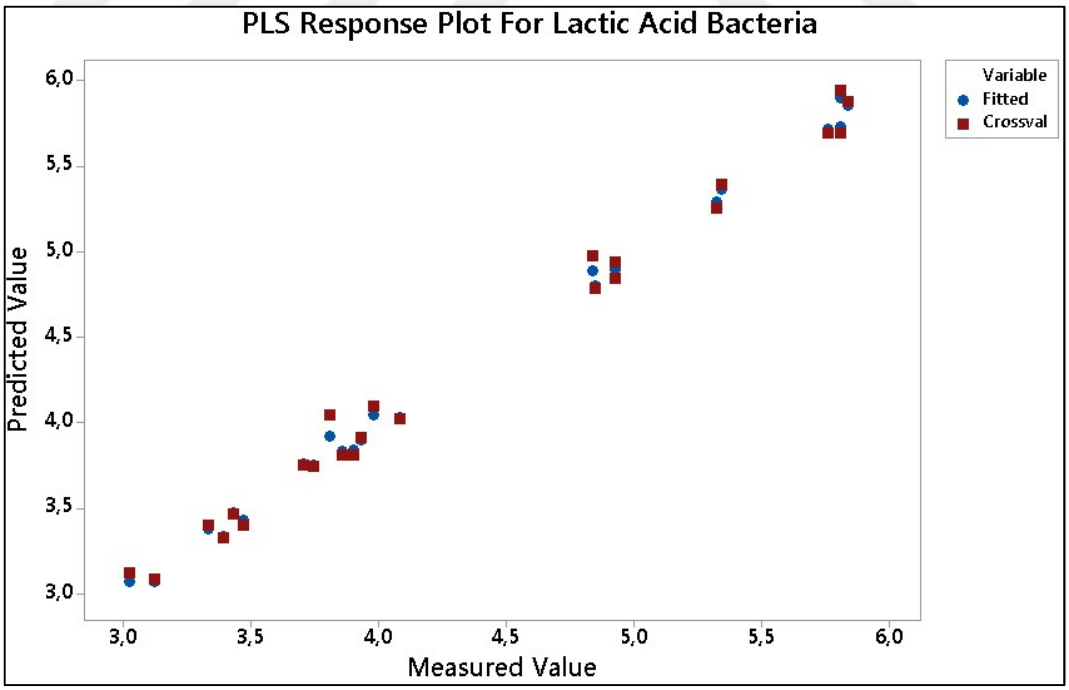
Figure 4.19 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of the total viable counts as estimated from the volatile organic compounds by PLS-R model for 4°C.

The plot for standardized residual versus predicted lactic acid bacteria is shown in Figure 4.20a. It is evident that distribution of residuals is irregular and 66.7% percentage of data exist between the accepted limits. Using GC-MS data by PLS-R

model is not suitable to predict the LAB number. Magnitute of residuals are higher at low number of LAB counts, generally below 4.5 log cfu/g. Over and underestimated zones were equal and homogenously distributed above ad below 0.



(a)



(b)

Figure 4.20 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of the lactic acid bacteria as estimated from the volatile organic compounds by PLS-R model for 4°C.

Model selection and validation parameters for *Pseudomonas* spp, TVC and lactic acid bacteria at 4°C are given in Table 4.26. Variance in model can be explained with 99.4% percentage for all microorganism counts with 10-component model.

Table 4.26 : Model selection and validation parameters for microorganisms at 4°C.

***Pseudomonas* spp.**					
Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred.)
1	0.509004	25.0928	0.499151	29.620	0.408787
2	0.768574	9.4809	0.810762	13.226	0.73601
3	0.835933	1.2821	0.97441	2.1526	0.957034
4	0.926625	0.9362	0.981314	1.7102	0.965865
5	0.944024	0.5493	0.989037	1.0780	0.978483
6	0.965372	0.5021	0.989978	0.9948	0.980144
7	0.981345	0.3553	0.992908	0.8775	0.982485
8	0.985423	0.1402	0.997202	0.5059	0.989902
9	0.990645	0.1240	0.997525	0.5193	0.989634
10	0.994581	0.0762	0.998479	0.3517	0.99298
Total Viable Counts					
Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred.)
1	0.509004	6.42278	0.594862	7.6049	0.520296
2	0.768574	1.77833	0.887826	2.55161	0.839049
3	0.835933	0.34913	0.977978	0.64039	0.959605
4	0.926625	0.34104	0.978488	0.60845	0.96162
5	0.944024	0.06511	0.995893	0.13669	0.991378
6	0.965372	0.05424	0.996579	0.12535	0.992093
7	0.981345	0.03531	0.997773	0.10195	0.993569
8	0.985423	0.03351	0.997886	0.10017	0.993681
9	0.990645	0.02985	0.998117	0.12137	0.992344
10	0.994581	0.02942	0.998144	0.12815	0.991917
Lactic Acid Bacteria					
Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred.)
1	0.509004	2.15123	0.894002	2.58532	0.872613
2	0.768574	0.39137	0.980716	0.60963	0.969962
3	0.835933	0.33334	0.983575	0.5567	0.97257
4	0.926625	0.3270	0.983888	0.55622	0.972594
5	0.944024	0.17621	0.991318	0.30526	0.984959
6	0.965372	0.10946	0.994607	0.33931	0.983281
7	0.981345	0.0634	0.996876	0.26803	0.986793
8	0.985423	0.06331	0.996881	0.2269	0.98882
9	0.990645	0.04491	0.997787	0.17043	0.991602
10	0.994581	0.04212	0.997925	0.17457	0.991398

All storage conditions did not alter the growth of *E. coli* bacteria. Coliform bacteria count was not affected until the last day of storage. At the end of the storage, significant increase was observed only at dynamic storage conditions (Table 4.27).

Table 4.27 : *E. coli* and coliform bacteria counts of fresh meat during storage at isotermal and dynamic conditions of 4°C storage temperature.

E. coli			
Days	**4°C Isothermal Condition**	**4°C / t_{open}= 300 sn, n_{cycle}=20**	**4°C / t_{open}= 600 sn, n_{cycle}=40**
0	1.15±0.21	1.15±0.21	1.15±0.21
1	0.95	0.95	0.95
3	0.95	0.95	0.95
5	0.95	0.95	0.95
7	0.95	0.95	0.95
coliform			
Days	**4°C Isothermal Condition**	**4°C / t_{open}= 300 sn, n_{cycle}=20**	**4°C / t_{open}= 600 sn, n_{cycle}=40**
0	2.98	2.98	2.98
1	2.35	2.31	2.25
3	2.46	1.72	2.72
5	1.13	0.98	0.97
7	2.79	3.65	3.05

Microbiological growth at 0°C storage temperature is given in Table 4.28. Total viable counts and *Pseudomonas* spp. had different growth characteristics compared to 4°C storage temperature. The lag time of total viable count is longer than *Pseudomonas* spp. that is called as spesific spoilage organism for raw meat. *Pseudomonas* spp. adapts to colder conditions rapidly. Total viable counts number started to increase wtih the beginning of day 5 significantly. Non-isothermal conditions affected the total viable count and *Pseudomonas* spp. minorly. Based on the recommended levels, total viable count of meats that were stored at isothermal and non-isothermal conditions was reached to limits at the same times. However, no significant difference was observed between two different dynamic conditions during all storage period both for total viable counts and *Pseudomonas* spp. Total viable counts at the end of the storage period were 6.20, 6.88 and 6.83 log cfu/g for condition 1, 2 and 3, respectively. *Pseudomonas* spp. count were 6.13, 6.76 ve 6.88 log cfu/g. When all results of two storage condition were assessed, *Pseudomonas* spp. is more accurate to evaluate the shelf life than total viable counts at cold conditions.

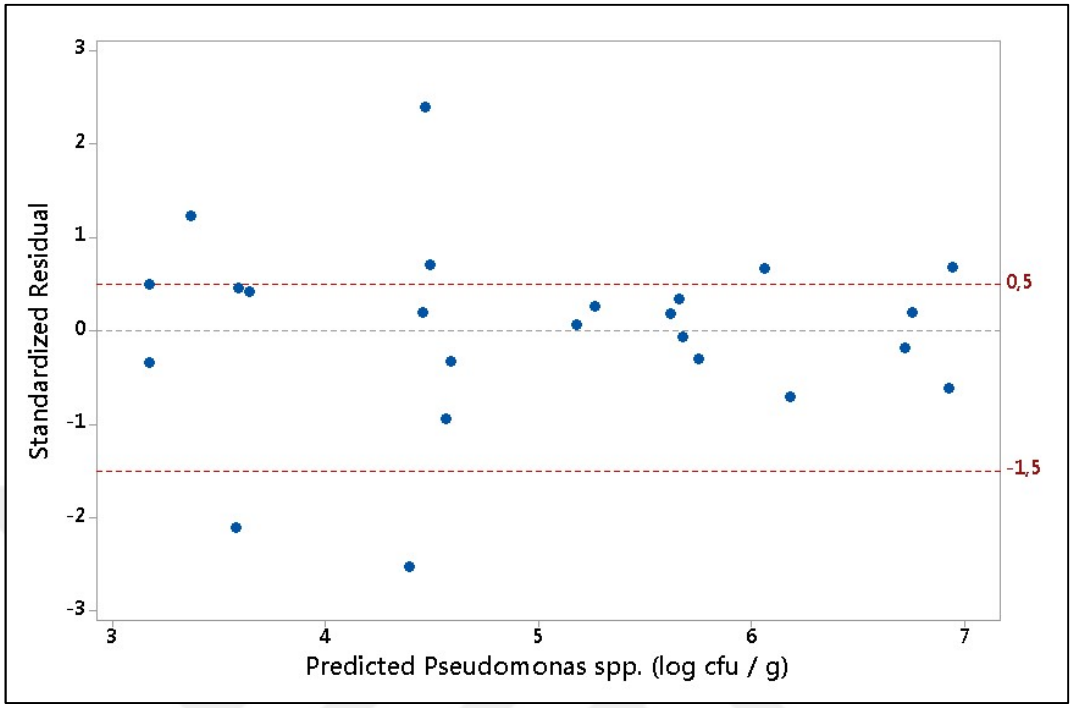
Table 4.28 : Microbiological growth of fresh meat during storage at isotermal and dynamic conditions of 0°C storage temperature.

Total Viable Counts			
Days	**0°C Isothermal Condition**	**0°C / t_{open}= 300 sn, n_{cycle}=20**	**0°C / t_{open}= 600 sn, n_{cycle}=40**
0	$5.12\pm0.09_{d}^{a}$	$5.12\pm0.09_{c}^{a}$	$5.12\pm0.09_{c}^{a}$
1	$4.59\pm0.07_{c}^{b}$	$4.85\pm0.12_{d}^{a}$	$4.82\pm0.02_{d}^{a}$
3	$4.97\pm0.06_{c}^{a}$	$4.91\pm0.01_{d}^{a}$	$4.97\pm0.11_{c}^{a}$
5	$5.74\pm0.27_{b}^{b}$	$5.84\pm0.18_{b}^{ab}$	$5.98\pm0.08_{b}^{a}$
7	$6.20\pm0.00_{a}^{b}$	$6.87\pm0.21_{a}^{a}$	$6.83\pm0.13_{a}^{a}$
Pseudomonas spp.			
Days	**0°C Isothermal Condition**	**0°C / t_{open}= 300 sn, n_{cycle}=20**	**0°C / t_{open}= 600 sn, n_{cycle}=40**
0	$3.65\pm0.20_{e}^{a}$	$3.65\pm0.20_{d}^{a}$	$3.65\pm0.20_{e}^{a}$
1	$3.20\pm0.23_{d}^{c}$	$3.62\pm0.55_{d}^{a}$	$3.41\pm0.17_{d}^{b}$
3	$4.54\pm0.34_{c}^{a}$	$4.63\pm0.25_{c}^{a}$	$4.47\pm0.09_{c}^{a}$
5	$5.28\pm0.30_{b}^{b}$	$5.73\pm0.28_{b}^{a}$	$5.68\pm0.21_{b}^{a}$
7	$6{,}13\pm0{,}06_{a}^{b}$	$6{,}76\pm0{,}19_{a}^{a}$	$6{,}88\pm0{,}13_{a}^{a}$
Lactic Acid Bacteria			
Days	**0°C Isothermal Condition**	**0°C / t_{open}= 300 sn, n_{cycle}=20**	**0°C / t_{open}= 600 sn, n_{cycle}=40**
0	$5.02\pm0.17_{a}^{a}$	$5.02\pm0.17_{a}^{a}$	$5.02\pm0.17_{a}^{a}$
1	$4.15\pm0.24_{c}^{b}$	$4.46\pm0.20_{c}^{a}$	$4.64\pm0.09_{b}^{a}$
3	$4.39\pm0.07_{c}^{b}$	$4.75\pm0.02_{b}^{ab}$	$4.81\pm0.13_{b}^{a}$
5	$4.77\pm0.04_{b}^{a}$	$4.96\pm0.20_{ab}^{a}$	$4.89\pm0.19_{ab}^{a}$
7	$4.81\pm0.29_{ab}^{a}$	$4.85\pm0.49_{ab}^{a}$	$4.78\pm0.21_{b}^{a}$

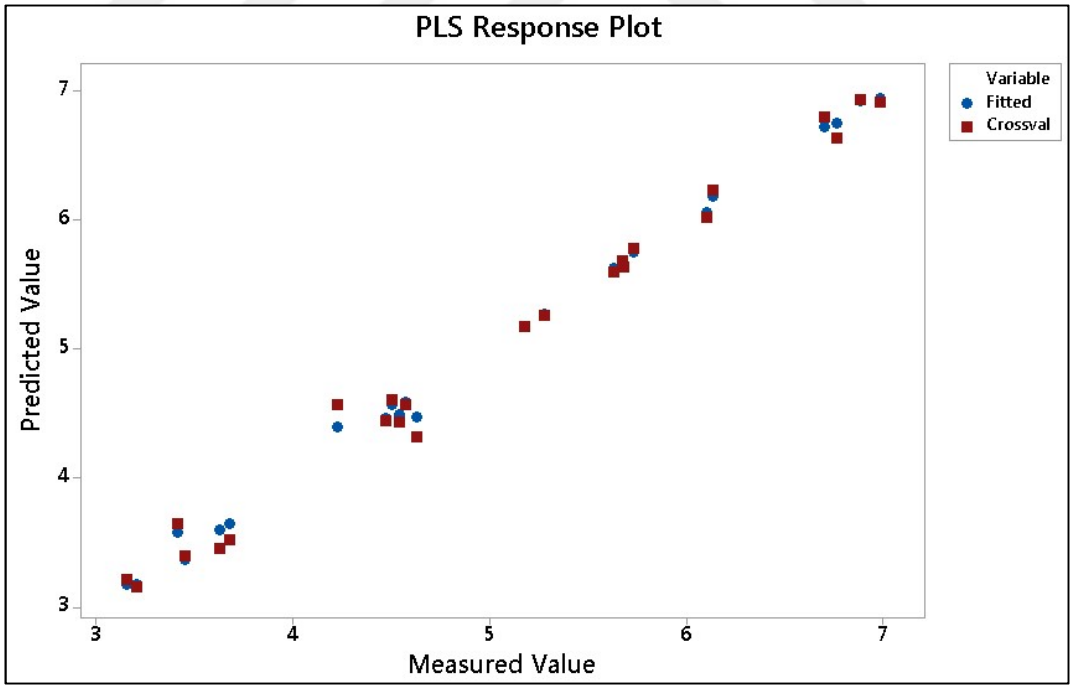
- *The different letters with subscript in the same column means the difference is significant between days for a condition ($p<0.05$).*
- *The different letters with superscript in the same line means the difference is significant between conditions for a day ($p<0.05$).*

Based on the PLS-R prediction model applied for *Pseudomonas* spp counts, predicted errors (log) distributed homogenously between 0.5 and 1.5 log with %70.83 of all values (Figure 4.21a). This percentage is identical to the result that was obtained for 4°C storage temperature and this means that *Pseudomonas* spp. can be predicted well with GC-MS data using PLS-R model for colder temperatures. Hovewer, the prediction quality is better in higher microorganism numbers above 5 log cfu/g against to 4°C storage temperature. Data in accepted range distributed homogenously around 0. Residuals were found to be higher between 3 and 5 log levels. As seen on the response plot of measured values versus predicted values (Figure 4.21b), linearity seems disorganized between 4 and 5 log cfu/g. Remaining predicted values that were

generated with GC-MS data by PLS-R model were very well fitted with measured values for *Pseudomonas* spp.



(a)

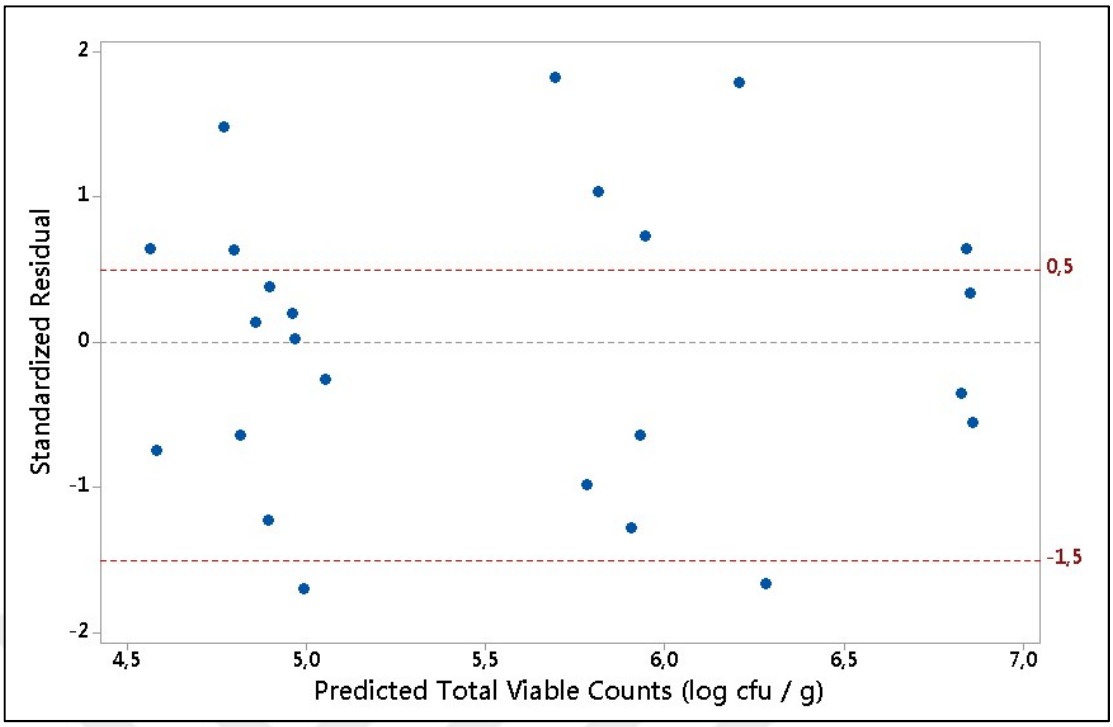


(b)

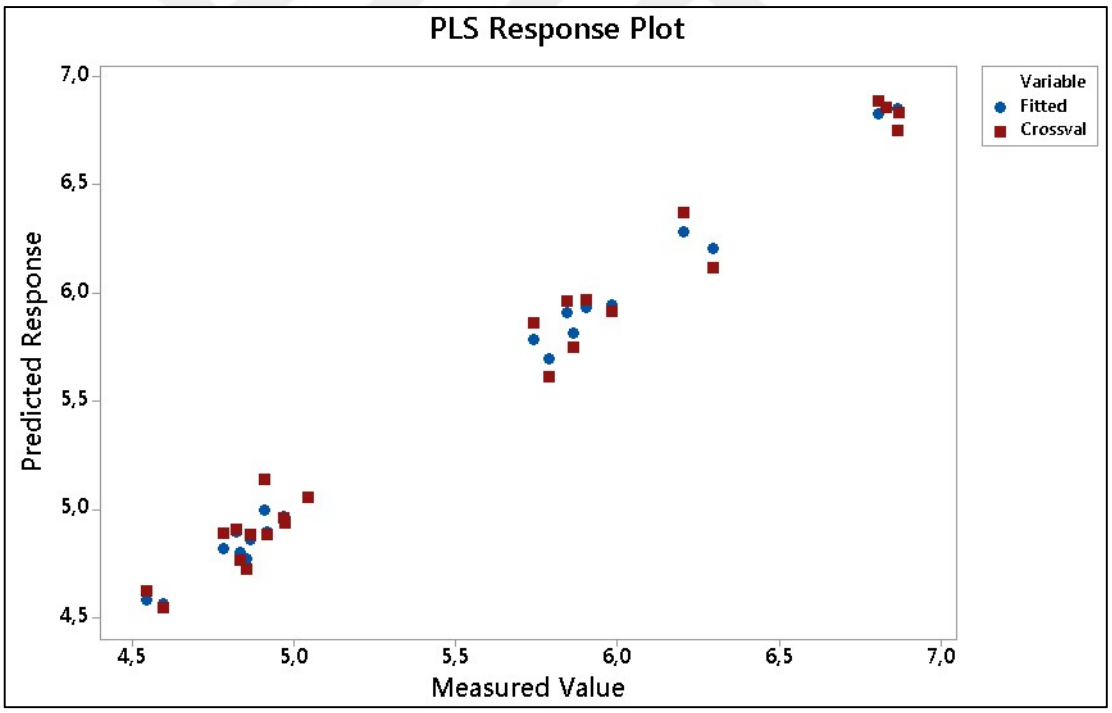
Figure 4.21 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of *Pseudomonas* spp. as estimated from the volatile organic compounds by PLS-R model for 0°C.

Like *Pseudomonas* spp., prediction quality of total viable count at 0°C was very similar to the results obtained at 4°C storage temperature. Based on the standardized residual values (log) for predicted total viable counts, most of the predicted errors (log) were out of accepted ranges (0.5 and 1.5 log) with 62.5% percentage of all data (Figure 4.22a). All the results of total viable counts both for two storage temperature indicated that prediction quality of total viable counts with PLS-R model was low by using volatile organic compund data. The prediction variance distributed evenly at any counts of microorganism. Underestimate and overestimate zones are equally distributed around 0. As seen on the response plot of clustered values (Figure 4.22b), only highest predicted values of PLS-R model above 6.5 log are well fitted with measured values.

Due to colder conditions at 0°C storage temperature, no significant growth of LAB was observed. LAB count remained constant during whole storage period. The change in LAB level in 4°C conditions was more evident compared to 0°C temperature. Additionally, no significant differantiation was observed between three different conditions based on time. LAB count at the last day of storage was around 5.5 log which was below the accepted spoilage level (10^7 cfu/g) indicated by Koutsoumanis et al. (2008) and Limbo et al. (2010). The plot for standardized residual versus predicted lactic acid bacteria is shown in Figure 4.23a. The distribution of residuals was irregular and low percentage of data (62.5%) fall between accepted limits. Using GC-MS data by PLS-R model, it was not suitable to predict the LAB number, especially for lower temperatures. Additionally, it can be seen in the response plot (Figure 4.23b) that the linearity was very low and variable.

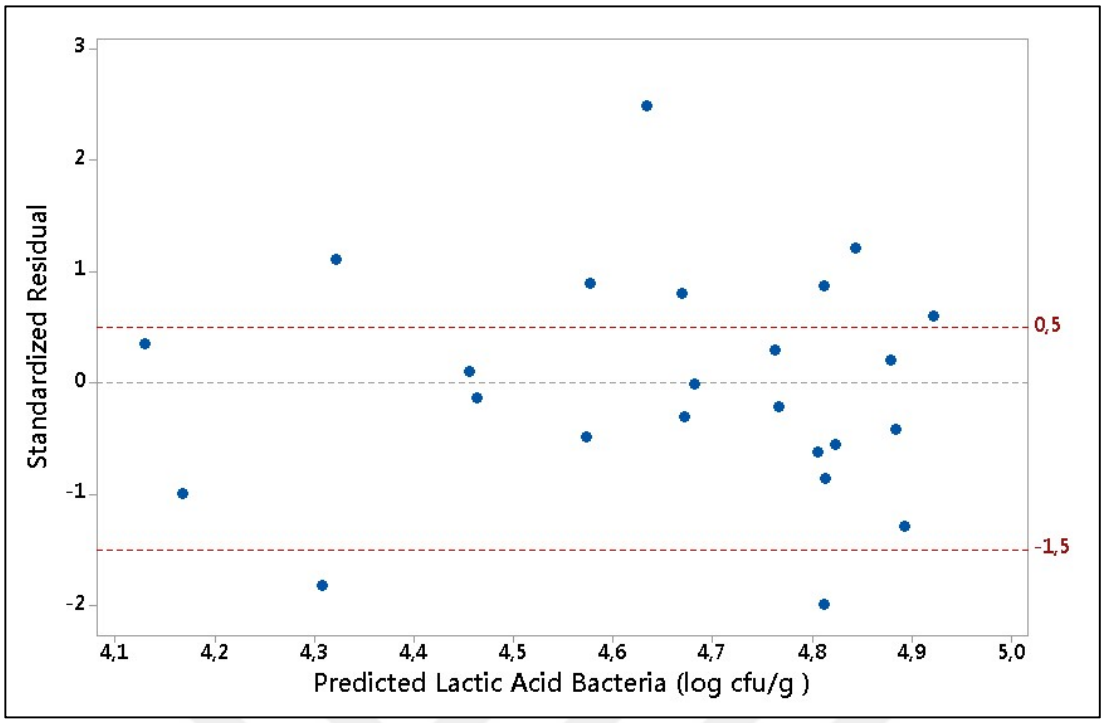


(a)

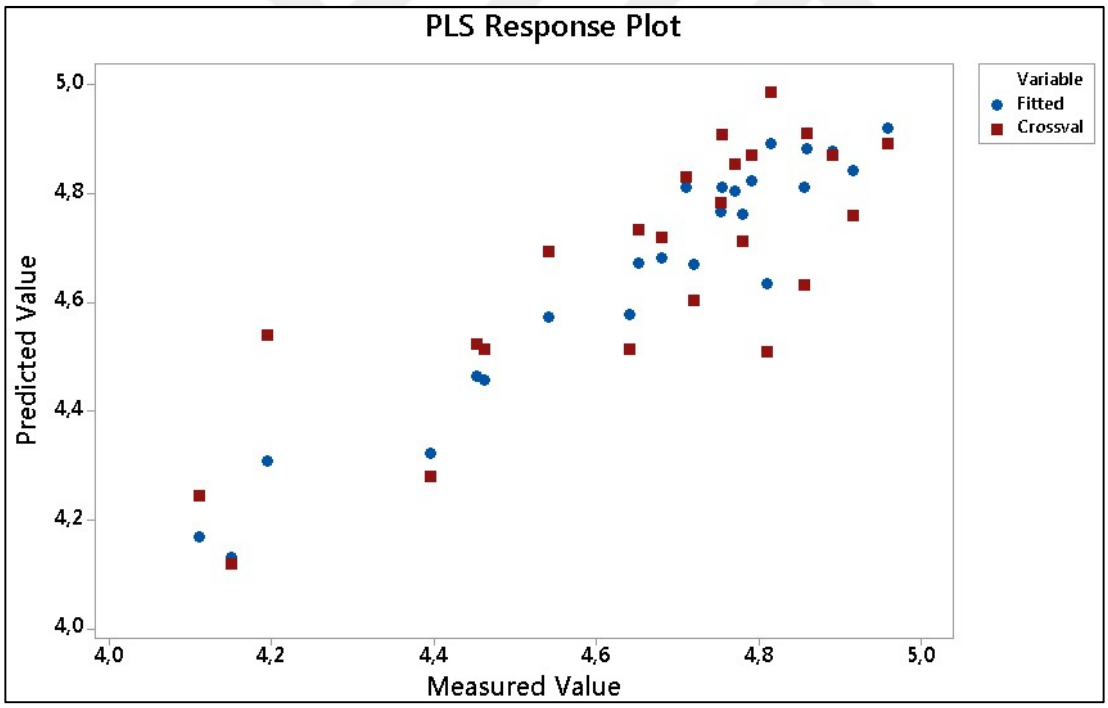


(b)

Figure 4.22 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of the total viable counts as estimated from the volatile organic compounds by PLS-R model for 0°C.



(a)



(b)

Figure 4.23 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of the lactic acid bacteria as estimated from the volatile organic compounds by PLS-R model for 0°C.

Model selection and validation parameters for *Pseudomonas* spp, TVC and lactic acid bacteria at 0°C are given in Table 4.29. Variance in model can be explained with 99.2% percentage which is very similar to the result obtained for 4ºC for all microorganism counts with 10-component model.

Table 4.29 : Model selection and validation parameters for microorganisms at 0°C.

***Pseudomonas* spp.**					
Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred)
1	0.534784	4.34075	0.875252	5.52714	0.841156
2	0.711755	2.21649	0.9363	2.94757	0.91529
3	0.82931	1.95602	0.943786	2.89743	0.916731
4	0.866662	1.88014	0.945967	2.82613	0.91878
5	0.923443	1.35302	0.961116	2.45901	0.929331
6	0.936023	1.29713	0.962722	2.7661	0.920505
7	0.973807	0.26563	0.992366	0.83272	0.976069
8	0.986436	0.24537	0.992948	0.69682	0.979974
9	0.99130	0.12766	0.996331	0.45085	0.987043
10	0.992996	0.10689	0.996928	0.40524	0.988354
Total Viable Counts					
Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred)
1	0.534784	1.11394	0.923323	1.3943	0.904026
2	0.711755	1.04798	0.927864	1.32711	0.90865
3	0.82931	0.68023	0.953177	1.02021	0.929775
4	0.866662	0.5205	0.964172	0.93923	0.935349
5	0.923443	0.31338	0.978429	0.69722	0.952008
6	0.936023	0.17743	0.987787	0.65527	0.954896
7	0.973807	0.09342	0.99357	0.27491	0.981077
8	0.986436	0.09248	0.993634	0.2626	0.981924
9	0.99130	0.09142	0.993707	0.27918	0.980783
10	0.992996	0.06105	0.995797	0.26792	0.981558
Lactic Acid Bacteria					
Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred)
1	0.534784	0.926214	0.310298	1.09721	0.182969
2	0.711755	0.565294	0.579056	0.76089	0.433408
3	0.829310	0.547929	0.591986	0.76791	0.428176
4	0.866662	0.541112	0.597063	0.78678	0.414128
5	0.923443	0.536667	0.600373	0.99935	0.255841
6	0.936023	0.275068	0.795172	0.96121	0.284240
7	0.973807	0.268932	0.799740	0.62030	0.538092
8	0.986436	0.214401	0.840347	0.52423	0.609633
9	0.991300	0.198494	0.852192	0.57038	0.575270
10	0.992996	0.092816	0.930885	0.47857	0.643631

According to Table 4.30, the growth of *E. coli* bacteria was slightly affected by the storage time. There was no significant effect of dynamic conditions against isothermal condition. Coliform bacteria count was not affected during storage.

Table 4.30 : *E. coli* and coliform bacteria counts of fresh meat during storage at isotermal and dynamic conditions of 4°C storage temperature.

E. coli			
Days	**0°C Isothermal Condition**	**0°C / t_{open}= 300 sn, n_{cycle}=20**	**0°C / t_{open}= 600 sn, n_{cycle}=40**
0	3.11±0.05	3.11±0.05	3.11±0.05
1	2.71±0.33	2.94±0.13	2.85±0.20
3	3.10±0.28	2.93±0.04	3.05±0.04
5	4.64±0.08	4.46±0.02	4.67±0.04
7	3.79±0.16	4.00±0.00	4.14±0.10
coliform			
Days	**0°C Isothermal Condition**	**0°C / t_{open}= 300 sn, n_{cycle}=20**	**0°C / t_{open}= 600 sn, n_{cycle}=40**
0	2.07±0.10	2.07±0.10	2.07±0.10
1	0.98±0.03	0.98±0.03	1.03±0.05
3	0.98±0.03	1.13±0.25	1.13±0.21
5	1.30±0.43	1.80±0.14	1.58±0.08
7	1.59±0.83	1.80±0.45	1.89±0.17

4.2.8 Effects of isothermal and non-isothermal cold storage conditions on color parameters of meat

The color of fresh meat is the most important quality attribute affecting the consumers' preferences to purchase it. Discoloration affects the consumer negatively (Mancini & Hunt, 2005). Minolta color measurement results of meats stored at 4°C storage temperature is given in Table 4.31. Lightness (L*) did not alter during storage, even under temperature fluctuation conditions (p>0.05). Mean L* value was around 39 similar to the results that were obtained by Lanza et al. (2009) and Lorenzo and Gomez (2012). However, Lorenzo and Gomez (2012) found that L value increased through the storage period. On the contrary, Bingol and Ergun (2011) reported a decrease in L value. The redness (a*) value decreased significantly for all conditions during storage (p<0.05), especially after day 3. Decreasing in redness (a*) value indicates the conversion of myoglobin to metmyoglobin (Lorenzo and Gomez, 2012). Cut-off value of a* value is generally accepted as ≤14. Initial value was around 23 and generally higher than the other researches reported (Lanza et al, 2009; Franco et al, 2011). Slight differences were observed between isothermal and non-isothermal conditions. On the

last days of the storage, redness value was slightly lower at non-isothermal conditions than isothermal conditions. Hovewer, there was no statistically significant difference between two non-isothermal conditions. Redness threshold limit was exceeded after day 5 for all conditions.

Table 4.31 : The color parameters of beef for isothermal and non-isothermal conditions at 4°C.

Color Parameters	Days	4°C Isothermal Condition	4°C / t_{open}= 300 sn, n_{cycle}=20	4°C / t_{open}= 600 sn, n_{cycle}=40
L*	0	39.21_{a}^{a}	39.29_{a}^{a}	39.18_{a}^{a}
	1	37.29_{a}^{a}	37.70_{a}^{a}	38.74_{a}^{a}
	3	38.24_{a}^{a}	39.21_{a}^{a}	38.44_{a}^{a}
	5	39.52_{a}^{a}	39.58_{a}^{a}	38.55_{a}^{a}
	7	40.85_{a}^{a}	38.87_{a}^{a}	38.94_{a}^{a}
a*	0	23.93_{a}^{a}	23.46_{a}^{a}	23.23_{a}^{a}
	1	22.21_{a}^{a}	20.88_{a}^{b}	20.91_{a}^{b}
	3	18.76_{ab}^{b}	19.55_{ab}^{a}	18.64_{ab}^{b}
	5	17.02_{b}^{b}	17.88_{b}^{a}	17.54_{b}^{ab}
	7	13.64_{b}^{a}	13.19_{b}^{a}	12.61_{b}^{b}
b*	0	7.38_{c}^{b}	7.91_{bc}^{a}	7.44_{b}^{ab}
	1	9.70_{a}^{a}	9.56_{a}^{a}	8.94_{a}^{a}
	3	8.79_{b}^{a}	8.84_{ab}^{a}	8.69_{a}^{a}
	5	7.29_{c}^{a}	7.50_{c}^{a}	7.11_{b}^{a}
	7	8.52_{b}^{a}	8.64_{b}^{a}	8.50_{a}^{a}
Hue Angle (°)	0	17.14_{c}^{b}	18.63_{c}^{a}	17.75_{d}^{ab}
	1	23.60_{bc}^{b}	24.61_{b}^{a}	23.15_{bc}^{b}
	3	25.11_{b}^{a}	24.33_{b}^{a}	25.00_{b}^{a}
	5	23.18_{bc}^{a}	22.76_{bc}^{ab}	22.07_{c}^{b}
	7	32.00_{a}^{b}	33.22_{a}^{ab}	33.97_{a}^{a}
Saturation Index	0	25.04_{a}^{a}	24.76_{a}^{a}	24.39_{a}^{a}
	1	24.24_{a}^{a}	22.96_{b}^{b}	22.74_{b}^{b}
	3	20.72_{b}^{b}	21.45_{c}^{a}	20.56_{c}^{b}
	5	18.51_{c}^{b}	19.39_{d}^{a}	18.93_{d}^{ab}
	7	16.09_{d}^{a}	15.77_{e}^{ab}	15.21_{e}^{b}

- *The different letters with subscript in the same column means the difference is significant between days for a condition ($p<0.05$).*
- *The different letters with superscript in the same line means the difference is significant between conditions for a day ($p<0.05$).*

The initial yellowness (b*) value was around 7.5 in this study. The yellowness (b*) value increased over storage but there was not a characteristic increasing trend. These results were in consistent with the study of Lorenzo and Gomez (2012). However, some researchers found decrease in b* value during storage (Bingol and Ergun, 2011;

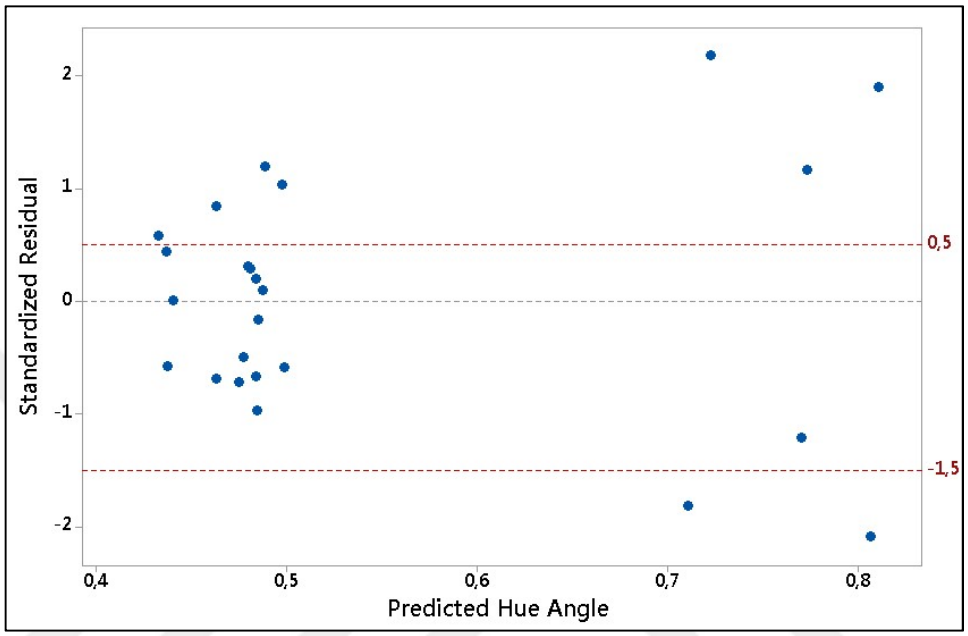
Esmer et al, 2011). No significant differences were measured between all conditions in this study. Hue angle [arctangent (b*/a*)] is also a very useful color parameter to indicate the changes in color over time which also shows the percieved color by human eye. Higher hue values indicate redder product (Howe et al, 1982; Korteil et al, 2015). In this study, hue angle value increased over time for all conditions significantly (p<0.05), especially at the last days of the storage. Hue angle for non-isothermal conditions compared to isothermal conditions was significantly different at the last days of the storage time. Hue angle cut-off value may be assumed as around 26°. This cut-off value was exceeded only at the last day of storage for all conditions. Saturation index (or Chroma) explains the intensity of a color on the products. Higher the saturation index, higher the saturation of the principle hue of meat (AMSA, 2012). It represents to the dominance of hue in the color. In this study, saturation decreased over time for all conditions. On the last days of storage, saturation index value was slightly lower for non-isothermal conditions than isothermal conditions. There was no difference between two non-isothermal conditions in the other color parameters. According to one-leave out validation method for hue angle values of meat stored at 4°C storage temperature, variance in model can be explained with 86% percentage for 5-component model when it was assessed together with lowest error and PRESS value between all components (Table 4.32).

Table 4.32 : Model selection and validation parameters for hue angle at 4°C.

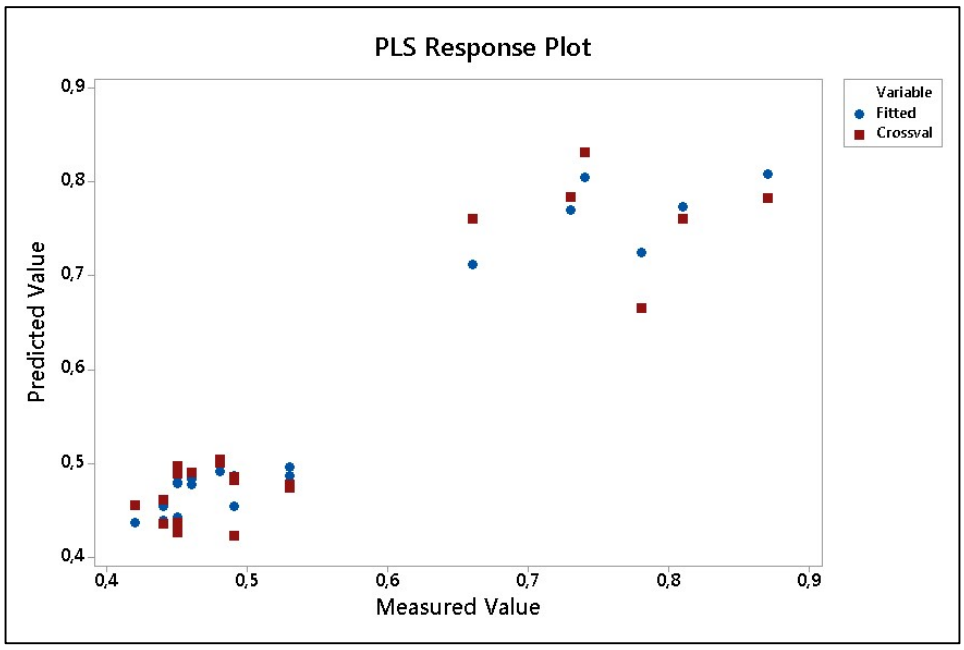
Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred.)
1	0.521818	0.0651887	0.847974	0.080351	0.812615
2	0.7704	0.0357715	0.916578	0.049289	0.885053
3	0.833673	0.0317579	0.925938	0.048344	0.887257
4	0.921427	0.0272719	0.936399	0.058783	0.862912
5	0.94259	0.0248913	0.941951	0.059712	0.860747
6	0.0236381	0.944874	0.06344	0.852052	
7	0.0204599	0.952286	0.068661	0.839876	
8	0.0192798	0.955038	0.071607	0.833007	
9	0.0183479	0.957211	0.090972	0.787845	
10	0.0120622	0.97187	0.105047	0.755021	

According to 5-components PLS-R model, standardized residual values versus predicted hue angle values are given in Figure 4.24a. Predicted errors were presented mostly between 0.5 and 1.5 log with 62.5% for predicted hue angle values of 4°C conditions. Since the accepted percentage was above 70%, this ratio had slightly lower

prediction quality to predict hue angle using GC-MS data. It is seen that the predicted hue angle values distributed equally under and over-estimates zone with homogenous distribution around "0". Lower the hue angle, better the prediction quality of hue angles by GC-MS predictors. As seen in the PLS response plot of hue angle, linearity was better in high hue angle values (Figure 4.24b).



(a)



(b)

Figure 4.24 : a) Standardized residuals vs. predicted values, b) PLS plot of predicted value vs. measured value of the hue angle as estimated from the volatile organic compounds by PLS-R model for 4°C.

Minolta color parameters of meats over time stored at 0°C storage temperature are given in Table 4.33. Lightness (L*) did not change during storage for all storage conditions (p>0.05) similar to the result of 4°C storage temperature as expected. Mean of initial L* value was around 43.5 which was higher than the L* value of meat stored at 4°C temperature. The redness (a*) value decreased significantly for all conditions during storage (p<0.05), especially after day 5. However, the decreasing rate was slower than the a* value of meat at 4°C temperature. Main cause of this was the slowing down of microbial growth and oxidation reactions at relatively lower temperatures. Decreasing in redness (a*) value indicates the conversion of myoglobin to metmyoglobin (Lorenzo and Gomez, 2012). It is indicated that there is a strong correlation between surface discoloration and total bacteria on the meat (Stringer et al, 1969). Robach and Costilow (1961) reported that most of the aerobic bacteria, especially *Pseudomonas aeruginosa*, *Ps. Fluorescens* and *Ps. Geniculata* found on meat deplete the O_2 concentration and this cause discolouration on surface.

Cut-off value of a* value (≤14) was not reached during all storage period for all conditions. a* value was more stable at 0°C. Initial value was around 21. Slight differences were observed between isothermal and non-isothermal conditions. On the last days of storage period, redness value was lower at non-isothermal condition 2 with more frequent open-close cycles than the other conditions. Redness threshold limit was not exceeded and all meats were accebtable at the end of storage time. The initial yellowness (b*) value was around 9 and it did not change over storage significantly (p>0.05). Non-isothermal conditions did not differentiate the b* value of meat than isothermal conditions similar to results that were obtained in 4°C temperature. Hue angle value increased after initial loading at day 0 significanlty however it did not change over the rest of the storage time for all conditions. The difference of non-isothermal conditions compared to isothermal conditions was seen at the last day of storage significantly. Hue angle cut off value is accepted as around 26°. This cut-off value was exceeded very early after day 0 due to high yellowness value. As expected, saturation decreased more slowly over time for all conditions compared to 4°C storage temperature. Non-isothermal conditions affected saturation index significanlty (p<0.05) after day 3 compared to isothermal conditions. Differences between two non-isothermal conditions were observed at the last day of storage period.

Table 4.33 : The color parameters of beef for isothermal and non-isothermal conditions at 0°C

Color Parameters	Days	0°C Isothermal Condition	0°C / t_{open}= 300 sn, n_{cycle}=20	0°C / t_{open}= 600 sn, n_{cycle}=40
L*	0	43.74_{a}^{a}	43.28_{a}^{a}	43.73_{a}^{a}
	1	44.58_{a}^{a}	44.03_{a}^{a}	43.44_{a}^{a}
	3	43.19_{a}^{a}	42.99_{a}^{a}	44.17_{a}^{a}
	5	44.52_{a}^{a}	46.37_{ab}^{a}	43.76_{a}^{b}
	7	43.00_{a}^{a}	43.26_{a}^{a}	43.71_{a}^{a}
a*	0	21.10_{a}^{a}	20.99_{a}^{a}	21.52_{a}^{a}
	1	21.97_{a}^{a}	20.30_{a}^{ab}	20.85_{ab}^{ab}
	3	18.84_{b}^{b}	19.71_{ab}^{a}	19.79_{b}^{a}
	5	20.34_{ab}^{a}	17.18_{b}^{b}	17.45_{c}^{b}
	7	18.55_{b}^{a}	18.49_{b}^{a}	16.77_{c}^{b}
b*	0	8.58_{d}^{c}	8.92_{b}^{b}	9.57_{bc}^{a}
	1	11.12_{a}^{a}	9.73_{a}^{ab}	9.93_{ab}^{a}
	3	9.97_{c}^{ab}	9.51_{ab}^{b}	10.21_{a}^{a}
	5	10.20_{b}^{a}	8.42_{b}^{c}	9.01_{c}^{b}
	7	9.96_{c}^{a}	9.99_{a}^{a}	8.85_{c}^{b}
Hue Angle °	0	22.13_{c}^{c}	23.02_{d}^{b}	23.97_{c}^{a}
	1	26.86_{b}^{a}	25.60_{c}^{b}	25.47_{b}^{b}
	3	27.88_{a}^{a}	25.75_{bc}^{b}	27.28_{ab}^{a}
	5	26.62_{b}^{b}	26.11_{b}^{b}	27.31_{ab}^{a}
	7	28.24_{a}^{a}	28.37_{a}^{a}	27.82_{a}^{a}
Saturation Index	0	23.59_{a}^{a}	23.46_{a}^{a}	23.59_{a}^{a}
	1	24.62_{a}^{a}	22.51_{b}^{b}	23.09_{a}^{b}
	3	21.31_{bc}^{b}	21.91_{bc}^{ab}	22.27_{ab}^{a}
	5	22.76_{b}^{a}	19.14_{d}^{b}	19.64_{bc}^{b}
	7	21.06_{c}^{a}	21.02_{c}^{a}	18.96_{c}^{b}

- *The different letters with subscript in the same column means the difference is significant between days for a condition ($p<0.05$).*
- *The different letters with superscript in the same line means the difference is significant between conditions for a day ($p<0.05$).*

Prediction R-Sq value and selected component value using GC-MS data was very low than the prediction quality of the model for 4°C storage conditions (Table 4.34). According to one-leave out validation method for hue angle values of meat stored at 0°C storage temperature, variance in model can be explained with 4.4% percentage for only 1-component model. The main reason of this could be the change in color parameters very slightly despite the changes in volatile compounds over time. Due to very low expression of model for Hue angle at 0ºC conditions, no PLS response plot was drawn.

Table 4.34 : Model selection and validation parameters for hue angle at 0°C.

Components	X Variance	Error	R-Sq	PRESS	R-Sq (pred)
1	0.540728	0.0146351	0.2720070	0.0192135	0.0442672
2		0.0115144	0.4272400	0.0301202	0.0000000
3		0.0105984	0.4728060	0.0271345	0.0000000
4		0.0095507	0.5249200	0.0266087	0.0000000
5		0.0089483	0.5548860	0.0319039	0.0000000
6		0.0085740	0.5735020	0.0334820	0.0000000
7		0.0081595	0.5941240	0.0356541	0.0000000
8		0.0074626	0.6287890	0.0384354	0.0000000
9		0.0068135	0.6610780	0.0421581	0.0000000
10		0.0063617	0.6835520	0.0433638	0.0000000

4.3 Pearson Correlation Coefficients of All Quality Parameters versus GC-MS Data for 4°C

The Pearson correlation analysis assess the linear relationship of two continuous variables. The accumulation of some detected volatile compounds is considered to be the results of some microbiological and biochemical changes. The linear relationhip between measured quality parameters (TBA value, pH value, hue angle, microbiological counts and overall acceptability) and volatile compounds data were investigated with Pearson correlation in 95% confidence. These correlation coefficients are given in Table 4.35 for 4°C storage temperature.

For microbiological counts, *Pseudomonas spp.* was associated positively with 2-butandione, 3-hydroxy-2-butanone, 3-methyl-1-butanal and 1-pentanol compounds with high correlation coefficients between 0.719-0.866. *Pseudomonas spp.* are the main specific spoilage organism (SSO) in meat (Dainty et al, 1983) and it is known that *Pseudomonas spp* has capability to produce some alcohols and aldehydes (Nychas et al, 2014). These correlation coefficients were in consistent with the indications of Insausti et al. (2002) and Casaburi et al. (2015) that presented that 3-methylbutanal and 1-pentanol were formed by *Pseudomonas* spp. 2-butandione and 3-hydroxy-2-butanone also showed high correlation with *Pseudomonas spp*. in this study. It is generally known that, 2-butandione and 3-hydroxy-2-butanone are formed by *Brochotrix termosphacta* (Whitfield, 2003). Similarly, total viable count also

correlated with the same volatile compounds as in *Pseudomonas spp*.. Above-mentioned volatiles can be recommended to be used for the prediction of microbial spoilage and shelf life of meat. Ketones can be produced by lactic acid bacteria beside *Brochotrix termosphacta* (Ardö, 2006).

Table 4.35 : The Pearson correlation coefficients (r) between measured quality parameters and detected volatiles for 4°C temperature.

VOCs	*Pseudomonas* spp.	Total Viable Count	Lactic Acid Bacteri	pH	TBA	Overall Acceptability	Hue angle
2-propanone	-0.025	-0.065	-0.366	-0.637	-0.344	0.129	**-0.795**
Dimetyl sulphide	**-0.865**	**-0.869**	**-0.900**	**-0.825**	**-0.826**	**0.878**	-0.687
Etanethiol	-0.158	-0.251	-0.452	-0.650	-0.449	0.319	**-0.747**
2-butanone	**-0.854**	**-0.890**	**-0.912**	**-0.884**	**-0.856**	**0.900**	**-0.772**
2-butanamine	-0.152	-0.171	-0.200	-0.055	-0.349	0.187	0.015
Dimetilamine	-0.096	-0.156	-0.460	-0.673	-0.404	0.210	**-0.762**
2,3-butandione	**0.768**	**0.798**	0.656	0.404	0.624	**-0.761**	0.064
Ethyl acetate	0.536	0.588	**0.814**	**0.921**	**0.860**	-0.650	**0.918**
3-hydroxy-2-butanone	**0.866**	**0.913**	**0.900**	**0.738**	**0.892**	**-0.911**	0.462
2-pentanone	0.432	0.526	0.536	0.333	0.556	-0.517	0.017
Hexanal	0.105	0.035	-0.241	-0.488	-0.226	0.028	-0.670
3-methyl-1-butanal	**0.767**	**0.724**	0.574	0.377	0.658	-0.715	0.242
Pentanal	0.231	0.114	-0.237	-0.423	-0.200	-0.064	-0.451
1-pentanol	**0.719**	**0.790**	**0.952**	**0.945**	**0.979**	**-0.830**	**0.782**
1-octen-3-ol	0.384	0.319	0.012	-0.279	-0.012	-0.245	-0.516
2-propanamine	0.043	-0.011	-0.168	-0.202	-0.141	0.007	-0.056
2-ethyl-1-hexanol	0.317	0.334	0.172	-0.072	0.184	-0.295	-0.387
3-metil-1-butanol	0.448	0.486	0.490	0.517	0.317	-0.475	0.412
1-hexanol	0.312	0.354	0.241	-0.004	0.236	-0.320	-0.357
2,3-octandione	0.308	0.367	0.260	-0.018	0.222	-0.313	-0.338
2-heptanone	0.588	0.657	**0.713**	0.601	**0.736**	-0.671	0.289
2-propanamine	0.446	0.497	0.649	**0.786**	0.533	-0.522	**0.772**
Methyl acetate	0.540	0.598	**0.825**	**0.960**	**0.819**	-0.653	**0.958**
Dimetyl disulphide	0.448	0.502	0.647	**0.782**	0.503	-0.524	**0.767**
Ethyl butanoate	0.524	0.579	**0.812**	**0.935**	**0.849**	-0.639	**0.943**
Ethyl 3-methyl butanoate	0.531	0.587	**0.819**	**0.943**	**0.838**	-0.647	**0.947**
3-octanone	0.535	0.593	**0.810**	**0.950**	**0.781**	-0.642	**0.955**
Dimetyl trisulphide	0.535	0.591	**0.822**	**0.950**	**0.836**	-0.648	**0.961**
Ethyl hexanoate	0.537	0.594	**0.826**	**0.955**	**0.834**	-0.653	**0.956**
1-undecene	0.535	0.595	**0.802**	**0.943**	**0.736**	-0.641	**0.933**
Methyl hexanoate	0.500	0.552	**0.783**	**0.895**	**0.848**	-0.614	**0.911**
Isoamyl acetate	0.521	0.575	**0.807**	**0.925**	**0.837**	-0.638	**0.926**
Isobutyl acetate	0.536	0.593	**0.822**	**0.948**	**0.822**	-0.652	**0.947**

Consistence with this, in this study, high correlation was obtained between lactic acid bacteria and 3-hydroxy-2-butanone. An interesting issue was the very high correlation of lactic acid bacteria with esters. Most of the esters were formed in the end of the storage period and most of the conducted researches in literature did not indicate direct relationship of lactic acid bacteria with ester formation. Dimethyl sulphide and 2-butanone correlated negatively with most of the parameters and it was considered that dimethyl sulphide can be related with the fresh meat flavor.

Hexanal did not show any relationship with TBA value unexpectedly, despite the expressions of Perez et al. (2008). According to sensory evaluations, there was a negative correlation between overall acceptability and 2-butandione, 3-hydroxy-2-butanone and 1-pentanol volatiles with high coefficients of -0.761, -0.911and -0.830, respectively. Hue angle together with TBA value showed very high correlations around 0.9 with ester compounds that were formed at the end of the storage period. However, most of these esters are known as the metabolites of microbial esterase activity of microorganisms (Talon et al, 1998) and correlation coefficients of ester compounds with microbial counts were low. Conclusively, 3-hydroxy-2-butanone and 1-pentanol gave more consistent and stable results with most of the quality parameters and can be accepted as the best volatile compounds to be used for spoilage indicator for around 4°C storage conditions.

4.4 Pearson Correlation Coefficients of All Quality Parameters versus GC-MS Data for 0°C

The linear relationhip between measured quality parameters (TBA value, pH value, hue angle, microbiological counts and overall acceptability) and volatile compounds were calculated for 0°C storage temperature conditions in 95% confidance. The correlation coefficients are given in Table 4.36 for 0°C storage temperature. The correlation coefficients above 0.7 (accepted threshold value) are shown in bold.

Table 4.36 : The Pearson correlation coefficients (r) between measured quality parameters and detected volatiles for 0°C temperature.

VOCs	*Pseudomonas* spp.	Total viable counts	Lactic acid bacteria	pH	TBA	Overall acceptability	Hue angle
2-propanone	0.696	0.639	**0.726**	0.569	**0.806**	**-0.707**	0.099
Dimetyl sulphide	**-0.700**	**-0.795**	-0.402	-0.432	-0.626	0.614	-0.364
Etanethiol	-0.206	-0.414	0.082	-0.024	-0.014	0.112	-0.131
2-butanone	**-0.820**	**-0.819**	-0.523	-0.492	**-0.711**	**0.762**	-0.465
2-butanamine	-0.194	-0.402	0.028	-0.021	-0.078	0.084	-0.075
Dimetilamine	0.671	**0.743**	0.224	0.598	0.619	-0.687	0.391
2.3-butandione	**0.759**	**0.819**	0.292	0.624	0.643	**-0.745**	0.469
3-hydroxy-2-butanone	**0.778**	**0.843**	0.333	0.568	0.646	**-0.760**	0.491
2-pentanone	**0.748**	0.688	0.669	0.579	**0.851**	**-0.777**	0.186
Hexanal	-0.303	-0.479	0.065	0	-0.098	0.155	-0.232
3-methyl-1-butanal	**0.797**	**0.780**	0.626	0.43	**0.765**	**-0.742**	0.279
Pentanal	0.441	0.510	0.207	0.156	0.393	-0.459	0.386
1-pentanol	**0.910**	**0.868**	**0.721**	**0.805**	**0.939**	**-0.952**	0.432
1-octen-3-ol	**0.818**	**0.770**	0.672	**0.825**	**0.903**	**-0.916**	0.333
2 ethyl-1-hexanol	**0.775**	**0.844**	0.320	0.678	**0.706**	**-0.773**	0.284
3-metil-1-butanol	**0.748**	**0.778**	0.435	0.413	0.535	-0.681	0.503
1-hexanol	**0.864**	**0.914**	0.459	0.624	**0.738**	**-0.832**	0.497
2.3-octandione	0.061	0.028	0.358	-0.214	-0.037	0.002	0.001
2-heptanone	**0.739**	**0.787**	0.361	0.435	0.525	-0.680	0.502
3-octanone	0.673	**0.743**	0.214	0.610	0.619	-0.683	0.379
Carbon disulphide	**-0.930**	**-0.858**	-0.685	-0.584	**-0.834**	**0.888**	-0.537
Heptanal	**-0.704**	-0.559	-0.486	-0.451	**-0.719**	**0.728**	-0.449
Nonanal	-0.176	-0.164	-0.185	-0.376	-0.301	0.331	-0.123
1-heptanol	0.676	**0.745**	0.218	0.629	0.624	-0.691	0.398
1-undecene	0.553	0.628	0.218	0.323	0.498	-0.563	0.365
Thiourea	0.630	**0.705**	0.234	0.478	0.577	-0.649	0.409

For 0°C conditions, total viable count and *Pseudomonas spp* were correlated with the similar volatiles. 2-Butandione and 3-hydroxy-2-butanone, 3-methyl-1-butanal and 1-pentanol compounds similarly showed high correlations with microbial counts around 0.7-0.8 Pearson coefficients as observed with 4°C condition. 2-Butandione and 3-hydroxy-2-butanone also showed high correlation with *Pseudomonas spp*.. Alcohol compounds were more dominant than the other components on linear consistency with increase in *Pseudomonas spp.* and total viable count numbers compared to the results obtained at 4°C condition. 3-Methyl-1-butanol, 1-octen-3-ol, 2-ethyl-1-hexanol and 1-hexanol that were identified in this study are found in deteriorated meat and are mainly caused by *Pseudomonas spp.* (Insausti et al, 2002). Different than 4°C temperature

conditions, lactic acid bacteria only correlated positively with 1-pentanol due to the slower growth of lactic acid bacteria on colder conditions, which were lower than 4°C temperature. Dimethyl sulphide, 2-butanone and heptanal showed negative correlations with most of the quality parameters, attributed to the fresh meat flavor in consistency with overall acceptability. There was a strong correlation of overall acceptability with 2-butandione, 3-hydroxy-2-butanone, 2-heptanone, 1-pentanol, 3-methyl-1-butanol, 1-octen-3-ol, 2-ethyl-1-hexanol and 1-hexanol volatiles with high coefficients around 0.70-0.90. It indicates that overall acceptability changes mainly depending on the microbial changes. Since no changes were observed in hue angle parameter, no linear relationship was observed with any of the volatile compounds. As a result, it can be concluded that the relationship between some microbiological and biochemical changes with different types of volatiles highly depend on temperature. Based on the data, 1-pentanol, 3-methyl-1-butanol, 1-octen-3-ol, 2-ethyl-1-hexanol and 1-hexanol were more correlated volatiles with most of the quality parameters and they can be evaluated as spoilage indicators for around 0°C storage conditions.

5. CONCLUSIONS

Evaluation of meat volatiles is an important subject in order to design spoilage sensors to inform consumers if meat is spoiled or not. Since the reliable measurement of volatiles in headspace is important for designing a sensor, preconcentration techniques for meat matrix should be investigated before analysis.

In this thesis, the volatile organic compounds of raw beef meat preserved under isothermal and non-isothermal cold storage conditions in domestic refrigerators at two different storage temperatures (0°C and 4°C) were investigated to simulate the home habits on cold storage. Together with this, establishing the relationships with other quality parameters (lipid oxidation, surface color, pH, sensory evaluation and microbiological assesments) were aimed. GC-MS data that was obtained for different conditions were classified with Principal Component Analysis (PCA) and volatile organic compound data were subjected to factorial discriminant analysis for the classification quality of fresh, semi-fresh and spoiled meat. For quantitative analysis, partial least squares regression (PLS-R) model was used to predict the lipid oxidation, hue angle, pH value, and microbiological counts by using GC-MS data.

In the first part of this study, different product loading amount for a control volume loading factor) effect was studied for two volatile sampling techniques: 1) Solid phase microextraction and 2) Gas flushing on Tenax tube with Thermal Desorption (TD) to investigate the interactions and relationship between sample amount, type of analyte and extraction method. It was focused on the effect of loading factor (sample amount: total volume ratio) in the container where the meat samples were stored by comparing the two preconcentration techniques such as Headspace (HS)-Solid Phase Microextraction (SPME) and gas flushing on Tenax tube. GC-MS and thermal desorber (TD)-GC-MS systems were used for the further analysis of volatile organic compounds. Loading factor can be defined as the ratio of the sample amount of meat in the chamber to the total volume of chamber. The interaction of volatiles in headspace with adsorbent fiber is dependent on the amount of product in a specified volume due to the changes in the partial pressure and volatility of analytes. Additionally, differentiation quality of different adsorbent is possible due to the variety

in chemical structure of volatiles. Three different loading factors were studied such as 0.025 kg/L, 0.05 kg/L and 0.10 kg/L. Both of two preconcentration techniques identified similar volatile organic compounds. The volatile organic compounds identified in the early stages of meat spoilage include alcohols, aldehydes, ketones, esters and amine compounds. Loading factor affected the extraction efficiency on fiber and Tenax significantly. The volatile profiles in different loading factor levels did not change significantly, but the intensity of volatiles was affected with the extraction technique and loading factor. Optimum loading factor for the best efficient extraction depended on the type of the volatile compound. The total peak area of volatiles with Tenax (~390x10^6 AU at 0.05 kg/L) were higher than SPME sampling (~290x10^6 AU at 0.05 kg/L) in each loading factor. Although optimum loading factor depended on the type of volatile, 0.05 kg/L loading factor resulted the most efficient extraction by the two preconcentration techniques for most of the volatiles generally. It was noticed that the competitiveness was more existing in SPME due to the stationary conditions. Related with this, humid headspace may be a problem for SPME especially at high sample amounts. Higher recovery of analytes was obtained by thermal desorber-Tenax sampling for all studied loading factor due to the forced flashing of all headspace. The application of Tenax sampling method for volatile analyses of meat can be extensively studied in future studies. The average of recovery percentages with gas flushing on Tenax were between 70%-110% accepted as suitable for further analysis. It was continued with gas flushing on Tenax tube sampling technique coupled with TD-GC-MS identification system in the second part of the study by selecting loading factor as 0.05 kg/L.

In the second part of the study, research was conducted to investigate the spoilage-causing odor compounds of raw beef stored under isothermal and non-isothermal conditions (15 min. and 30 min. intervals between cooler door open-close cycles) at two temperatures (0°C and 4°C) together with sensory evaluations in a domestic refrigerator. Fresh beef samples stored under aerobic conditions were monitored with both analytical methods, and several volatile compounds were found to be possible chemical indicators. For 0˚C conditions, shelf life (overall acceptability < 4.0) of samples was longer than all conditions of 4˚C. The overall acceptability of beef samples severely decreased at the fluctuating conditions compared with isothermal temperature condition of 4˚C and 0˚C after 24 h and 72 h, respectively. Total number

of detected volatiles was less at 0°C conditions for 7 days storage period due to the retarded spoilage and effect of increasing open-close cycle was more differentiable. 2,3-butandione, 3-hydroxy-2-butanone and 1-pentanol were common volatiles that increased significantly at spoilage period and were affected under dynamic conditions. According to principal component analysis (PCA) classification method, 2,3-butandione, 3-hydroxy-2-butanone, 1-pentanol, 2-pentanone, 3-methyl-1-butanal were classified as the common indicators of spoilage both for 0°C and 4°C conditions in PCA analysis. Discriminant analysis were in consistency with PCA results for classification performance of GC-MS data on shelf life prediction as fresh, semi-fresh and spoiled state. Estimation of all quality parameters by using GC-MS volatile compounds data were modelled with partial least square regression (PLS-R) prediction models. *Pseudomonas spp*. counts were predicted well with GC-MS data by using PLS-R model compared to other analytic parameters. Based on the PLS-R prediction model, predicted errors (log) distributed homogenously between 0.5 and 1.5 log with %70.83 of all data for 0°C and 4°C storage temperatures for *Pseudomonas spp.* counts. According to pearson correlation coefficients, hexanal did not show any relationship with Thiobarbutyric acid (TBA) value unexpectedly both for 0°C and 4°C. Hue angle (°) together with TBA value showed very high correlations around 0.9 with ester compounds that formed at the end of the storage period. There was a strong negative correlation between overall acceptability scores and 2-butandione, 3-hydroxy-2-butanone and 1-pentanol volatiles with high coefficients of -0.761, -0.911 and -0.830, respectively for 4°C storage condition. For 0°C temperature, there was a strong correlation of overall acceptability with 2-butandione, 3-hydroxy-2-butanone, 2-heptanone, 1-pentanol, 3-methyl-1-butanol, 1-octen-3-ol, 2-ethyl-1-hexanol and 1-hexanol volatiles with high coefficients around 0.7-0.9. It shows that overall acceptability changes mainly are affected by microbial changes. Since no changes were observed in hue angle parameter, no linear relationship was observed with any of volatile compounds.

In raw meat, various volatile metabolites are formed due to the spoilage reactions. However, assessment of meat freshness is limited for consumers to use it quickly to ensure the freshness and avoid wastage. Beside this, decrease in meat quality is inevitable due to causing factors such as temperature setting, door open-close cycle of refrigerator which result with huge fluctuations in temperature. Defining volatiles are

essential for developing odor sensors to give advice about meat freshness for consumers. Therefore, this study was conducted to investigate the spoilage-causing odor compounds of raw beef at constant and fluctuating cold storage conditions in domestic refrigerators.

For future work, the scope of the related study can be expanded to different types of meat and the effect of different internal characteristic such as pH levels, fat content, origin of meat and the effects of different external conditions such as different temperature and packaging conditions can be investigated. Similar analogy with the model of this study can be established for different instrumentations such as spectroscopic, colorimetric and sensor devices to discover different spoilage indicator tools.

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PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

- **Met, A.** and Şahin Yeşilçubuk, N. (2017). Comparison of Two Volatile Sampling Techniques Based on Different Loading Factors in Determination of Volatile Organic Compounds Released from Spoiled Raw Beef. *Food Analytical Methods*. 10, 2311-2324. Springer. DOI: 10.2017/s12161-017-0805-6.
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