ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

Naidandorj DAMDIN

THE INFLUENCE OF RAW MATERIALS, COMMERCIAL YEAST AND NITROGEN ADDITION ON CIDER QUALITY

DEPARTMENT OF FOOD ENGINEERING

ADANA, 2019

ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

THE INFLUENCE OF RAW MATERIALS, COMMERCIAL YEAST AND NITROGEN ADDITION ON CIDER QUALITY

Naidandorj DAMDIN

PhD THESIS

DEPARTMENT OF FOOD ENGINEERING

We certify that the thesis titled above was reviewed and approved for the award of degree of the Doctor of Philosophy Science by the board of jury on 09/07/2019.

……	…….....	……...
Prof. Dr. Hüseyin ERTEN	Prof. Dr. İbrahim EKİZ	Prof. Dr. Turgut CABAROĞLU
SUPERVISOR	MEMBER	MEMBER
……...	……	
Prof. Dr. Serkan SELLİ	Prof. Dr. Haşim KELEBEK	
MEMBER	MEMBER	

This PhD Thesis is written at the Food Engineering Department of Institute of Natural and Applied Sciences of Çukurova University.
Registration Number:

Prof. Dr. Mustafa GÖK
Director
Institute of Natural and Applied Sciences

A part of this Study was financially supported by Scientific Çukurova University Academic Research Projects Units.
Project No: FDK-2017-874

ABSTRACT

PhD THESIS

THE INFLUENCE OF RAW MATERIALS, COMMERCIAL YEAST AND NITROGEN ADDITION ON CIDER QUALITY

Naidandorj DAMDIN

ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF FOOD ENGINEERING

Supervisor : Prof. Dr. Hüseyin ERTEN
Year: 2019, Pages: 135
Jury : Prof. Dr. Hüseyin ERTEN
: Prof. Dr. İbrahim EKİZ
: Prof. Dr. Turgut CABAROĞLU
: Prof. Dr. Serkan SELLİ
: Prof. Dr. Haşim KELEBEK

In this study, the effects of the nitrogen addition and commercial dry yeast on cider fermentation obtained from fresh and concentrated apple juice were investigated. Fresh apple juice was produced from *Golden Delicious* cultivar. Experiments were performed by spontaneously and inoculated with commercial dry yeast. Fermentations were performed at 18°C. Fermentations were daily monitored by a drop in specific gravity and pH. Also, the enumeration of yeast population was done. According to the results obtained, nitrogen addition led to increase for the utilisation of sugar and fermentation rates. Specific gravity dropped faster in nitrogen added musts compared to others. The maximum numbers of total yeast and non-*Saccharomyces* yeast in nitrogen added trials were counted as 9.62 log CFU/mL and 6.28 log CFU/mL on day 2, respectively. The pH values were slightly decreased and ranged from 3.12-3.27 at the end of the fermentation. Extraction of aroma compounds in ciders was made by using liquid-liquid extraction method. Identification and amount of aroma compounds were made using GC-MS-FID. 55 aroma compounds were determined, including 13 higher alcohols, 13 ethyl esters, 5 acetate esters, 9 volatile acids, 3 six-carbon alcohols, 5 phenolic compounds, 4 lactones and 3 carbonyl compounds. The overall quantity of aroma substances were identified as 85.18 mg/L in C1, 89.14 mg/L in C2, 72.8 mg/L in C3, 74.65 mg/L in C4, 75.26 mg/L in C5, 66.92 mg/L in C6, 62.4 mg/L in C7 and 70.3mg/L in C8 cider ($p<0.05$). According to the results of sensory analysis, C7 cider was ranked the highest followed by C6 and C5. Fruity and floral odours were dominant aroma in the ciders according to sensory analysis. Finally, C7 cider was valued as the best one.

Key Words: Cider, Golden Delicious, nitrogen, yeast, aroma compounds, cider quality

ÖZ

DOKTORA TEZİ

ELMA ŞARABININ KALİTESİ ÜZERİNE HAMMADDE, TİCARİ MAYA VE AZOT İLAVESİNİN ETKİSİ

Naidandorj DAMDIN

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
GIDA MÜHENDİSLİĞİ ANA BİLİM DALI

Danışman : Prof. Dr. Hüseyin ERTEN
Yıl: 2019, Sayfa: 135
Jüri : Prof. Dr. Hüseyin ERTEN
: Prof. Dr. İbrahim EKİZ
: Prof. Dr. Turgut CABAROĞLU
: Prof. Dr. Serkan SELLİ
: Prof. Dr. Haşim KELEBEK

Bu çalışmada, azot ve ticari kuru maya ilavesinin taze ve konsantre elma sularının fermantasyonları üzerine etkisi araştırılmıştır. Taze elma suyu üretiminde *Golden Delicious* çeşidi kullanılmıştır. Denemeler 18°C'deki ortamda spontan olarak ve kuru maya ilave edilerek gerçekleştirilmiştir. Alkol fermantasyonu günlük olarak özgül ağırlık ve pH'daki düşüş ile izlenmiştir. Ayrıca, maya sayımı yapılmıştır. Elde edilen sonuçlara göre azot ilavesi, şeker kullanımını ve fermantasyon hızını arttırmıştır. Azot ilavesi yapılan denemelerde özgül ağırlık azot kullanılmayan denemelere göre daha hızlı düşmüştür. En yüksek toplam maya ve *Saccharomyces* olmayan maya sayısı fermantasyonun ikinci gününde sırasıyla 9.62 log kob/ml ve 6.28 log kob/ml olarak tespit edilmiştir. pH değerlerinde hafif bir düşüş görülmüş ve fermantasyon sonunda pH değerleri 3.12-3.27 arasında bulunmuştur. Elma şaralarında aroma ekstraksiyonu sıvı-sıvı ekstraksiyon metodu kullanılarak yapılmış, aroma miktarları ve tanımlanmaları ise GC-MS-FID cihazı kullanılarak belirlenmiştir. Elma şaraplarında 13 adet yüksek alkol, 13 adet etil ester, 5 adet asetat ester, 9 adet uçucu asit, 3 adet altı karbonlu alkol, 5 adet fenolik bileşik, 4 adet lakton ve 3 adet karbonil bileşik olmak üzere 55 adet aroma bileşeni tanımlanmıştır. Aroma bileşenlerinin toplam miktarı C1 denemesinde 85.18 mg/L, C2'de 89.14 mg/L, C3'de 72.8 mg/L, C4'te 74.65 mg/L, C5'te 75.26 mg/L, C6'da 66.92 mg/L, C7'de 62.4 mg/L ve C8'de ise 70.3 mg/L olarak bulunmuştur ($p<0.05$). Duyusal analiz sonuçlarına göre C7 en beğenilen şarap olurken bunu sırasıyla C6 ve C5 denemeleri izlemiştir. Aroma profil üzerinde meyvemsi olma ve çiçek kokusu özellikleri etkili bulunmuştur. Sonuç olarak C7 denemesinden elde edilen elma şarabı en iyi şarap olarak ön plana çıkmıştır.

Anahtar kelimeler: Elma şarabı, Golden Delicious, azot, maya, aroma bileşenleri, elma şarabı kalitesi

GENİŞLETİLMİŞ ÖZET

Bu çalışmada, azot ilavesi ve ticari kuru maya kullanımının elma şarabı fermantasyonu üzerine etkisi 8 farklı deneme kurularak incelenmiştir. Denemelerde taze ve konsantre elma suları kullanılmıştır. Alkol fermantasyonu sırasında pH ve özgül ağırlıktaki azalma ayrıca maya sayımı ile izlenmiştir. Ayrıca, örneklerin fiziko kimyasal özellikleri ve aroma bileşikleri araştırılmıştır.

Alkol fermantasyonunda kullanılan taze elma suyunun bileşimi şu şekildedir: briks 15.0, pH 3.48, malik asit cinsinden toplam asitlik 7.05 g/L, toplam şeker 155.36 g/L, özgül ağırlık 1.060, esmerleşme indeksi 0.1683, serbest amino asit 108.48 mg/L, renk L* 33.19, a* -1.53, b* 4.97, Hue değeri -72.88, Chroma değeri 5.22. Alkol fermantasyonunda kullanılan konsantre elma suyunun bileşimi ise; briks 14.6, pH 3.50, malik asit cinsinden toplam asitlik 6.45 g/L, toplam şeker 154.1 g/L, özgül ağırlık 1.059, esmerleşme indeksi 0.2395, serbest amino asit 102.92 mg/L, renk L* 32.70, a* -0.59, b* 14.63, Hue değeri -87.69, Chroma değeri 14.64 şeklindedir.

Elde edilen örneklerin kodlandırılmaları şöyledir: C1: DAP ilavesi olmadan taze elma suyunda spontan olarak fermentasyonu, C2: DAP ilavesi olmadan taze elma suyunda ticari kuru maya kullanılarak fermentasyonu, C3: DAP ilavesi ve taze elma suyunda ticari kuru maya kullanılarak fermentasyonu, C4: DAP ilavesi ve taze elma suyunda spontan olaran fermentasyonu, C5: DAP ilavesi olmadan konsantre elma suyunda spontan olaran fermentasyonu, C6: DAP ilavesi olmadan konsantre elma suyunda ticari kuru maya kullanılarak fermentasyonu, C7: DAP ilavesi ve konsantre elma suyunda spontan olaran fermentasyonu ve C8: DAP ilavesi ve konsantre elma suyunda ticari kuru maya kullanılarak fermentasyonu belirtmelitedir.

Elde edilen sonuçlara göre elma şırasının fermantasyon başında özgül ağırlığı 1.065'tir. C3 ve C8 denemelerinde fermantasyon 16 günde tamamlanmıştır. Şeker miktari taze ve konsantre elma şıranın ilk başlangıcında 155.36 g/L ve 154.1 g/L olarak belirlenmiştir. Toplam şekerleri fermentasyonun sonunda 1.59 g/L ile 5.38 g/L arasında değişmiştir. Tüm denemelerde fermantasyonlar 16-25 gün içerisinde tamamlanmıştır. Fermantasyon gelişiminin DAP ilavesine bağlı olduğu belirlenmiştir.

Örneklerin pH değerleri alkol fermantasyonun başlangıcında 3.48 olarak belirlenmiştir. pH değerleri fermantasyon sırasında hafif bir düşüş göstermiş ve fermantasyon sonunda 3.12-3.26 arasında bulunmuştur. En düşük pH değeri C3 kodlu elma şarabında 3.12 olarak bulunurken en yüksek değer C6 kodlu denemede 3.27 olarak belirlenmiştir. Sonuç olarak, özgül ağırlık değerleri 0.9872-0.9884 arasında bulunmuştur.

Mikrobiyolojik analiz sonuçlarına göre toplam *Saccharomyces* olmayan maya sayıları azot ilave edilmeyen ve spontan olarak yürütülen fermantasyonun başlangıcında 3.48-3.71 log kob/mL arasında değişirken en yüksek sayı olarak 6.82-6.90 log kob/mL arasında bulunmuştur. Azot ilaveli spontan fermantasyon ile gerçekleştirilen denemede ise toplam *Saccharomyces* olmayan maya sayıları başlangıçta 4.00-4.12 log kob/mL arasında değişirken, en yüksek olarak 6.83-7.00 log kob/mL değerine ulaşmıştır. Azot ilave edilen ve *S. cerevisiae* kullanılan şıralarda bu mayaların sayıları uzun süren sabit faz boyunca yavaş bir şekilde artmıştır. Ancak, *Saccharomyces* olmayan mayalar fermantasyonun 6 ve 7. günlerinde ölmüştür. Azot ilave edilmeden ticari maya kullanılarak kurulan denemelerde toplam maya sayısı fermantasyon başlangıcında 5.94-6.49 log kob/mL arasında değişirken en yüksek rakama fermantasyonun 2. gününde 7.19-7.61 log kob/mL olarak ulaşmıştır. Toplam *Saccharomyces* olmayan maya sayısı 5.30-5.40 log kob/mL arasında değişirken en yüksek sayıya 6.13-7.0 log kob/mL olarak fermantasyonun 2. günü rastlanmıştır. Azot ve ticari maya ilavesi kullanılan şırada toplam maya sayısı fermantasyon başlangıcında 6.30-6.65 log kob/ml arasında değişirken fermantasyonun 2. gününde en yüksek seviyesine ulaşmış ve 9.54-9.62 log kob/mL olarak tespit edilmiştir. Toplam *Saccharomyces* olmayan maya sayısı fermantasyon başlangıcında 5.12-5.83 log kob/mL olarak belirlenirken en yüksek sayıya yine fermantasyonun 2. günü 6.08-6.28 log kob/mL olarak ulaşılmıştır. Yukarıda belirtilen sonuçlara göre mikrobiyal popülasyon tüm fermantasyon denemelerinde DAP ilavesine bağlı olarak değişmiştir.

Taze elma suyu kullanılarak da elma şarabı üretilmiştir. Şaraplarda pH 3.12-3.25; malik asit cinsinden toplam asitlik 6.87-7.01 g/L, esmerleşme indeksi 0.0493-0.0614, tanen 0.137-0.378 g/L, etil alkol % 8.49-8.75, özgül ağırlık 0.9868-0.9883, karbon dioksit % 0.138-0.245, kükürt di oksit 30.84-38.43 mg/L, uçucu asit 0.26-0.55 g/L, toplam şeker 1.59-5.38 g/L, kalıntı şeker 1.49-5.29 g/L, malik asit 2.96-

3.98 g/L, laktik asit 0.23-0.59 g/L, asetik asit 0.01-0.03 g/L, renk L* 44.66-75.41, a* -0.18-(-1.09), b* 3.28-4.62, Hue değeri 86.09-86.90, Chroma değeri 3.29-4.63 arasında bulunmuştur. Elma suyu konsantresinden de elma şarabı yapılmıştır. Şaraplarda pH 3.24-3.27, malik asit cinsinden toplam asitlik 6.65-6.86 g/L, esmerleşme indeksi 0.1332-0.0641, tanen 0.046-0.086 g/L, etil alkol % 8.13-8.37, özgül ağırlık 0.9884-0.9887, karbon di oksit % 0.05-0.143, kükürt di oksit 36.33-42.68 mg/L, uçucu asit 0.22-0.74 g/L, toplam şeker 2.13-4.18 g/L, kalıntı şeker 1.97-4.05 g/L, sitrik asit 0.08-0.11 g/L, malik asit 3.80-4.25 g/L, laktik asit 0.31-0.53 g/L, asetik asit 0.25-0.95 g/L, renk L* 41.28-55.41, a* -0.67-(-2.42), b* 4.92-10.63, Hue değeri 66.00-86.14, Chroma değeri 5.38-10.82 arasında belirlenmiştir.

Aroma ekstraksiyonunda sıvı sıvı ekstraksiyon metodu kullanılmış ve 8 farklı elma şarabı için aroma maddelerinin tanımlanıp miktarlarının belirlenmesinde GC-MS-FID cihazı kullanılmıştır. Tüm analizler 3 tekerrürlü gerçekleştirilmiştir. Toplamda 55 aroma maddesi tanımlanmıştır. Bunlardan 13'ü yüksek alkoller, 13'ü etil esterler, 5'i asetat esterler, 9'u uçucu asitler, 3'ü altı karbonlu alkoller, 5'i fenolik bileşikler, 4'ü laktonla ve 3'ü de karbonil bileşiklerdir.

Aroma bileşenlerinin toplam miktarı C1 denemesinde 85.18 mg/L, C2'de 89.14 mg/L, C3'de 72.8 mg/L, C4'te 74.65 mg/L, C5'te 75.26 mg/L, C6'da 66.92 mg/L, C7'de 62.4 mg/L ve C8'de ise 70.3 mg/L olarak bulunmuştur ($p<0.05$). En yüksek miktar C2 denemesinde belirlenmiştir. GC-MS-FID analiz sonuçlarına göre en önemli aroma aktif bileşik etil hekzanoat, etil 4-hidroksi bütanoat, etil oktanoat ve izoamil asetat olarak C2 ve C7 denemelerind.

Toplam yüksek alkol miktarları, 38.77-70.25 mg/L arasında değişmiştir. En yüksek 2-fenil etanol miktarı C4 denemesinde 19.6 mg/L olarak belirlenmiştir. En düşük 2-fenil etanol miktarı ise 12.05 mg/L olarak C6 denemesinde bulunmuştur.

Üretilen elma şaraplarında toplam ester miktarları 3.9-9.3 mg/L arasında bulunmuştur. En yüksek ester miktarı C7 denemesinde görülmüştür. Toplamda 9 adet uçucu asit 5.3-12.4 mg/L arasında belirlenmiştir. En yüksek uçucu asit miktarı C7 denemesinde en düşük miktar ise C1 denemesinde görülmüştür.

Altı karbonlu alkollerin miktarı tüm denemelerde 0.3-2.9 mg/L arasında değişmiştir. En düşük ve en yüksek cis-3-heksenol miktarı C7 (21.7 µg/L) ve C1 (39.9 µg/L) elma şarabında tespit edilmiştir. En yüksek ve en düşük altı karbonlu

alkol miktarları C3 ve C7 şaraplarında belirlenmiştir. En düşük ve en yüksek miktarda 1-heksanol C7 (265.9 µg/L) ve C1 (2897.9 µg/L) elma şarabında bulunmuştur.

Toplam fenolik bileşen miktarları 0.7-1.4 mg/L arasında değişmiştir. En düşük ve en yüksek 4-alilfenol ve 2,6-dimetoksi fenol miktarı C7 (59.4 µg/L), C6 (37.4 µg/L) ve C1 (244.3 µg/L), C2 (254.6 µg/L) şarplarında belirlenmiştir. En düşük ve en yüksek 4-allyguaiacol miktarı C8 (139.2 µg/L), C7 (231.7 µg/L) ve C5 (996.4 µg/L), C1 (351.2 µg/L) şaraplarında hesaplanmıştır. En düşük ve en yüksek 2-metoksi-4-vinilfenol miktarı C4 (173.8 µg/L), C7 (203.4 µg/L) ve C7 (437 µg/L), C3 (385.4 µg/L) şaraplarında saptanmıştır. Elma şaraplarında lakton miktarları 0.26-0.63 mg/L arasında değişmiştir. En yüksek lakton miktarına C1 şarabında rastlanırken bunu C7 şarabı takip etmiştir. En yüksek γ-butirolakton miktarı C1 (522.5 µg/L) ve C7 (499.8 µg/L) elma şarabında tespit edilmiştir. En düşük γ-butirolakton miktarı C3 (147.0 µg/L) ve C8 (188.7 µg/L) elma şarabında belirlenmiştir. Karbonil bileşiklerinin miktarı 0.08-0.47 mg/L arasında değişmiştir. En yüksek karbonil bileşen miktarı C8 denemesinde en düşüğü ise C7 denemesinde belirlenmiştir.

Aroma profili ve lezzet açısından şarapların duyusal özellikleri değerlendirilmiştir. Koku elma şaraplarında önemli bir kalite ölçüsüdür. Bu bakımdan en yüksek koku puanı 6.81 ile C1 elma şarabında belirlenmiş ve ikinci sırada ise C7 şarabı yer almıştır. C7 kodlu elma şarabı tat açısından en yüksek puanı (6.10) almış ve bunu 4.73 puan ile C1 kodlu şarap takip etmiştir. Tatlılık açısından 3.40 ile en yüksek puanı C5 kodlu şarap alırken, ekşilik (asitlik) açısından en yüksek puanı 6.16 ile C3 kodlu şarap almıştır. Acılık ve burukluk bakımından en yüksek puanları sırasıyla 3.54 ve 3.26 olmak üzere C1 kodlu elma şarabı almıştır. Genel kabul edilebilirlik açısından en yüksek puanı 7.17 ile C7 şarabı almıştır. Panelistler elma şaraplarının meyve, çiçek, baharat, karamel, ot, bitkisel, kimyasal veya iştah açıcı şeklinde aroma karakterlerinide belirlemişlerdir. Meyvemsi karakter elma şarapları için önemli bir özelliktir. Bu açıdan en yüksek puan C1 şarabında 7.38 iken bunu C7 şarabı takip etmiştir.

Bu sonuçlar değerlendirildiğinde C7 kodlu elma şarabının diğer denemelere göre daha fazla beğenildiği ortaya çıkmıştır.

ACKNOWLEDGEMENTS

I would like to explain my honest thankfulness to my supervisor Prof. Dr. Hüseyin ERTEN for the unlimited support of my PhD study, and also his tolerance, imagination, and great knowledge. His recommendations really helped me in the whole of my research and writing up this thesis. I could not have guessed to having a better mentor for my PhD study.

Also, I would like to thank advisor Prof. Dr. Turgut CABAROĞLU for his suggestions and support during my PhD study.

I would like to thank the respectable thesis committee, Prof. Dr. İbrahim EKİZ, Prof. Dr. Turgut CABAROĞLU, Prof. Dr. Serkan SELLİ and Prof. Dr. Haşim KELEBEK for their imaginative statements and encouragement, also for the good questions which give me to open my mind.

I would like to acknowledge to the Scientific Research Project of Çukurova University (BAP), Project No: FDK-2017-874, which gave financial support for my PhD study.

My sincere thanks to Res. Assist. Bilal AĞIRMAN, Dr. Z. Dilan ÇELİK, Res. Assıst. Dr. Erdal AĞÇAM, Res. Assist. Gamze GÜÇLÜ and also, technician Mesut ÖZDEN, Serap DURAN and Hayri SORAL and other people, who provided me with all things and who give me to enter the laboratory and facilities. Without their any support it could not be possible to perform this researching work.

I would like to say that a huge thanks to my family, especially my parents, my wife and daughters for their enormously supporting on my mentality during this PhD.

CONTENTS **PAGE**

LIST OF TABLES **PAGE**

LIST OF FIGURES **PAGE**

LIST OF ABBREVIATIONS

µg	: Microgram
µL	: Microliter
µm	: Micrometer
ABV	: Alcohol by Volume
AD	: Anno Domini
AJC	: Apple Juice Concentrate
ANOVA	: Analysis of variance
BC	: Before Christ
BI	: Browning Index
C	: Chroma
CAGR	: Compound Annual Growth Rate
CFU	: Colony Forming Unit
CO_2	: Carbon Dioxide
DAP	: Diammonium Phosphate
F	: Furfural
S	: Significance
FAN	: Free Amino Nitrogen
FAO	: Food and Agriculture Organization
g	: Gram
GL	: Gallon Litre
GC-FID	: Gas Chromatography-Flame Ionization Detector
GC-MS	: Gas Chromatography-Mass Spectrophotometry
H	: Hue
HMF	: Hydroxymethylfurfural
HPLC	: High Performance Liquid Chromotography
Kg	: Kilogram

L	: Litre
LRI	: Liner Retention Index
M	: Mole
mg	: Milligram
min	: Minute
mL	: Millilitre
mmol	: Milimole
N	: Normal
nd	: not detected
nm	: Nanometer
NCR	: Ninhydrin Color Reagent
ns	: not significant
^{o}C	: Centigrade degree
PDA	: Photo Diode Detector
PPM	: Parts Per Million
RID	: Refractive Index Detector
S.	: *Saccharomyces*
Sec.	: Second
SO_2	: Sulphur Dioxide
SG	: Specific Gravity
TTA	: Total Titratable Acidity
TSS	: Total Soluble Solids
UK	: United Kingdom
US	: United State
UV	: Ultra Violet
v/v	: Volume per volume
w/w	: weight/weight

1. INTRODUCTION

Cider is a fermented alcoholic drink, produced by the fermentation of apple juice. However, in North America, it is called "hard cider", whereas apple cider is a non-alcoholic unfiltered apple juice with a distinct sweet-tart flavour. Cider making process is gone two successive biological fermentation stages. The first one is the yeast fermentation in which sugar is converted to ethanol and carbon dioxide (CO_2) as many compounds and also into flavour compounds such as higher alcohols, esters, carbonyl compounds and organic acids etc., by the yeasts. The second one is called the malolactic fermentation in which L(-)-malic acid is converted to L(+)-lactic acid and carbon dioxide (CO_2) by the lactic acid bacteria being in the apple juice or in the natural fermentation (Donalies et al., 2008). Ethanol is the most important alcohol formed in cider and constitutes 10-13%. It is crucial or the stability, aging, and sensory properties of wine (Rowles, 2010).

The most significant compound for alcoholic drinks, including ciders, is ethanol ranging from 4 to 13 % (v/v) (Donalies et al., 2008 and Rowles, 2010). The medium temperature is affects the characteristic properties of alcoholic and malolactic fermentation processes. It affects the level of acetic acid, which influences the organoleptic qualities of cider in negative terms and increases with growing temperature. The decrease in the concentrations of higher alcohols and ethanol with growing temperature, mainly formed during yeast metabolism, was observed (Herrero et al., 2006). Among the process parameters that influence the progression of fermentation, temperature can alter the rate of fermentation by affecting assimilation of nitrogen and sugar, and consequently has a significant effect on final wine quality (Malherbe et al., 2004).

Production of ciders at low temperature leads to the floral and fruity aroma, and also preserves a high level of volatile aroma compounds (Beltran et al., 2008). At low temperatures, sugars are fermented slowly compared to high-temperature fermentation, which allows a faster yeast growth (Yilmaztekin et al., 2013).

The level of the assimilable nitrogen in the apple juice varies from 27 to 574 mg/L, is considered the main limiting factor for the growth of the yeast (Drilleau, 1990; Cruz et al., 2002). Nitrogen is also required in the stationary phase (80 % of the alcoholic fermentation) to motivate protein synthesis, especially for sugar consumption in the alcoholic fermentation (Bely et al., 1994). Nitrogen utilization varies among commercial yeasts (Julien et al., 2000; Colombié et al., 2007). Metabolism of amino acids can influence the effectiveness of the alcoholic fermentation and the quality of the wines (Salmon and Barre, 1998).

The aroma compounds influence the characteristic flavour of cider that defines the quality and significance of cider (Satora et al., 2008; Pino and Queris 2011). Apple varieties are also very important for the production of good quality ciders (Boylston et al., 2003; Lee et al., 2013).

The production of volatile compounds during the cider making is affected by the fermentation parameters, the strain of yeast, fermentation conditions, and so on. Different aroma components lead to the development of particular and special flavours of different kinds of cider. Cider producers usually evaluate the flavour of cider and more based on technological parameters. Especially those that affect the fermentation process and apple varieties (Villiere et al., 2015).

Nowadays cider market is the fastest growing in some European countries including Czech Republic, Romania, Russia and Turkey etc. (European Cider Trend 2016).

This study aims to investigate the effect of the main parameters on alcoholic fermentation and the process of cider production. Cider fermentation obtained from fresh and concentrated apple juice was carried out at 18°C. The combined effect of the following factors were examined.

- ✓ Effect of commercial yeast usage on fermentation;
- ✓ Effect of the nitrogen contents;
- ✓ Effect of usage of concentrated apple juice.

2. LITERATURE REVIEW

2.1. History of cider

Arthey and Ashurds (1996) reported that the first simple alcoholic drinks were made in the Roman Empire, which was the occupation of England in 55 BC. The legend of Celtic admired the “holy apple” in 77 AD. Also, Pliny the Elder mentioned drinks made from the juice of the apple. In the third century of AD, apple cider was drunk all over European countries. In the 4th century St. Jerome used sicera (means cider) to define alcoholic beverages, which were made from apple.

In the 18th and 19th centuries, apple wine was famous throughout Europe and was drunk broadly in North America. Even though prohibition laws were established in the USA after the American civil war to limit the uses of hard cider, it started being reproduced in the 1990s in North America. Cider alcohol strength in America ranges between 1.2 and 8.5% (v/v), and in traditional English ciders, from 3.5 to 12%. Apples are the general raw material for the cider making production and the traditional categorization for English cider apples are sharp, bitter sharp, bittersweet and sweet, which depends on their acidity and tannin. Also, a coalition of factors has led to excessive production of apples in the USA (US Apple Association, 2008).

2.2. Production and uses

Cider is produced from cider apples, which are bitter, sharp and sour. But, North America plants give dessert apples. Dessert apples are deficient in acidity and tannin levels found in cider apples and are recognized unsuitable for producing hard cider. However, economics dictate the manufacture of hard cider from dessert apples in the US. As scales of production rise from microbreweries to larger scales, there will be a need to analyse and predict fermentation performance. Most of the

apple dropped to 25-35 % in the commercial classification process, as well as manufacturing apple varieties, are used as raw material in the processing of apple juice, cider (Lea & Drilleau, 2003; Brown, 2012).

For the last 10 years, interest has increased because of marketing innovations associated with product innovation. The global growth rate is starting to decrease, following another global increase in cider and perry trades in 2013. However, growth rates kept an important level of positive growth in 2014-2017 (Fig.2.1). Cider and Perry's classification size share globally increased slightly in 2014-2017, and then remained at over a 1% volume share in 2017 (Euro Monitor International, 2016).

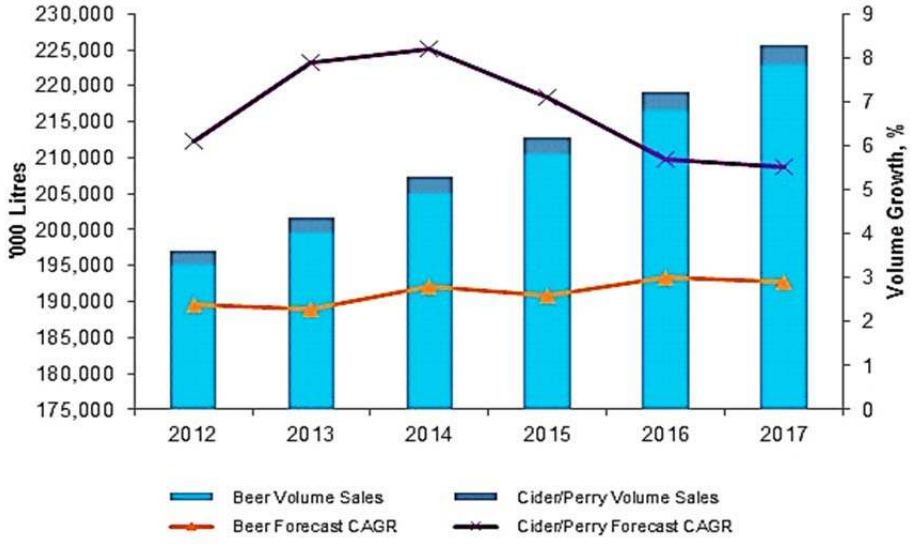


Figure 2.1. Global cider VS Beer market share 2012-2017 (Euro Monitor International, 2016)

Among fruits crops, apple represents the 3th place worldwide with 3.03 million tons annually in Turkey (FAO, 2017). Apples are planted in various regions over Turkey, which is an around 50 % of all purchases apple product comes from three regions; Isparta, Karaman and Niğde. Those regions are settled in the

southern part of Central Anatolia and the Northern Mediterranean Regions. Market apples are also grown in Antalya, Eregli, Denizli, Yalova and Amasya. Main apples are red delicious (Starking) and Golden delicious in Turkey. Amasya is the most common native species, which constitutes about 10 % of total production. The Granny Smith, Fuji, Gala, Jonagold, and Braeburn varieties are also grown. Traditionally about 90% of Turkey's apple production is used as fresh products. About 5 % is used into juice, canned goods, vinegar or dried products, and less than 4 % are exported (Global Agricultural Information Network, 2015).

2.3. Raw materials of cider production

Apples are important raw material for apple wine producing. The traditional classification for English cider apples is shown in Table 2.1.

Table 2.1. The traditional classification for English cider apples (Andrew et al.., 2003)

Group	Acid (%)*	Tannin (%)**
Sharp	> 0.45	< 0.2
Bitter sharp	> 0.45	> 0.2
Bittersweet	< 0.45	> 0.2
Sweet	< 0.45	< 0.2

* as malic acid equivalents;
** as tannic acid equivalents;

A characteristic feature of cider apple, especially French and English bittersweets, is the comparatively high concentration of polyphenols, called 'tannin'. Even though present ciders are usually weaker in tannin than in the past, tannin still makes a significant difference to the overall mouth feel of the drink and prevents it from becoming weaker. The polyphenols make the main difference in flavour, colour, press ability and also have weak antimicrobial (Lea, 1984).

In some circumstances, cider apple is defined by the vintage attribute, which is an interest to the small traditional producer. Vintage attributes usually

give more complex and pleasant flavours to the cider. However, the vintage cultivars usually have lower yields and are more difficult to plant. UK cultivars are given in Table 2.2.

Table 2.2. Common cider apple cultivars in UK (Andrew et al., 2003)

Character	Early Season	Mid/Late Season
Sharp/Bitter sharp	Breakwells Seedling	Brown's Apple
	Backwell Red*	Frederick*
		Crimson King*
		Kingston Black*
	Foxwhelp	Stoke Red*
Bittersweet	Ashton Bitter	Dabinett*
	Ellis Bitter	Chisel Jersey
	Major*	Harry Masters Jersey*
	Tremlett's Bitter	Yarlington Mill*
	Taylors	Michelin
		Vilberie
		Medaille d'Or*
Sweet		Sweet Coppin*
		Sweet Alford*
		Northwood*

* - Denotes vintage quality cultivars

Canbaş et al., (2000) produced low, normal and high alcoholic strength apple wines from Golden and *Starking Delicious* cultivars. For the low alcoholic apple wines, the fermentation was stopped by cooling and addition of SO_2 when the alcohol degree reached 2.5-4 % (v/v). For the high alcoholic apple wines, sugar was added into the apple juices. The results showed that Starking delicious was the most suitable apple cultivar for cider making and normal alcoholic apple wine was 8 % (v/v).

2.3.1. Juice preparation

At these days, UK cider producers are widely using apple juice concentrate (AJC). But it is permitted to a limited amount in France. Presently UK cider producers widely use additions such as fermentable sugars from cane, beet or hydrolysed starch. Fresh apple should be fully ripe before cider producing. It is

usually stored for several weeks after harvesting so that all the starch can be converted into sugar. Apples must be classified and cleaned before the pressing to remove rotten fruit and orchard debris which have adverse effects on microbiological status and ultimate cider quality. Traditional juice preparation use pack presses, but the larger factories use horizontal piston presses and belt presses. For juice preparation, the apples must be poured into tanks of fibreglass, high-density polyethylene, stainless steel for pre-fermentation. The juice should be prepared before fermentation. The traditional spontaneous clarification of juice by "keeving" ("maceration et cuvage" in France) was obsoleted in England. In modern English cider producing the fermentable sugar sources (juice, AJC and syrups) are mixed to the required level. It must be at the specific gravity 1.080-1.120 in order to give a final alcohol strength of 10-15 % (Beech 1993, Lea 1994).

If fast fermentation is needed nutrients are added to complete the fermentation. Unlike the traditional "keeving" where nutrients are removed from the juice to ensure a slow fermentation. It is enough to add ca 250 ppm ammonium sulphate or phosphate, and thiamin at 0.2 ppm. Nutrients of pantothenate, pyridoxine and biotin were also used. This addition of nutrients is especially important if the apple juice is prepared with fermentable adjuncts (which do not contain any nutrients) or with AJC since the Maillard reaction during concentrate storage decreases nutrient levels and produces yeast inhibitors such as 5-hydroxymethylfurfural (HMF). If clarified concentrates and adjuncts are to be fermented, a source of insoluble solids is often helpful. This allows the yeast cells a solid surface on which to rest, and from which ethanol and CO_2 can be liberated to the medium. The addition of sterol can be useful to promote the healthy growth of yeast cell walls. Also, it is supported by yeast aeration after pitching (Ewart, 1995).

Many cider producers usually add a pectolytic enzyme for preparing before the fermentation of fresh juice. Also, prevents post-fermentation sediments. Sometimes pectolytic enzymes are added initially to the mash. This is done to improve press ability and to enhance the yield (Lea, 1991; 1994).

The most important supplement is sulphur dioxide, which is used in modern UK cider production as well as in wine producing. The efficiency of sulphur dioxide is dependent on the pH of juice. Therefore cider producers are always adjusted pH under 3.8 by the addition of malic acid before the sulphur dioxide addition for making cider. The amounts of SO_2 can be calculated from Table 2.3.

Table 2.3. SO_2 addition required to cider apple juices (Andrew et al., 2003)

pH	Addition required (mg/L)
3.0 - 3.3	75
3.3 - 3.5	100
3.5 - 3.8	150

Apple contains ascorbic acid. It is similar to L-xylosone which is a strong sulphite binder. Juices are also produced from depectinased apple juice concentrate. But it is just a weak sulphite binder. Its influence only becomes significant at the high concentrations (Jarvis and Lea, 2000).

Bozoğlu et al., (2015) studied on the effect of operating parameters on ethanol concentration in apple wine production process. They examined temperature, pH, and sulphur dioxide concentration. The examined temperatures were 18°C and 25°C, pH 3.0-4.0 and 50-150 ppm for sulphur dioxide. The results report that the influence of the tested parameters on ethanol was good. High temperature supported a faster fermentation rate than low temperature. Low levels of pH and high levels of sulphur dioxide inhibited yeast and other unwanted microorganisms.

2.4 Fermentation process

2.4.1. Yeast selection

There is no added external source of yeast in traditional cider making. Because the apples themselves contain a mixed yeast microflora which can be at the level of $5x10^4$ cells per gram of collected fruit. Spontaneous fermentation starts within a few hours if the temperature of the juice is above 10^oC. Also, there is no added sulphite in traditional cider fermentation.

The first few days are dominated by the non-*Saccharomyces* species, such as *K. apiculata* and *M. pulcherrima*. A rapid evolution of gas and alcohol is produced. They also cause a distinctive range of flavours, characterised by ethyl acetate, butyrate and other esters. As the alcohol level rises from 2 to 4%, these primary fermenters begin to die off and the fermentation is dominated by *S. uvarum*. If sulphur dioxide is added to the original juice, the non-*Saccharomyces* yeasts and most bacteria are died off. This provides a way for the *Saccharomyces* species to multiply after a lag phase of several days, and the fermentation proceeds to dryness with a more similar and better microflora than an unsulphured juice. Also, there is less secondary infection by film yeasts and acetifying bacteria (Beech, 1993).

Since the 1980's the use of active dried wine yeast has become almost common in the UK cider manufacturers. A combined inoculum *S. uvarum* and *S. bayanus* are often used in the fermentations that the primary yeast contributes to quickly starting fermentation. But the secondary yeast makes better with the fermentation to dryness of the high alcohol bases which is now accepted throughout cider manufacturing. This dried yeast requires no pre-propagation and is easily hydrated in warm water before pitching into the juice. There is some concern that fermentations dominated totally by *Saccharomyces* are requiring in estery cider character (Apiculatuston) in Germany, and that the role of *K. apiculata* is significant it (Scholten, 1992). Thus, in France the need for a mixed microflora is regarded as axiomatic and recent experimentation has focused on mixed inocula of

e.g. *M. pulcherrima* and *S. uvarum* in an attempt to produce a complex and traditional flavour but under closer microbiological control (Bizeau et al., 1992).

Modern French cider makers still use traditional methods and fermentation is done at low temperature that important residual sugar remains in the final apple wine. (Drilleau, 1990). But English apple winemaking practice is almost totally the opposite. In most cases, there is a suitable temperature range of 15-20°C. Thus a portion of the fermenting juice is sometimes heated to 25°C. High temperature practice lead to formation of cider containing 10-12 % (v/v) ethanol within the 2 weeks. However, at these days, low temperature cider making is preferred.

Valles et al., (2007) mentioned about yeast species associated with the spontaneous fermentation of cider. In their study, yeast colonies isolated from apple juice of Asturias (Spain). The yeast strains were identified by restraint fragment length polymorphism analysis of the 5.8S rRNA gene and internal transcribed sequences (ITS). Non-*Saccharomyces* yeasts (*Hanseniaspora* genus and *M. pulcherrima*) dominated in must. The species of *Saccharomyces* were predominant species as *S. cerevisiae* and *S. bayanus. S. bayanus* at the beginning and the middle of the fermentation process, and reaching a percentage of isolation between 33% and 41%, whereas *S. cerevisiae* took over the process in the final stages of fermentation. Meanwhile, the species of *H. valbyensis* was always isolated by the end of fermentations in the 2001 harvest.

2.4.2. Nitrogen contents

The free nitrogen content of the apple juice, usually varies from 59 to 330mg/L, which is average of 120 mg/L, is supposed the main limiting factor for cell growth of the yeast and fermentative activity during the beginning stage of alcoholic fermentation (Alberti et al., (2011).

Total nitrogen (<75 mg/L) mixed with low concentrations of dissolved oxygen (<2.5mg/L) can lead to stuck fermentations in apple wine processing. Thus, some apple winemakers add ammonia to the apple must. It can prevent an

incomplete alcoholic fermentation (Alberti et al., 2011; Barbosa et al., 2012). But, the addition of oxygen can be done by the bubbling of the must. It should be avoided to oxidize essential nutrients (Alberti et al., 2014).

The necessary nitrogen content is required in the stationary phase (up to 80%) in the alcoholic fermentation (Bely et al., 1994). Also, it will continue to improve the microbial metabolism and reach up to 10% of the yeast biomass on a dry weight (Julien et al., 2000; 2001). Some factors such as the composition of apple juice and the kind of the yeast strain can affect the assimilation of the nitrogenous compounds (Julien et al., 2000; Colombié et al., 2007). The metabolism of amino acids can impact the efficiency of the alcoholic fermentation and the quality of apple wine (Salmon and Barre, 1998).

The fraction of nitrogen in apple juice contains 86-95% of total amino acids as asparagine, glutamine, aspartic acid, glutamic acid and serine, and they are immediately assimilated by yeasts. The apple plants with high fertilizers can contain the nitrogen compounds up to 5 times higher than usual apple juice (Lequere and Drilleau, 1998). The amount of nitrogen in apple juices can be low (<75 mg/L) or high (>150 mg/L) depending on the duration of the plantation and the amount of the fertilizers used in it (Nogueira et al., 2003). These factors influence the growth of the yeast and affect the fermentation time, active of aroma production and microbiological instability in typically gasified apple wine (Garde-Cerdán and Ancin-Azpilicueta, 2008).

Kelkar and Dolan (2012) studied on modelling the effects of initial nitrogen content and temperature on fermentation kinetics of hard cider. They used diammonium phosphate (0, 100, 300, 600 ppm) in juice. Fermentation temperature trails were 11°C, 17°C and 22°C. They were examined the effect of yeast cell concentration, nitrogen, sugar, ethanol concentration, temperature and original nitrogen content. The results were best-fitted on the fermented at 22°C and DAP 100 ppm. They were also approved by Alberti et al., (2011) who studied on apple wine processing with different nitrogen contents.

The aspartic acid, asparagine and glutamic acid varies from 57.10 to 81.95% in all apple juices. These three amino acids were produced with high consumption (>90%) during fermentation in all the apple wines. The cider produced with low nitrogen content noted the stuck fermentation and about 50% less content of volatile compounds, which were 3-methyl-1-butanol (isoamyl alcohol) and esters. High contents of amino acids in apple musts are important for the final product of fusel alcohols and most of the esters by aromatic yeasts during the apple wine fermentation (Santos et al., 2015).

Kinetic parameters in these models could be dependent on yeast and different fermentation methods at the same time. Recently mechanical patterns in oenology were introduced by Cramer et al., (2002) and Nobile et al., (2003). The usual mechanical principles are introduced to explain the alcoholic fermentation kinetics. Nitrogen is the mainly growth-limiting nutrient for the yeast cell mass (Cramer et al., 2002; Nobile et al., 2003), utilization of sugar and ethanol production is equivalent to the viable yeast cell concentration (Bailey and Ollis, 1986), the death scale of cells is equivalently simple to the alcohol content (Ansaney-Galeote et al., 2001).

With the process parameters that affect the method of fermentation, the temperature can change the frequency of fermentation by pretending assimilation of sugar and nitrogen, and the result has an important impact on final wine quality (Malherbe et al., 2004).

2.4.3. The malolactic fermentation

Traditional cider makers are very generally do malolactic fermentation. The major beneficial organism affecting this change appears to be the heterofermentative *coccus* (*Leuconostoc oenos*) in grape wines, although other *Lactobacillus* species may also perform the malolactic fermentation. It is favoured by a lack of sulphiting during fermentation and storage, and by a certain amount of nutrient release from yeast autolysis when the cider stands un-racked on its lees. In

French cider making where the primary fermentation is very slow, the malolactic change may occur concurrently with the yeast fermentation, whereas in UK cider making it is most likely to occur once the yeast fermentation has finished and the cider is in bulk store (Drilleau, 1992).

The simple external characteristic of the malolactic change is the decarboxylation of malic to lactic acid with the release of CO_2. The total acidity decreases and the flavour becomes more complex. The malolactic fermentation is usually considered as trouble and is not approved in modern UK cider making. Thus, the current conditions do not like it, since sulphide is commonly used before and after fermentation and the ciders do not stand on their yeast lees for a long time. Although, spoilage of stored apple ciders by rod-shaped lactic acid bacteria is not usual and often shows itself nowadays by blockage of membrane filters during final packaging. However, the organisms affected in this case are not regularly those which are associated with the traditionally good effect of the malolactic change. The effect of malolactic fermentation on the aroma quality of cider was studied using *Leuconostoc mesenteroides* subsp. *mesenteroides* Z25 at 25°C for 12 days after the end of alcoholic fermentation by *S. cerevisiae*. Strain Z25 presented good activity at the beginning MLF with 10 % alcoholic concentration. Higher content of malic acid gave negative organoleptic properties to the apple wine. It was decreased significantly from 4.0 g/L to 0.25 g/L after malolactic fermentation. The concentration of lactic acid increased from 0.99 g/L to 3.50 g/L. The acetic acid content of the apple wine was 0.74 g/L. Z25 produced the cider with more volatile compounds and enough adjustment of organic acids. It was used by *L. mesenteroides* subsp. *mesenteroides* strain Z25 to start the MLF of apple wine improving the flavour quality of the cider produced (Zhao et al., 2014).

2.4.4. Sulphite binding

Sulphur dioxide is added into apple juice. Pyruvate, α-ketoglutarate and acetaldehyde are all essential metabolic intermediates in the result of ethanol by

yeast. However, they are all carbonyls which bind to sulphur dioxide. The remains at the end of fermentation will affect instantly on the ability of any sulphite, which is added to the apple wine for storage. Acetaldehyde occurs the powerful binder of sulphite. Until all this part is bound, no free-sulphite practiced can remain in the apple wine. The other carbonyls bind less strongly and consequently can co-exist partially un-bound in balance with free SO_2 (Andrew et al., 2003). The percentage of cider carbonyls which are bound at the level of 50 mg/L free SO_2 as given in Table 2.4.

Table 2.4. Binding of SO_2 to juice carbonyls (Andrew et al., 2003)

Compound	Percentage of carbonyl that is bound	Typical level in cider (mg/L)	Bound SO_2 contribution (mg/L) due to that carbonyl
Naturally present			
Glucose	0.11	7000	8
Galacturonic acid	4.4	1000	15
L-xylosone	36	20	4
Acetaldehyde	99.8	25	35
Pyruvate	83	20	12
A-keto glutarate	58	15	4
From bacterial contamination			
5-keto fructose	70	*not*	
2,5 diketo gluconic acid	64	*be present*	
Overall bound SO_2			78
TOTAL SO_2 (bound + free)			128

2.4.5. Colour of cider

The colour of cider is defined in juice oxidation (degradation) and in point of fact it is possible to make white high tannin apple wines if oxidation is fully restrained (Lea and Timberlake, 1978). The impact of pulp and juice oxidation on juice colour sets the first appearance of the apple juice. It is expected to the quinoidal oxidation products of phloridzin, epicatechin and the procyanidins. After

the yeast fermentation, the original colour of fresh juice reduces by 50%. The colour from concentrate decreases only 10%, so during fermentation, since the carbonyl-amino chromophores from Maillard browning are resistant to this reducing action (Lea 1984; 1991).

2.4.6. Flavour of cider

Cider is a mixture of aroma and taste. The heavily tannic flavours of traditional ciders are much less in demand it and the procyanidins are noticeable in modern factory ciders only as a part of the general mouthfeel. The volatile aroma of cider is in most role qualitatively equal to that of any other fermented drinks as wine, perry, and beer etc.., and obtains to a large range from the yeast using widely known bio-synthetic pathways (Berry, 1995).

If they contain the highest concentration of esters and, to a lesser extent, fusel alcohols, carboxylic acids, ketones and aldehydes give fruity odours by yeast during fermentation. (Herrero et al., 2006; Rita et al., 2011; Gamero et al., 2011). Amino acids detected in the original apple must are the most important precursors and intermediates for the biosynthesis of many volatile compounds (Ardö, 2006).

Volatile aroma compounds were examined in apple juice (*Auksis*), yeast and fermented apple juices. Fermentations were done at 16±1°C and 23±1°C. The main aroma compounds were determined as 2-hexenal, acetic acid butyl ester. In fermented apple juices, essential aroma compounds were determined as 2-hydroxyethyl hydrazine, 3-methyl-1-butanol, hexanoic acid ethyl ester, acetic acid and hexyl ester (Rita et al., 2011).

Fermentation temperature was strongly affected by yeast metabolism on during processes, aroma and wine quality. They were 9 key aroma compounds (ethyl acetate, isobutyl acetate, isopentylacetate, ethyl caprylate, ethyl 4-hydroxybutanoate, isobutylalcohol, isopentylalcohol, 3-methylthio-1-propanol, and benzeneethanol) in cider importantly raised with the accretion of fermentation temperature from17 to 20°C. However, 8 out of the 9 key aroma compounds with

the exception of ethyl 4-hydroxybutanoate. It reduced when the temperature goes up to 20 to 26°C. Fermentation temperature was at 20°C, which was supposed to be the most fitting condition using the chosen yeast strain (*S. cerevisiae* AP05) for cider making (Peng et al., 2015). Cider flavour wheel is shown in Fig.2.2.

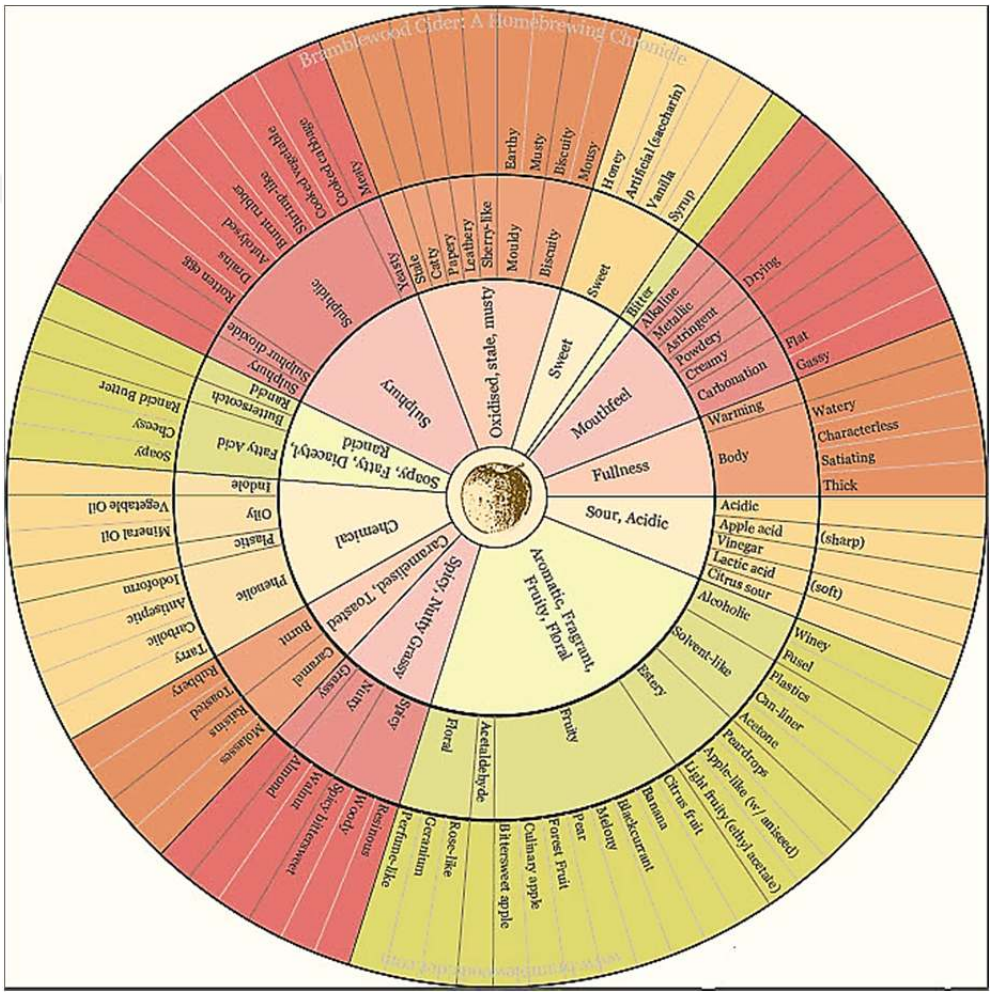


Figure 2.2. Cider flavour wheel (National Association of Cider Market, 1994)

2.5. Post fermentation process

2.5.1. Racking and storage

Fermentation of cider is finished after racking from the yeast sediment for storage. Clarification and racking process are done and some English cider producers do not do maturation and they give on the market. In the others, cider rests on their sediment for several weeks and rack into containers or oak barrels for a maturation period of several months (Scott and Swaffield, 1998). Through this period, a malolactic fermentation may/may not carried out and sulphur dioxide is not be added during storage. First clarification could be carried out by the natural settling of a well flocculating yeast, by centrifugation, by fining or by a mixture of all three. Typical fining agents are bentonite, gelatin, isinglass (chitosan) which is made from crab shell waste in North America. Gelatin is frequently used together with bentonite to boost floc formation. Germany cider makers popularly use to the extremely efficient gelatines, but opposite in the UK. Almost modern UK cider makers are mixed before the sale, although small traditional cider makers usually produced a little bit mixing and handling procedures at bottling than the primary producers. They form the high alcohol “base ciders” from which mix is presented by the cider maker's terms. Water is added to these bases. It could produce the exact alcoholic strength for retailer’s sale, including additions of sugar, artificial sweeteners, malic or other acids, allowed food additives and carbonation. Regularly in the UK regulations permit for cider, all those processes and additives are allowed by EU “horizontal” food’s law. In France and Germany, there is a specific “vertical” legislation which concerns to cider and is much more limiting (Andrew, et al., 2003).

Final filtration could be made just before and after mixing. They use to produce bright products by powder filters (coarse disposable films), followed by the near-sterile films (membrane filtration 0.5 μm) to remove all yeasts and bacteria. Ciders are carbonated and pasteurized. In some cider industries are still used in-bottle and cans. The most usage of PET (Polyethylene terephthalate)

bottles in most large industries, HTST (High-temperature short-time) method in a flow-through pasteurizer and chiller is needed to follow by near aseptic filling conditions. On the other hand, cold aseptic filling after sterile membrane filtration (0.2 μm) is used. Almost all cider producers will propose to add 50 ppm of sulphur dioxide at filling to give a stability level of 30 ppm free sulphur dioxide in the drink. It depends on the level of sulphite binders in the cider. For cans, the total level of sulphur dioxide is often 25 ppm. Ciders intended for canning are often specially fermented in the absence of sulphite.

There is a definite market for "naturally conditioned" cider in kegs or small plastic barrels. These ciders are usually produced from fully fermented dry ciders, which an additional charge of sugar and flocculating yeast has been added. The product is of course slightly hazy but may rest in good condition for several weeks due to the slow fermentation. Real "bubbling" ciders are prepared by fermentation in bottle followed by "disgorgement" of yeast from the neck. They are nowadays actually unknown in the UK although some traditional cider producers are trying to improve the method (Andrew, et al., 2003).

2.5.2. Cider spoilage by yeast

Zymomonas mobilis bacterium is resistant to sulphite that ferments sugar in bulk sweet ciders stored at pH>3.7. The characteristics of cider disorder are a restored fermentation, followed by a "raspberry" or "banana skin" aroma and deep white turbidity in the drink. Nowadays, it is practically unknown in English cider making since the ciders are usually at pH < 3.5 and are nevermore stored sweet. Another simple disorder is “ropiness”. It is caused by some lactic acid bacteria as *Lactobacillus* and *Leuconostoc* which synthesize a polymeric glucan. It happens to increase the viscosity of cider at low levels and looks oily form in the composition. The cider transfers to a slimy rope at higher concentrations of glucan when drained from a bottle. But, the taste is not much affected by it. If not too critical, ropy cider

can be corrected by moving quickly to break up the glucan chains, followed by the addition of 100 ppm sulphur dioxide to prevent more growth (Wilkins, 1990).

Protein sediments in bottled ciders are very rare because the original protein content of apple juice is so low (ca 100 ppm). If extra gelatine is added, sediments nearly always appear from over fining at some time in production. They are used to prepare concentrated apple juice, which is fined with gelatine and kieselsol. It contains almost large quantities of unstable protein still in the presence of colloidal silica. This will not be manifest in the concentrated apple juice itself since the protective effect of the high solids prevents agglomeration and flocculation occurrence. However, the unstable protein may eventually form a haze or sediment after fermentation and dilution. Many cider sediments also include significant amounts of polyphenols, in combination with proteins and polysaccharides. When the product is cooled, it may occur "shock out" a haze or sediment. Even in the absence of protein, such as hazes may still form at low pH due to the breaking and re-forming of carbon-carbon bonds between the procyanidin units, leading to the slow build-up of random polymers which eventually drop out of solution. Flavour spoils in ciders may occur from contamination. For instance, the presence of naphthalene where the tarred rope has been stored adjacent to a cider keg. However, many spoils are arisen from an imbalance in the natural flavour profile due to the microbiological process (Andrew, et al., 2003).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Raw materials

In this study, “fresh” and “concentrated” apple juices were used. Fresh apple juice was produced from Golden delicious (*Malus domestica*), and concentrated apple juice was obtained from Anadolu Etap Company in Mersin, Turkey.

3.1.2. Yeast

Actiflore BO213 (Laffort, France) commercial dry yeast (*Saccharomyces cerevisiae)* was used. The both of apple juice was inoculated as 20 g/hL of yeast. The yeast was rehydrated before inoculation.

3.1.3. Microbiology media

Malt Extract Agar (Merck, Germany) was used for in determination the total yeasts. L-lysine agar was for counting the non-*Saccharomyces* yeasts. 0.1% peptone water was used in diluting samples.

3.1.4. Tools and equipment used in experiments

The experiment was carried out in 10 L vessels at the winery, Faculty of Agriculture, Çukurova University, Adana, Turkey.

3.1.5. Tools and equipment used in analyses

Juice extractor was used for making a fresh apple juice. pH was determined by “Mettler Toledo pH/Ion S220” pH meter. The determination of total soluble solids (°Brix) was done by a refractometer RA-130, Kyoto Electronic, Japan). “Color Quest XE” HunterLab Fuse 3A/SB” was used to measure L*, a*, b* colour values. “Agilent technologies Cary 60 UV-Vis” spectrometer was used for free

amino nitrogen (FAN), browning index (BI) and tannin measurements. “JSR-JSIB 22T” model water bath was used for analyses. “Aqua Lytic AL654” incubator was used in microbiology counting. “Hirayama HA-300M IV" autoclave was used for sterilization. “Hettich universal 320R” centrifuge was used for centrifugation. “Dujardin-Salleron Vola.2000” automatic extractor was used for volatile acids. “Anton Paar DMA 4500 M” was used for ABV %, ethanol and specific gravity of cider. “Anton Paar carboQC” was used for CO_2 of cider.

Sugars, reducing sugars and organic acids of samples were determined by SPD-M20A, RID 10A, Shimadzu LC-20AD model HPLC.

Hydroxymethylfurfural (HMF) was determined by SPD-M20A diode array detector (DAD), Shimadzu LC-20AT model HPLC. Before the usage of the sample in HPLC was filtered by 0.45 μm and 0.22 μm (Millipore Millex-HV, Hydrophilic PVDF).

The analysis of the aroma compounds was carried out using "Agilent 6890N" gas chromatography and the associated mass spectrometer "Agilent 5975B VL MSD". “Millipore” simplicity 185 models ultrapure water device was used for preparing regents of chromatographic analysis.

3.2. Methods

3.2.1. Process of apple juice

Golden delicious apples were transferred to the Winery of Çukurova University and then washed and disintegrated and the extracted using home juice extractor. The extracted juice was poured into 10 L glass vessels (Fig.3.1), and the pectolytic enzyme (3 mL/hL, Pectinex, Novozymes, USA) was used for depectinization. Depectinization was done for 60 minutes at 45ºC and then cooled below 20ºC. Then 50 mg/L of sulphur dioxide (5% of SO_2) was added. Maceration process was done at 10ºC for 24 hours in order to separate the sediment. After that fresh apple juice was left for alcoholic fermentation (Fig.3.2).

Figure 3.1. Picture of fresh and concentrated apple juice after adjusting procedure.

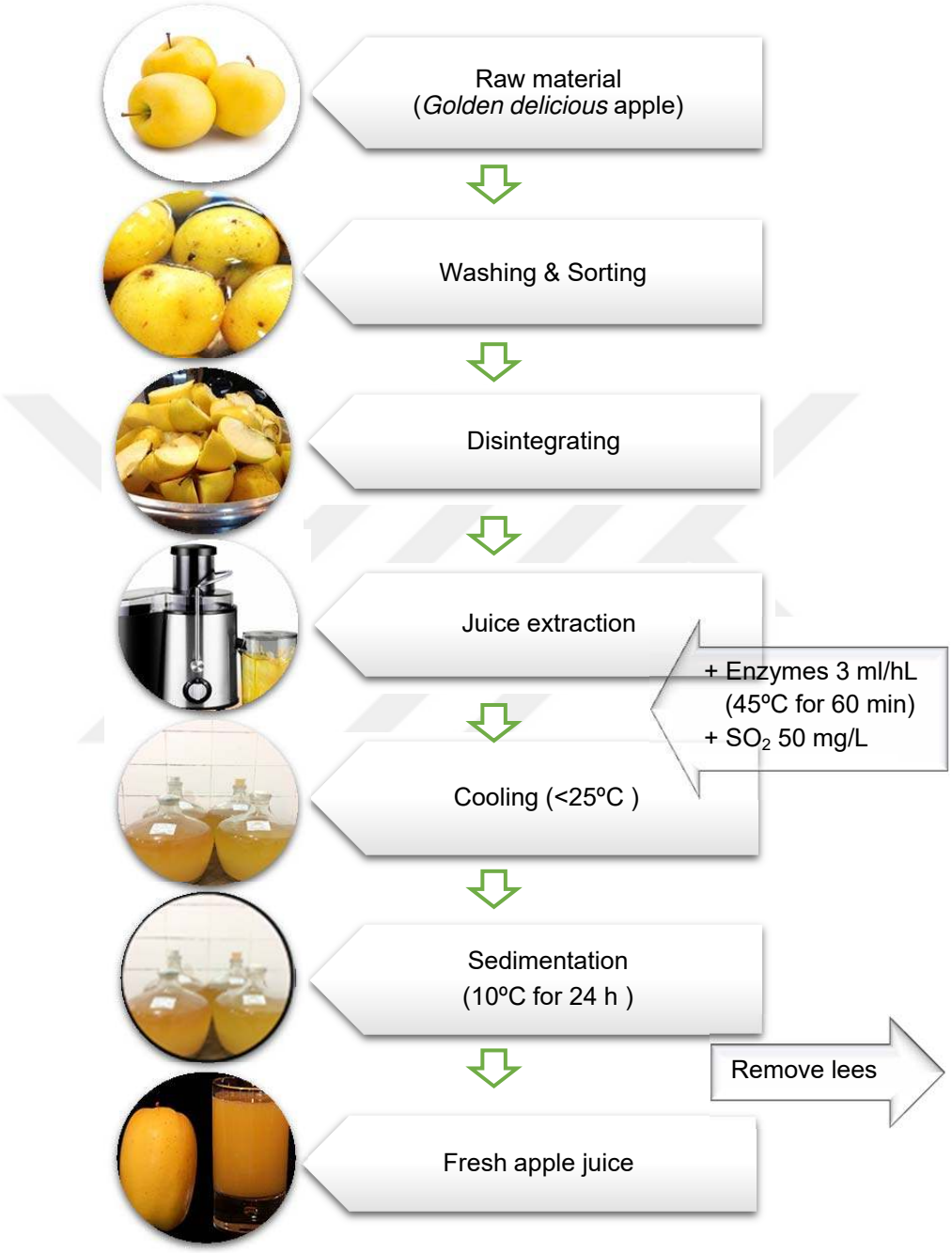


Figure 3.2. A flowchart of apple juice processing

3.2.2. Fermentation process of cider

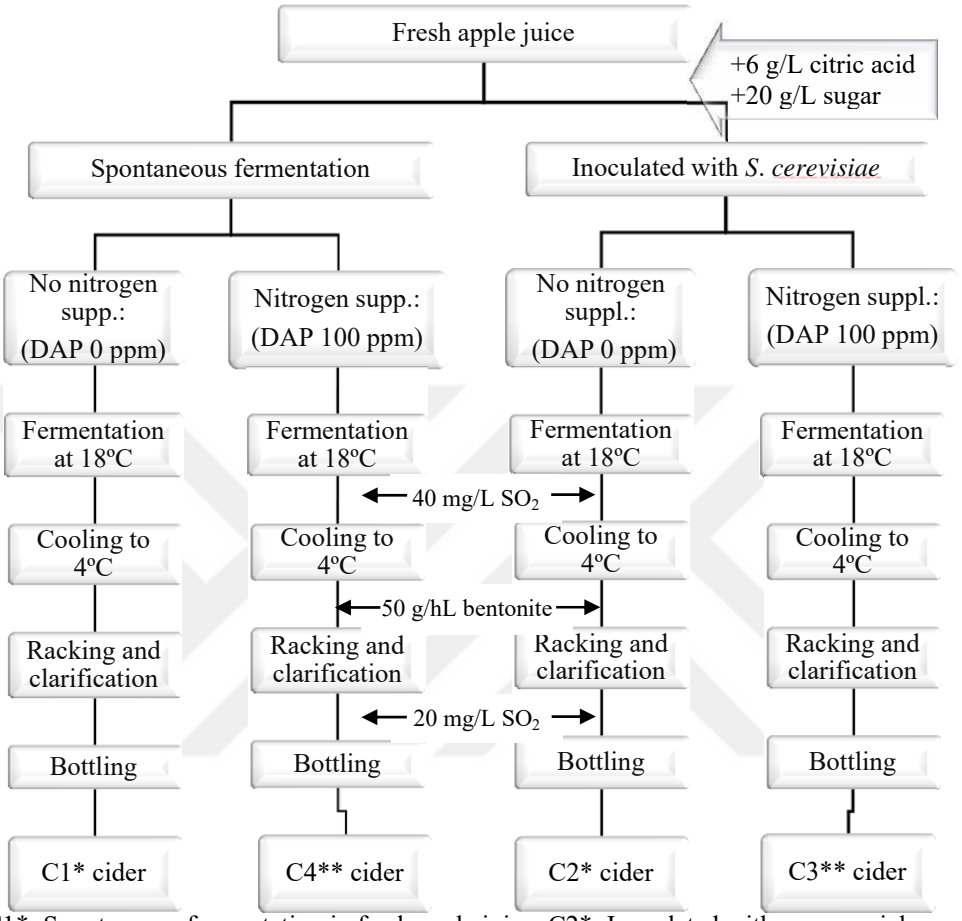
The apple juice (fresh and concentrated) was carried out in 10 L glass vessels. The apple juice concentrate was diluted at 1:6 ratio. 6 g citric acid and 20 g sugar each per litre were added before fermentation. All fermentation vessels were equipped with fermentation locks.

Actiflore BO213 (Laffort, France) commercial dry yeast (*Saccharomyces cerevisiae*) was inoculated as 20 g/hL. The commercial yeast was rehydrated before inoculation. Fermentation conditions were carried out at 18ºC (Fig.3.3).

SG, pH, sugars and enumeration of the yeast population was monitored during fermentation. After finishing fermentation, the ciders were cooled to 4ºC and 40 mg/L sulphur dioxide (5 %, SO_2) was added. Then ciders were racked and transferred to vessels. During the maturation, 50 g/hL bentonite was added (Fig.3.4 and Fig.3.5), racked and 20 mg/L sulphur dioxide (SO_2) was added before bottling (Fig.3.6).

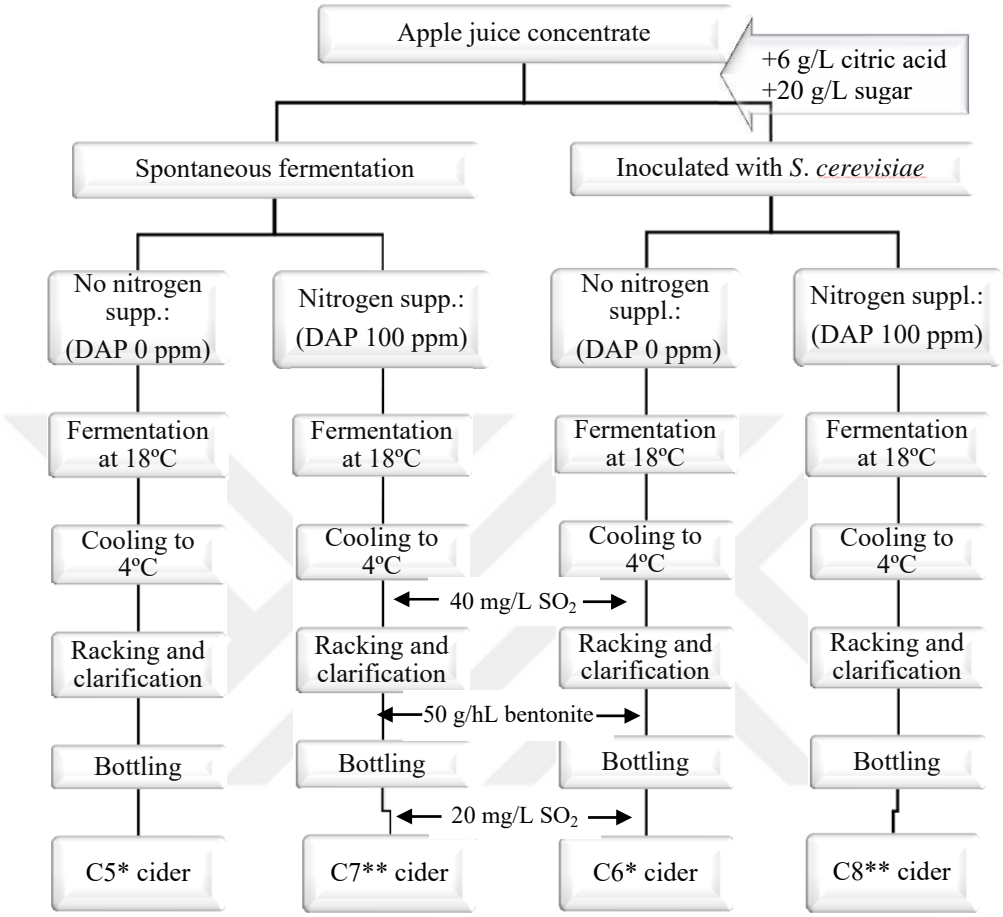
The samples were coded as follows: C1, spontaneous fermentation in fresh apple juice without supplementation of DAP; C2, inoculated with commercial yeast fermentation in fresh apple juice without supplementation of DAP; C3, inoculated with commercial yeast fermentation in fresh apple juice with supplementation of DAP; C4, spontaneous fermentation in fresh apple juice with supplementation of DAP; C5, spontaneous fermentation in apple juice concentrate without supplementation of DAP; C6, inoculated with commercial yeast fermentation in apple juice concentrate without supplementation of DAP; C7, spontaneous fermentation in apple juice concentrate with supplementation of DAP and C8, inoculated with commercial yeast fermentation in apple juice concentrate with supplementation of DAP. From C1 to C4 fermentations were done by fresh apple juice and other fermentations were done by apple juice concentrate.

Figure 3.3. Picture of alcoholic fermentations



C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; *- no supplementation of DAP; **- with supplementation of DAP;

Figure 3.4. Fermentation flowchart of fresh apple juice



C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8**: Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

Figure 3.5. Fermentation flowchart of apple juice concentrate

Figure 3.6. Picture of cider bottling process

3.2.3. Analyses of raw materials

Fresh and concentrated apple juices were analysed as follows:

3.2.3.1. Determination of pH

pH was determined by direct measure in a digital glass pH meter (Mettler Toledo pH/Ion S220) previously calibrated with three standard solutions at pH 4.0, 7.0 and 11.0. According to International Methods of Analysis (OIV-MA-AS2-01A).

3.2.3.2. Determination of specific gravity

Specific gravity was determined by pycnometer at 20°C, according to International Methods of Analysis (OIV-MA-AS2-01A).

3.2.3.3. Determination of total titratable acidity

The total titratable acidity of samples was determined by titrating method, according to International Methods of Analysis (OIV-MA-F1-05, 2011) expressed as malic acid.

3.2.3.4. Determination of browning index

Ethyl alcohol (5mL), was added to 5mL of sample, centrifuged (10 min; 7800 x g), and the absorbance of the supernatant was measured at 420 nm (Cohen., et al 1998).

3.2.3.5. Determination of colour (L*, a*, b*, Hue and Chroma values)

Samples were measured by the "ColorQuest" L*, a*, b* colour values, which was analysed by Hunter Lab. D65 illuminant device (Hunter, 2008).

3.2.3.6. Determination of sugars and organic acids

Sugars (sucrose, glucose, and fructose) and organic acids (citric, malic, lactic and acetic) were determined by HPLC. The liquid chromatographic apparatus (LC-20AD, Shimadzu, Kyoto, Japan) consisted of a pump system, an on-line degasser (DGU-20A_5), a column oven (CTO-10ASVP), a refractive index detector (RID 10A) for sugars, and a UV/Vis detector (SPD-20A) monitored at 210 nm for the analysis of organic acids (OIV-MA-AS311-03, 2003). HPLC conditions are shown in following:

HPLC conditions:

Mobile phase: 0.01 N sulphuric acid (H_2SO_4)

Column: Aminex HPX-87H (300 x 7.8 mm, Bio-Rad, Hercules, CA, USA)

Oven T ^oC: 50^oC

A loop injector: 20 µL

Running time: 30 min

Flow rate of mobile phase: 0.5 mL/min

UV wavelength: 210 nm (for organic acids)

Detector: refractive index detector - RID 10A

3.2.3.6. (1). Extraction procedure

950 µL of sample was mixed with 50µl of perchloric acid ($HClO_4$) and kept at 4℃ for 24 h. One mL of the supernatant was mixed with 85 µL of potassium hydroxide (5M, KOH) and centrifuged (80 RAD, 10 min, at 4℃; "Hettich universal 320R" Germany). Protein agglomerates were removed by centrifugation (80 RAD, 3-5 min, at 4℃). 100 µL of sample was mixed with 900 µL of distilled water (dilution 1:10 for only sugar analyses) and the supernatant was filtered through a 0.45 µm PVDF Syringe Filter (IsoLab) and injected into the HPLC system.

3.2.3.7. Determination of total soluble solids

The total soluble solids (°Brix) was determined using a refractometer RA-130.

3.2.3.8. Determination of free-amino nitrogen (FAN)

FAN was determined by Ninhydrin method (Baker, 1991).

3.2.3.8. (1). Preparation of reagents

a. Ninhydrin colour reagent (NCR)

   10 g of disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) was dissolved with 6 g of potassium dihydrogenphosphate (KH_2PO_4), 0.5 g of ninhydrin and 0.3 g of fructose in a total of 100 mL of distilled water. The pH must be between 6.6-6.8.

b. Dilution solution

   One gram of potassium iodide was dissolved in 300 mL distilled water and added 200 mL of 96 % ethanol.

c. Glycine stock solution

0.1072 g of glycine was dissolved in a total of 100 mL of distilled water. This solution was kept at 0°C.

d. Glycine Standard Solution:

One mL of the glycine stock solution was dissolved in 99 mL of distilled water. This standard solution was contained 2 mg/L amino nitrogen.

3.2.3.8. (2). FAN analysis procedure

One mL of the sample was diluted in 49 mL of distilled water. 2 mL of the diluted sample, the standard solution and the distilled water was pipetted into separate each test tubes. One mL of Ninhydrin colour reagent was added into each test tubes and mixed. Each test tubes were closed loosely in order to avoid evaporative losses and heated at 100 °C for 16 min. The solutions were cooled at 20 °C for 20 min. After that 5 mL of the diluted solution was added into test tubes and the absorbance of the supernatant was measured at 570 nm against the distilled water. Finally measured parameters were calculated in the following formula.

FAN Calculation:

$$\text{FAN [mg/L]} = \frac{AS-AB}{AG-AB} x2xF$$

AS= average absorbance of the sample;

AG = average absorbance of the glycine standard solution;

AB = average absorbance of the blank value (H_2O);

F = dilution factor of the sample;

2 = concentration of the glycine standard solution in mg/L;

3.2.3.9. Determination of Hydroxymethylfurfural (HMF)

HMF was determined by Shimadzu LC-20 AT model HPLC. Samples were extracted by Gökmen and Acar (1998). HPLC conditions are shown in follows:

HPLC conditions:

Mobile phase: Methanol/Water/Acetic acid (20/79/1) isocratic
Column: ACE 5 C18 (250 x 4.6 mm)
Oven T°C: 30°C
A loop injector: 20 µL
Running time: 15 min
Flow rate of mobile phase: 0.5mL/min
UV wavelength: 285 nm
Detector: Photo diode detector – PDA

3.2.3.9. (1). Extraction procedure

5 mL of sample was mixed with 10 mL of ethyl acetate ($C_4H_8O_2$) and poured into the separatory funnel and taken a limpid liquid (1). 10 mL of ethyl acetate was mixed with the sediment liquid and poured into the separatory funnel and taken a limpid liquid (2). 1 and 2 of the limpid liquids were mixed with 5 mL of sodium carbonate (1.5%, Na_2CO_3) and poured into the separatory funnel to taken a limpid liquid (3). 10 mL ethyl acetate was mixed with the sediment liquid and taken a limpid liquid (4). The limpid liquid (3) was mixed with a limpid liquid (4) and filtrated through the paper filter with sodium carbonate. After that solution was evaporated by rotary evaporator at 35℃. 4 mL of distilled water (pH4.0) was added into evaporation flask and dissolved for residues. The dissolved solution was filtrated through 0.45 µm PVDF Syringe Filter (IsoLab) and injected into the HPLC system.

3.2.4. Microbiological analysis

Samples were taken aseptically for yeast counts during fermentation. From each sample, 1 mL of sample was serially diluted as required in 0.1 % peptone water and 0.1 mL of diluted sample was spread on Malt Extract Agar and Lysine agar. Lysine agar was used for count non-*Saccharomyces* yeasts because it is a synthetic medium with glucose, vitamins, inorganic salts and L-lysine as the sole nitrogen source, and *Saccharomyces* are unable to grow on it. Plates were

incubated at 25°C for 3-5 days and total yeasts and non-*Saccharomyces* yeasts were counted. Each plate (MEA and L-lysine agar) was counted and results were expressed as CFU/mL (Campbell, 1988; Fleet, G.H and Heard, G.M, 1993).

3.2.5. Analyses on cider

The experimental ciders were analysed as follows:

3.2.5.1. General analyses

3.2.5.1. (1). Determination of pH

PH was determined by direct measure in a "Mettler Toledo pH/Ion S220" pH meter, according to compendium of international methods of analysis (OIV-MA-AS2-01A).

3.2.5.1. (2). Determination of specific gravity

Specific gravity was determined by hydrometer at 20°C, according to International Methods of Analysis (OIV-MA-AS2-01A).

3.2.5.1. (3). Determination of total titratable acidity

The total titratable acidity of samples was determined by titrating method, according to International Methods of Analysis (OIV-MA-F1-05, 2011) expressed as malic acid.

3.2.5.1. (4). Determination of browning index

5 mL of ethyl alcohol (C_2H_6O) was mixed with 5 mL of apple juice sample, centrifuged (10 min; 7800 x g), and the absorbance of the supernatant was measured at 420 nm (Cohen., et al 1998).

3.2.5.1. (5).Determination of colour (L*, a*, b*, Hue and Chroma values)

Samples were measured by the ColorQuest brand colour measuring system L*, a*, b* values, which was analysed in the Hunter Lab. D65 illuminant device (Hunter, 2008).

3.2.5.1. (6). Determination of sugars and organic acids

Sugars (sucrose, glucose, and fructose) and organic acids (citric, malic, lactic and acetic) were determined by HPLC. The liquid chromatographic apparatus (LC-20AD, Shimadzu, Kyoto, Japan) consisted of a pump system, an on-line degasser (DGU-20A5), a column oven (CTO-10ASVP), a refractive index detector (RID 10A) for sugars, and a UV/Vis detector (SPD-20A) monitored at 210 nm for the analysis of organic acids (OIV-MA-AS311-03, 2003).

3.2.5.2. Determination of alcoholic strength

Alcoholic strength was measured by using Anton Paar DMA 4500 M.

3.2.5.3. Determination of volatile acidity

Volatile acids were determined by "Dujardin-Salleron Vola.2000" automatic extractor, according to International Methods of Analysis (OIV-MA-AS313-02).

3.2.5.4. Determination of free and total sulphur dioxide (SO_2)

Sulphur dioxide was determined by a stream of oxygen. This system was oxidized by sparkling through a dilute and neutral solution of hydrogen peroxide. The sulphuric acid set was circumscribed by titration with a standard solution of sodium hydroxide (OIV-MA-AS323-04A, 2009).

3.2.5.5. Determination of CO_2 content

CO_2 was analysed by volume expansion technique (Anton Paar CarboQC, PFD).

3.2.5.6. Determination of tannins

Total tannins were determined by colorimetric method (Ribéreau-Gayon et al., 2006).

3.2.5.6. (1) Extraction procedure

One mL of the sample was dissolved in 49 mL of distilled water. 4 mL of the diluted sample was poured into 2 test tubes. 2 mL of distilled water was added, 6 mL of hydrogen chloride (12N, HCl) and mixed each test tube. One of the test tubes was heated at 100℃ in a water-bath for 30 min. The other one was not heated. After that heated test tube was cooled down. One mL of ethanol (95%, C_2H_6O) was added into each test tubes. The absorbance in a cuvette was measured at 550 nm. Finally, measured parameters were calculated in the following formula.

$LA_{(g/L)} = (D_2-D_1) \times 19.33$

D_2 : heated sample;

D_1 : not heated sample;

19.33 : corresponds to the molar extinction coefficient of the cyanidin obtained by the acid hydrolysis of the condensed tannins;

3.2.5.7. Determination of extracting solvent for aroma extraction by representative test

Extractions were carried out three different solvents (dichloromethane, pentane-dichloromethane and diethyl ether) in order to conclude the most suitable solvent to be used for the extraction of aroma substances on the ciders. Extraction

of aroma compounds was performed by liquid–liquid extraction (Fig.3.7). 40 mg of 4 nonanol as internal standard and 40 mL of solvent was pipetted into a 500 mL Erlenmeyer flask containing 100 mL of the sample before extraction. The content was mixed at 4°C for 30 min under nitrogen gas with a magnetic stir bar. The mixture was then centrifuged at 4°C (9000 rpm, 15 min). After the dehydration by sodium sulphate anhydrous (Na_2SO_4), the pooled organic extract was reduced to 1 mL using a Vigreux column at 48°C. All processes were repeated three times. The extracts were stored at -20°C in a glass vial equipped with a Teflon-lined cap before the analysis. Each sample was extracted in triplicate and the concentration of volatiles, as 4 nonanol equivalents were obtained as a means of three repetitions (Selli 2007, Selli and Kelebek 2011).

According to the results, the highest value was determined by sensory analysis in terms of aroma similarity and aroma intensity.

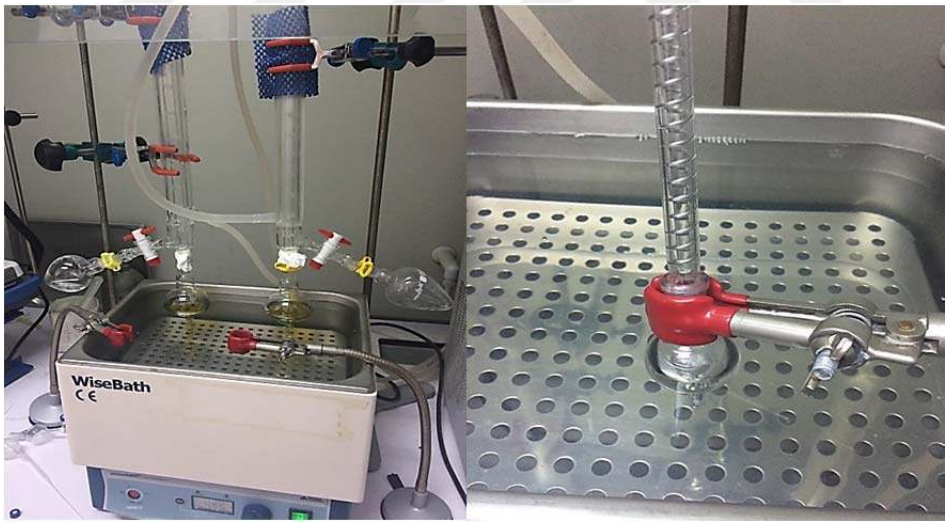


Figure 3.7. Aroma extraction and concentration system

The representative test was performed by a group of 9 panellists. Samples of ciders were presented to the panellists after being specially coded.

Extracts obtained from extractions carried out three different solvents were absorbed by sniffing and then put into coded glasses.

The aroma similarity and aroma intensity characteristics of the solvents were evaluated by panellists on a scale of 10 cm (Fig.3.8) in the representative test. The results are given in Table 3.1.

Table 3. 1. The result of the representative test.

Attributes	Solvent		
	Diethyl ether	Dichloromethane	Pentane-Dichloromethane
Aroma similarity	26.4	**77.8**	54.2
Aroma intensity	51.1	**69.1**	58.7

As seen from Table 3.1, dichloromethane of extracting solvent was the highest aroma similarity and aroma intensity score with 77.8 and 69.1. Considering this result, it was decided to use dichloromethane solvent for the aroma extraction.

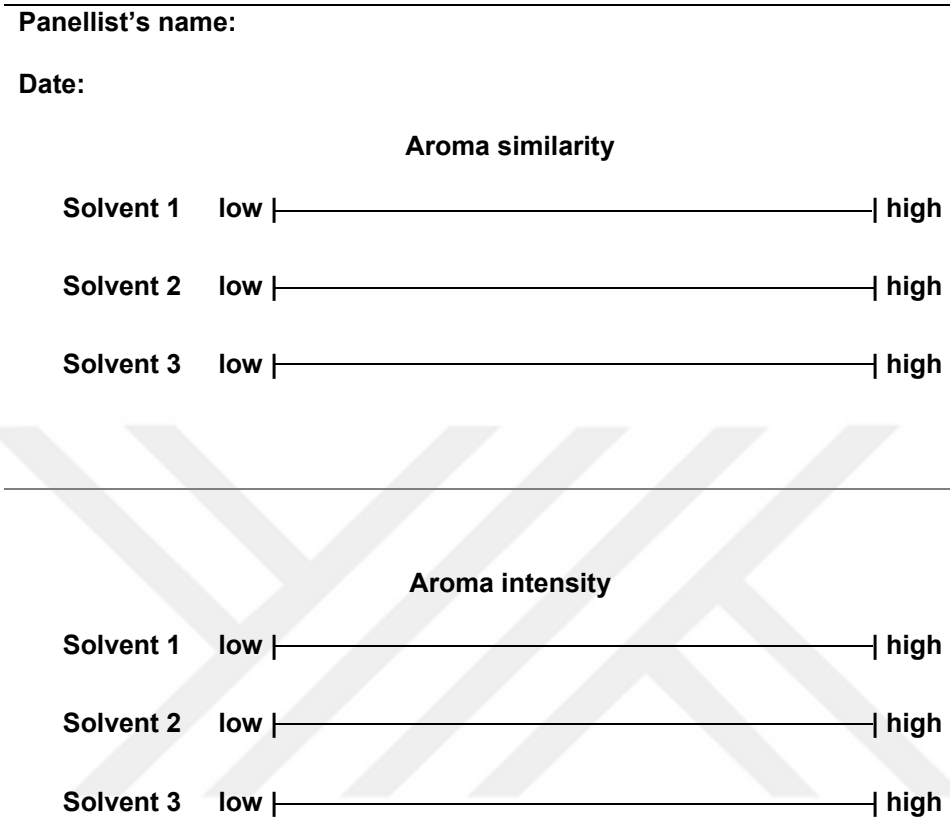


Figure 3.8. Representative test form

3.2.5.8. Determination of volatile aroma compounds (GC-FID, GC-MS)

The prepared samples were analysed by GC-FID, GC-MS. Analyses were executed three times for each sample (Priser et al., 1997, Selli 2007, Selli and Kelebek 2011).

3.2.5.8. (1) GC-FID, GC-MS conditions

Samples were analysed by using a gas chromatography "Agilent 6890N", Mass spectrometry "Agilent 5975B VL MSD" and olfactometry "Gerstel ODP-2".

In this system, the column output was equally divided by a special switch (Dean Switch). The first part was executed by the FID, the second part was executed by the MSD and the third part was executed by the olfactometer. At the same time, the sensitivity of the analysis was increased by carrying out quantification, identification and olfaction.

GC-FID analysis: The aroma compounds were measured using an "Agilent 6890N" with flame ionization detector at 250°C and a fused capillary column coated with DB-Wax (30 m X 0.25 mm) and 0.25 μm film thickness. The carrier gas was 'He' at a flow rate of 3.3 mL/min. Injector temperature was programmed at 220°C, detector temperature was at 250°C. The oven temperature was kept at 40°C for 4 min, and then increased to 250°C at 2°C/min. It was increased from 220 to 250°C at 2°C/min and was kept at 250°C for 20 min. One microliter of the sample was injected for each analysis.

GC-MS analysis: The aroma compounds was identified by GC-MS. "Agilent 5975B VL MSD" chromatograph was used with the column mentioned above. The temperature programs of the injector and oven were as described above. The flow rate of helium gas as the carrier was 3.3 mL/min. A mass spectrometer equipped with a quadruple detector was used for electron impact (EI). The temperature was 250°C. EI was recorded at 70 eV in the range 29–350 m/e at 1 sec intervals. The compounds were identified by comparing their retention times with those of reference compounds and by mass spectrum matching with database library spectra.

3.2.5.8. (2) Calculation of aroma concentration

Amounts of aroma substances after the identification of the pigments was calculated by the internal standard method using the following formula.

$C_c = (P_c / P_{in}) \times C_{in} \times RF \times CF$

C_c : Concentration of compound;

P_c : Peak area of the compound;

P_{in} : Peak area of internal standard;

C_{in} : Concentration of the internal standard (40 μg/100 mL)

RF : Response factor;

CF : Calculation factor (factor for conversion of sample quantity to litre: 10);

3.2.5.9. Sensory analysis

In this study, a sensory analysis was conducted using a panel of 11 panellists on aroma and flavour profile analysis using for 10 points, which is scored by the lowest (1 point) and the highest (10 points).

Form of sensory analysis is shown in Fig.3.9 and Fig.3.10. Sensory analysis was used by Cider Institute of North America (CINA). Samples of cider are shown in Fig.3.11 for using in sensory analysis.

Characteristics	The lowest ⟷ The highest
Fruity (apples/pears)	
Floral	
Spicy	
Caramelize	
Herbaceous	
Vegetative	
Chemical	
Piquant	

Figure 3.9. Aroma profile analysis form

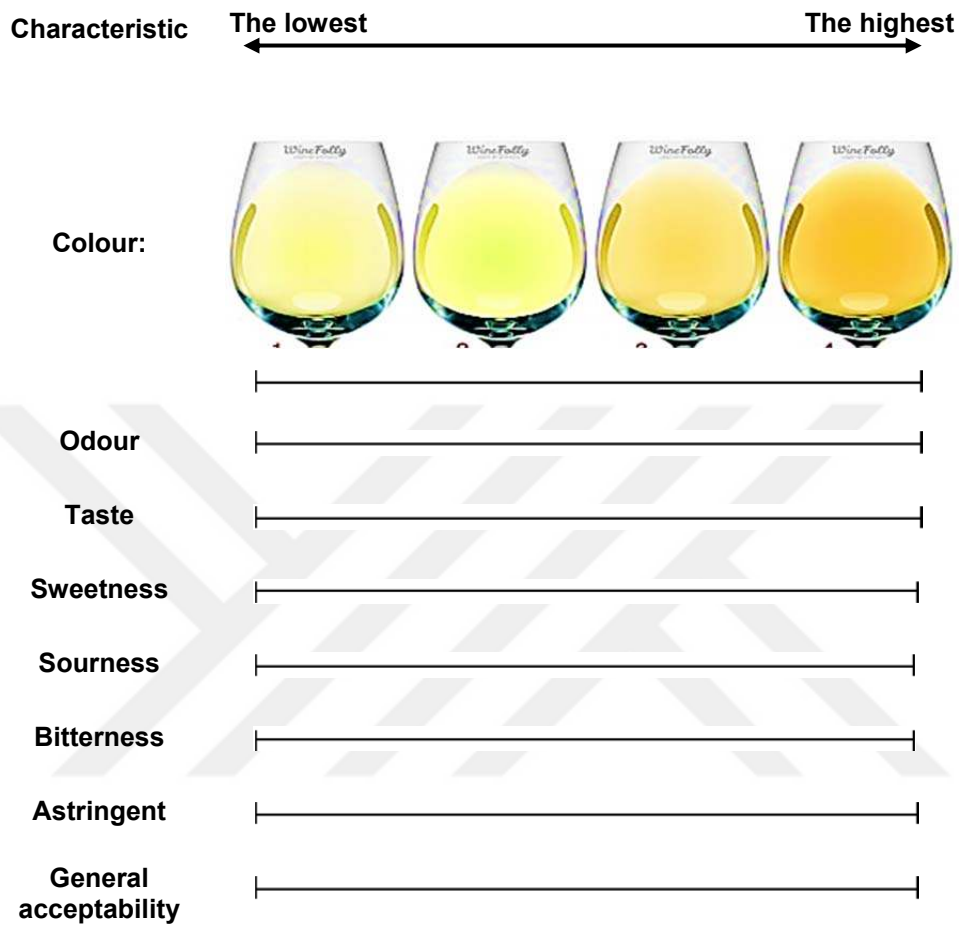


Figure 3.10. Flavour profile analysis form

Figure 3.11. Picture of experimental ciders for using in sensory analysis

3.2.5.10. Data analysis

All experiment data were reported that mean ± SD. The data were examined statistically by ANOVA and pair comparison of treatment means was achieved using Tukey's procedure at $P<0.05$ using the statistical software SPSS 20.0 for Windows.

4. RESULTS AND DISCUSSION

4.1. General composition of raw materials used in experiment

4.1.1. General composition of fresh apple juice

General composition of the fresh apple juice and adjusted juice is presented in Table 4.1. The fresh apple juice was supplemented with 20 gram of sugar (for final cider alcoholic reached to 8.5%), 100 ppm of DAP (Kelkar and Dolan, 2012) and 6 gram of citric acid per litre (Canbas et al., 2000) for preparing to cider fermentation. Samples of cider were analysed pH, free-amino nitrogen (FAN), total sugars and total titratable acidity (TTA). Because, they are essential values for alcoholic fermentation (Andrew et al., 2003). Significant differences ($p<0.05$) were observed amongst the examples as can been seen in Table 4.1.

Table 4.1. Composition of the fresh apple juice

Composition	Fresh juice	Adjusted juice
TSS (°brix)	12.8 ± 0.05	15.0 ± 0.06
pH	3.77 ± 0.06	3.48 ± 0.01
TTA[a] (g/L)	2.52 ± 0.18	7.05 ± 0.15
Total sugars (g/L)	132.4 ± 0.08	155.36 ± 0.06
SG	1.052 ± 0.18	1.060 ± 0.15
BI	0.1431 ± 0.0012	0.1683 ± 0.0011
FAN (mg/L)	68.96 ± 0.14	108.48 ± 0.16
HMF (mg/L)	0.031± 0.011	-
F (mg/L)	0.024 ± 0.002	-
Colour values:		
L*	33.90 ± 0.21	33.19 ± 0.19
a*	-1.33 ± 0.13	-1.53 ± 0.23
b*	6.76 ± 0.12	4.97 ± 0.18
Hue	-78.86 ± 0.18	-72.88 ± 0.20
Chroma	6.89 ± 0.25	5.22 ± 0.28

Data were reported as mean ± standard deviation of 3 replications. Results are the means of three repetitions. a: expressed as malic acid; TSS: Total soluble solids; TTA: Total titratable acidity; SG: Specific gravity; BI: Browning index; FAN: Free-amino nitrogen; HMF: Hydroxymethylfurfural; F: Furfural;

General composition of fresh apple juice is shown in Table 4.1. The general compositions were: total soluble solids, 12.8 ºBrix; pH, 3.77; total titratable acidity, 2.52 g/L (as malic acid); total sugars, 132.4 g/L; specific gravity, 1.052; browning index, 0.1431; free-amino nitrogen, 68.96 mg/L; hydroxymethylfurfural, 0.031 mg/L; furfural, 0.024 mg/L; colour L*, 33.90; a*, -1.33; b*, 6.76; Hue value, -78.86; Chroma value, 6.89.

The composition of the fresh apple juice (adjusted juice) was used in alcoholic fermentation as follows: total soluble solids, 15.0 ºBrix; pH, 3.48; total titratable acidity, 7.05 g/L (as malic acid); total sugars, 155.36 g/L; specific gravity, 1.060; browning index, 0.1683; free-amino nitrogen, 108.48 mg/L; colour L*, 33.19; a* , -1.53; b*, 4.97; Hue value, -72.88; Chroma value, 5.22.

The criteria for the evaluation of must was based on their oenological characteristics, as well as pH, total sugars, total titratable acidity and free amino nitrogen. In various studies pH values on cider musts were determined between 3.41-3.87, total sugars, 63.4-166g/L; total titratable acidity, 2.41-9.92g/L; free amino nitrogen, 59.00-155.81mg/L; colour L*, 51.66; a*, -1.56; b*, 15.77; (Canbaş et al., 2000; Rita et al., 2011; Li et al., 2015; Lobo et al., 2018).

4.1.2. General composition of apple juice concentrate

The general composition of apple juice concentrate is shown in Table 4.2. The general compositions were: total soluble solids, 15.0 ºBrix; pH, 3.78; total titratable acidity, 2.76 g/L (as malic acid); total sugars, 158.6 g/L; specific gravity, 1.061; browning index, 0.2468; free-amino nitrogen, 61.34 mg/L; hydroxymethylfurfural, 0.41 mg/L; furfural, 0.31 mg/L; colour L*, 36.90; a*, -0.82; b*, 15.42; Hue value, -86.95; Chroma value, 15.44.

Table 4.2. Composition of apple juice concentrate (AJC)

Composition	AJC	Adjusted juice
TSS (°brix)	15.0 ± 0.12	14.6 ± 0.11
pH	3.78 ± 0.08	3.50 ± 0.01
TTA[a] (g/L)	2.76 ± 0.13	6.45 ± 0.18
Total sugars (g/L)	158.6 ± 0.1	154.1± 0.2
SG	1.061 ± 0.08	1.059 ± 0.08
BI	0.2468 ± 0.0011	0.2395 ± 0.0014
FAN (mg/L)	61.34 ± 0.13	102.92 ± 0.12
HMF (mg/L)	0.41 ± 0.009	-
F (mg/L)	0.31 ± 0.003	-
Colour values:		
L*	36.90 ± 0.18	32.70 ± 0.15
a*	-0.82 ± 0.11	-0.59 ± 0.12
b*	15.42 ± 0.05	14.63 ± 0.1
Hue	-86.95 ± 0.21	-87.69 ± 0.19
Chroma	15.44 ± 0.04	14.64 ± 0.07

Data were reported as mean ± standard deviation of 3 replications. [a]: expressed as malic acid; TSS: Total soluble solids; TTA: Total titratable acidity; SG: Specific gravity; BI: Browning index; FAN: Free-amino nitrogen; HMF: Hydroxymethylfurfural; F: Furfural;

The composition of concentrated apple juice (adjusted juice) used in alcoholic fermentation as follows: total soluble solids, 14.6 ºBrix; pH, 3.57; total titratable acidity, 6.45 g/L (as malic acid); total sugars, 154.1 g/L; specific gravity, 1.059; browning index, 0.2395; free-amino nitrogen, 102.92 mg/L; colour L*, 32.70; a*, -0.59; b*, 14.63; Hue value, -87.69; Chroma value, 14.64.

In various studies pH values on the musts were determined between 3.41-3.87, total sugars, 63.4-166g/L; total titratable acidity 2.41-9.92g/L, free-amino nitrogen, 59.00-155.81mg/L; colour L*, 51.66; a*, -1.56; b*, 15.77 (Canbas et al., 2000; Rita et al., 2011; Li et al., 2015; Lobo et al., 2018).

4.2. Fermentation process

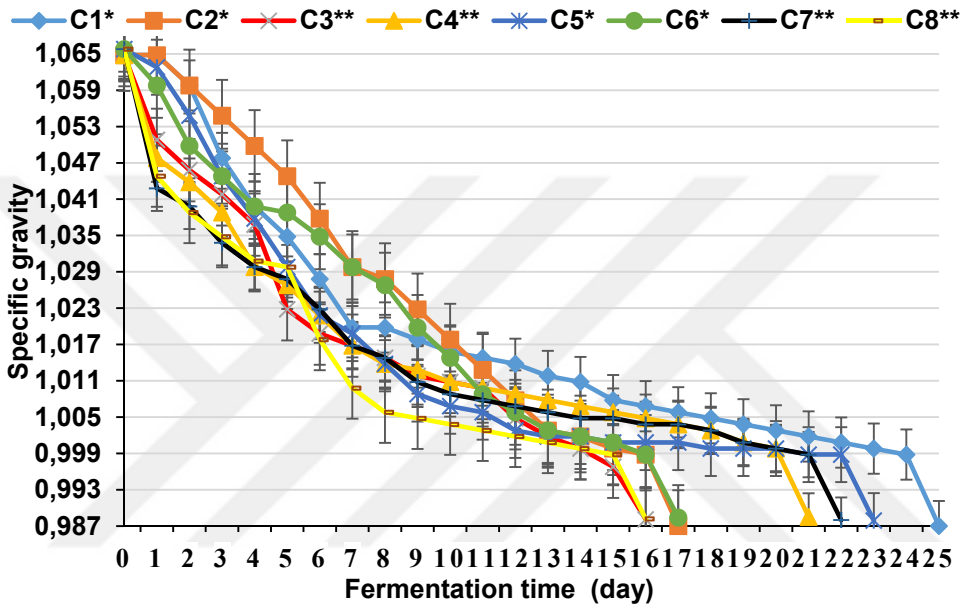
Fermentation processes were shown in Figure 3.4 and Figure 3.5. Alcoholic fermentations were daily monitored by a drop in specific gravity and pH. Enumeration of the yeast population was also done. Alcoholic fermentations were carried out at 18°C. The fermentations were coded as C1, C2, C3, C4, C5, C6, C7 and C8, with denoting cider. From C1 to C4 fermentation was done by fresh apple juice and other fermentations were done by apple juice concentrate. Alcoholic fermentations are shown in Figure 4.1.

Figure 4.1. Picture of alcoholic fermentation

4.2.1. The change in specific gravity and sugar content during fermentation

As seen from Figure 4.2, the specific gravity of apple musts was 1.065 at the beginning. Nitrogen addition led to increase for the utilisation of sugar. The specific gravity dropped faster in nitrogen added musts compared to others. C3 and C8 fermentations were completed on day 16.

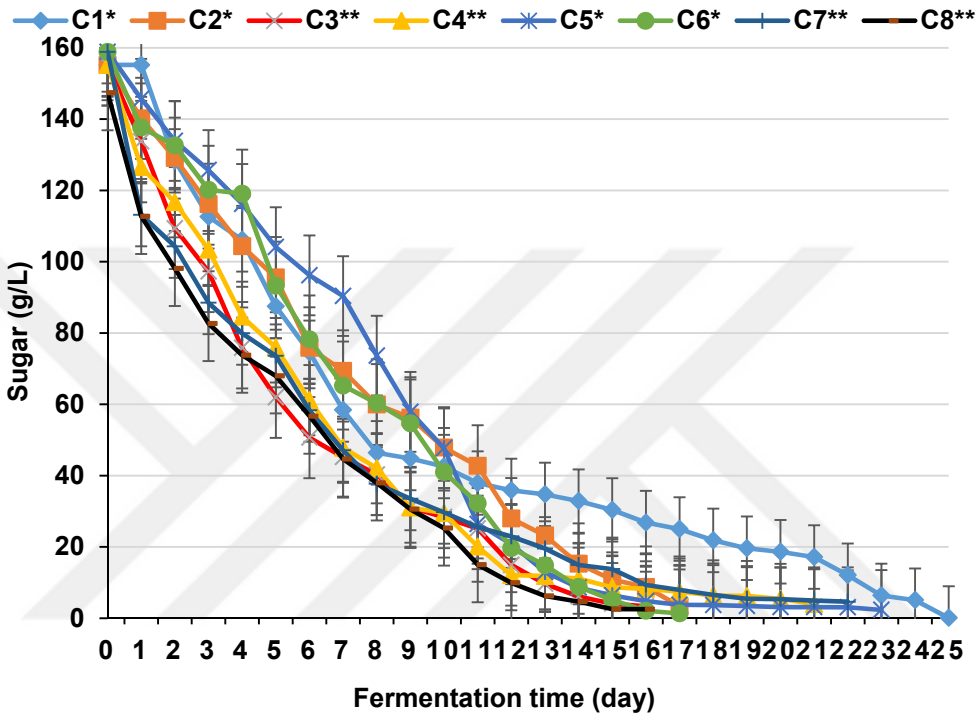
The specific gravity was slightly decreased during alcoholic fermentation. Finally, SG was reached to 0.9872-0.9884. The change in specific gravity of apple musts during fermentation is shown in Fig.4.2.



Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8**: Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;
Figure 4.2. The change in specific gravity of apple musts during fermentation

The change in sugar content of apple musts during fermentation is given in Figures 4.3. Initial sugar of fresh and concentrated apple musts varied between 155.36 g/L and 154.1 g/L at the beginning. Total sugars varied between 1.59g/L and 5.38g/L at the end of fermentation. The growth of fermentation was depended

on the supplementation of DAP. The all fermentations were completed between 16 to 25 days.



Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;
Figure 4.3.The change in sugar content of apple musts during fermentation

4.2.2. Microbiology development on the cider fermentation

The spontaneous fermentation no supplementation of nitrogen in the must showed an initial count ranged from 3.48 log CFU/mL to 3.71 log CFU/mL and reached to maximum count ranged from 6.82 log CFU/mL to 6.90 log/mL of total

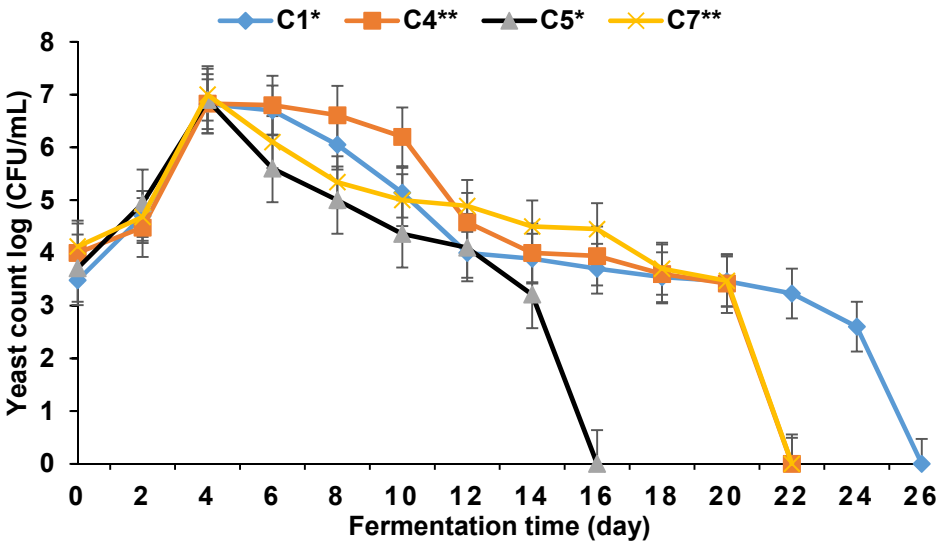
non-*Saccharomyces* yeasts. The spontaneous fermentation with supplementation of nitrogen in the must gave an initial count ranged from 4.00 log CFU/mL to 4.12 log CFU/mL and reached to maximum count ranged from 6.83 log CFU/mL to 7.0 log/mL of total non-*Saccharomyces* yeasts. Amounts of these yeasts grew up slowly at a long stationary phase in the must inoculated with *S. cerevisiae* with supplementation of nitrogen. The non-*Saccharomyces* species, however, died off by day 6 and 7. Development of total non-*Saccharomyces* yeasts in the must during spontaneous fermentation is shown in Figure 4.4.

Must inoculated by commercial yeast without nitrogen addition had the total yeast count ranged from 5.94 log CFU/mL to 6.49 log CFU/mL at the beginning of fermentation and reached to maximum count ranged from 7.19 log CFU/mL to 7.61 log CFU/mL on day 2, respectively. A total of non-*Saccharomyces* yeasts ranged from 5.30 log CFU/mL to 5.40 log CFU/mL and reached to maximum count ranged from 6.13 log CFU/mL and 7.0 log CFU/mL on day 2, respectively.

Must inoculated by commercial yeast with supplementation of nitrogen had the total yeast count ranged from 6.30 log CFU/mL to 6.65 log CFU/mL at the beginning of fermentation and reached to maximum count ranged from 9.54 log CFU/mL to 9.62 log CFU/mL on day 2, respectively. A total of non-*Saccharomyces* yeasts ranged from 5.12 log CFU/mL to 5.83 log CFU/mL at the beginning of fermentation and reached to maximum count ranged from 6.08 log CFU/mL and 6.28 log CFU/mL on day 2, respectively. Development of total yeasts and the total non-*Saccharomyces* yeasts in the must inoculated with commercial yeast during fermentation is shown in Figure 4.5

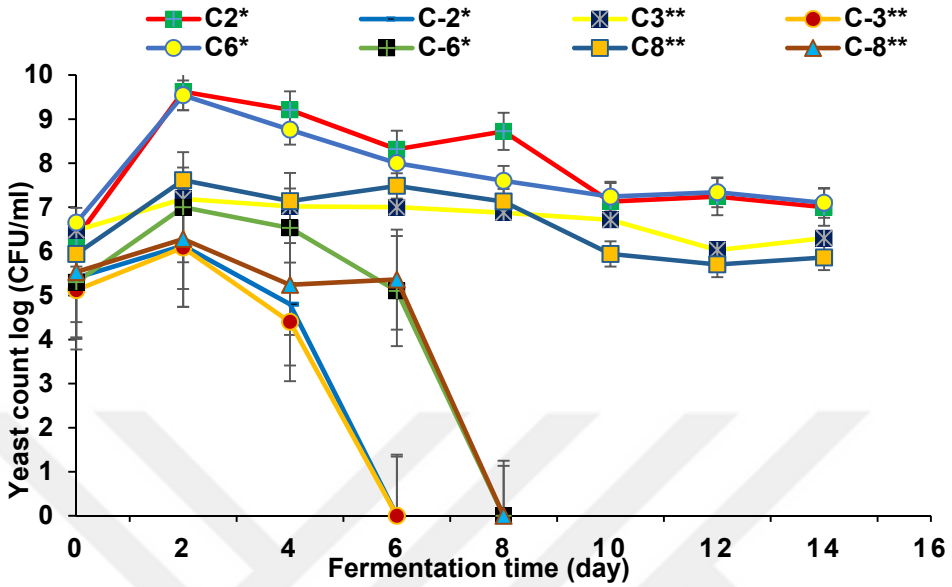
Alberti, et al., (2011) reported that the yeast population was reached a maximum of 8.66 x 10^7 cell/mL in 1.56 days. It was higher than the number usually found as 2 to 4 x 10^7 cell/mL.

Seguinot, et al., (2018) reported that the yeast population of additional DAP fermentation was reached a maximum of 6.79 x 10^7 cell/mL.



C1*: Spontaneous fermentation in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; *- no supplementation of DAP; **- with supplementation of DAP;

Figure 4.4. During the spontaneous fermentation of total non-*Saccharomyces* yeasts in apple must



C2*: Total yeast of the fresh apple juice inoculated with commercial yeast; C-2*: Total non-*Saccharomyces* yeasts of the fresh apple juice inoculated with commercial yeast; C3**: Total yeast of the fresh apple juice inoculated with commercial yeast; C-3**: Total non-*Saccharomyces* yeasts of the fresh apple juice inoculated with commercial yeast; C6*: Total yeast of apple juice concentrate inoculated with commercial yeast; C-6*: total non-*Saccharomyces* yeasts of apple juice concentrate inoculated with commercial yeast; C8**: Total yeast of apple juice concentrate inoculated with commercial yeast; C-8**: total non-*Saccharomyces* yeasts of apple juice concentrate inoculated with commercial yeast; *- no supplementation of DAP; **- with supplementation of DAP;

Figure 4.5. During the inoculated fermentation development of total yeasts and total non-*Saccharomyces* yeasts in apple must

4.2.3. The change in pH during fermentation

At higher pH the fermentation was controlled to microbial infection and at pH 4.0 or higher this could lead to off flavour problems. Many traditional bittersweet cider apples tend to be high in pH which is why they need blending with more acid fruit, preferably before fermentation (Andrew and Lea, 1997). Most ciders have a pH of between 3.3 and 4.1 (Russell, 1997).

The change in pH of apple juices during fermentation is shown in Figure 4.6. The pH values of the samples were determined 3.48 at the beginning of alcoholic fermentations. The pH values were slightly decreased and ranged from 3.12-3.26 at the end of the fermentation (Table 4.3). The lowest pH level was measured in the C3 as 3.12. The highest pH level was measured in C6 as 3.27. Considering that the highest total acidity value can be explained by the lowering of the pH in these experiments.

Table 4.3. pH levels of the experimental ciders

Cider	pH
C1*	3.25±0.01
C2*	3.22±0.01
C3**	3.12±0.01
C4**	3.13±0.01
C5*	3.26±0.02
C6*	3.27±0.03
C7**	3.25±0.01
C8**	3.24±0.02

Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

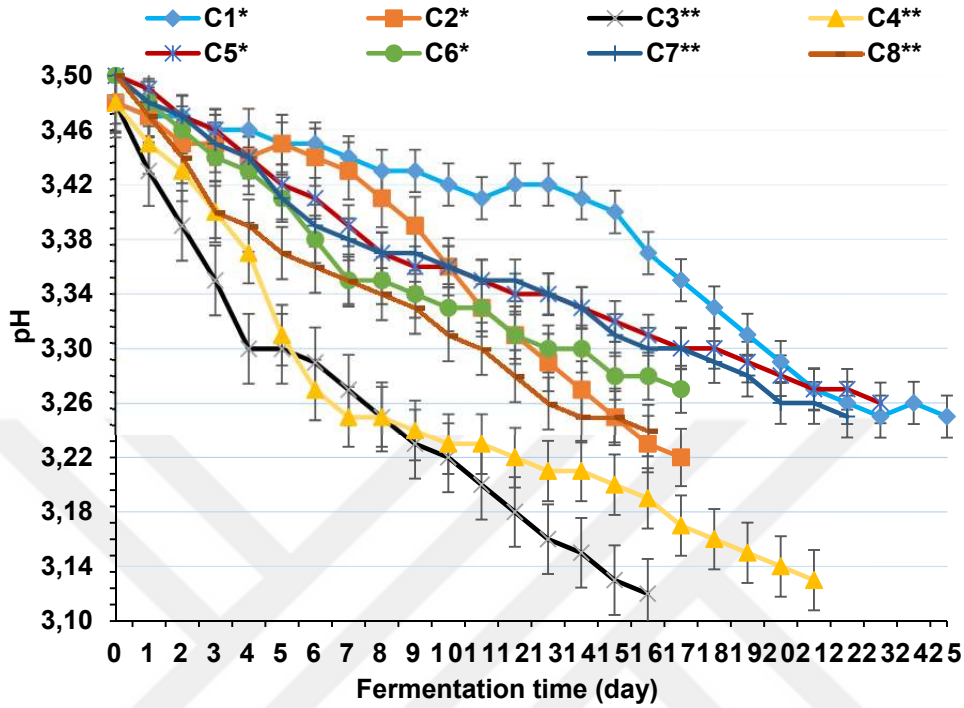


Figure 4.6. The change in pH of apple juices during fermentation
Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

The level of pH was determined between 2.71-3.27 (Hernandez-orte et al., 2006; Torrea et al., 2011).

Bozoğlu et al., (2015) reported that the highest amount of ethanol (8.25% v/v) was reached at pH 3 and 18°C. When pH value was at 4 and 3, the changes in ethanol with the difference in temperatures were decreased by 1.05 % (v/v) and 0.4 % (v/v).

4.3. General composition of ciders

Samples of cider were analysed as pH, total titratable acidity (TTA), browning index (BI), tannins, alcoholic strength (ABV, %), specific gravity (SG), carbon dioxide (CO_2, %), sulphur dioxide (SO_2, mg/L), volatile acid, total and residual sugars, organic acids (citric, malic, lactic, acetic), and colour values (L*, a*, b*, Hue and Chroma). Because these values are significant for alcoholic drinks (Andrew et al., 2003). Significant differences ($p<0.05$) were observed amongst the examples for all variables. General composition of ciders is given in Table 4.4 and 4.5.

As seen from Table 4.4 and 4.5, the value of PH varied from 3.12-3.27; total titratable acidity, 6.65-7.01 g/L (as malic acid); browning index, 0.0493-0.1641; tannins, 0.046-0.378 g/L; alcoholic strength, 8.13-8.75 %; specific gravity, 0.9868-0.9887; carbon dioxide, 0.05-0.245%; sulphur dioxide, 30.84-42.68 mg/L; volatile acid, 0.22-0.74 g/L total sugars, 1.59-5.38 g/L; residual sugars, 1.97-5.29 g/L, citric acid, 0.08-0.11 g/L, malic acid, 2.96-4.25 g/L, lactic acid, 0.23-0.59 g/L, acetic acid, 0.01-0.95 g/L; colour L*, 41.28-75.41; a* -0.18-(-2.42); b*, 3.28-10.63; Hue value, 66.00-86.90; Chroma value, 3.29-10.82.

Table 4.4. The general composition of the experimental ciders

Analysis	C1*	C2*	C3**	C4**
pH	3.25±0.01	3.22±0.01	3.12±0.01	3.13±0.01
TTA[a] (g/L)	6.87±0.02	6.90±0.01	7.01±0.02	6.99±0.01
BI	0.0493±0.0001	0.0546±0.0009	0.0507±0.0006	0.0614±0.0002
Tannins (g/L)	0.249±0.005	0.378±0.004	0.137±0.004	0.249±0.006
ABV (%)	8.75±0.01	8.55±0.01	8.53±0.01	8.49±0.01
SG	0.9868±0.0002	0.9873±0.0001	0.9882±0.0002	0.9883±0.0001
CO_2 %(w/w)	0.245±0.02	0.191±0.03	0.138±0.01	0.196±0.04
SO_2 (mg/L)	30.84±0.04	36.48±0.03	38.43±0.03	37.92±0.05
Volatile acid (g/L)	0.55±0.01	0.26±0.04	0.31±0.03	0.53±0.03
Sugars (g/L)				
Total	4.52±0.06	5.38±0.03	1.59±0.04	2.92±0.07
Residual	4.50±0.04	5.29±0.01	1.49±0.05	2.69±0.04
Organic acids (g/L)				
Citric acid	-	-	-	-
Malic acid	3.28±0.09	2.96±0.06	3.91±0.06	3.98±0.05
Lactic acid	0.59±0.04	0.43±0.08	0.23±0.05	0.43±0.06
Acetic acid	0.02±0.06	0.03±0.09	0.02±0.04	0.01±0.09
Colour analysis				
L*	59.61±0.16	75.41±0.11	51.74±0.15	44.66±0.18
a*	-0.25±0.1	-1.09±0.09	-0.29±0.17	-0.18±0.13
b*	4.62±0.06	4.48±0.18	4.25±0.13	3.28±0.07
Hue	86.90±0.17	76.32±0.12	86.09±0.08	86.85±0.15
Chroma	4.63±0.03	4.61±0.08	4.26±0.11	3.29±0.1

Data were reported as mean ± standard deviation of 3 replications. a: expressed as malic acid; TTA: Total titratable acidity; BI: Browning index; ABV: Alcohol by volume; SG: Specific gravity; CO_2: Carbon dioxide; SO_2: Sulphur dioxide; C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; *- no supplementation of DAP; **- with supplementation of DAP;

Table 4.5. The general composition of the experimental ciders

Analysis	C5*	C7**	C6*	C8**
pH	3.26±0.02	3.25±0.01	3.27±0.03	3.24±0.02
TTA[a] (g/L)	6.86±0.04	6.75±0.02	6.65±0.03	6.67±0.01
BI	0.1332±0.0005	0.1593±0.0004	0.1588±0.0005	0.1641±0.0004
Tannins (g/L)	0.086±0.004	0.085±0.001	0.046±0.002	0.054±0.003
ABV (%)	8.31±0.02	8.37±0.01	8.13±0.02	8.19±0.02
SG	0.9885±0.0005	0.9884±0.0006	0.9887±0.0002	0.9887±0.0004
CO_2 %(w/w)	0.05±0.04	0.143±0.01	0.06±0.02	0.102±0.01
SO_2 (mg/L)	42.68±0.08	38.2±0.09	36.33±1.01	37.3±1.04
Volatile acid (g/L)	0.22±0.08	0.74±0.07	0.27±0.03	0.62±0.04
Sugars (g/L)				
Total	2.92±0.05	4.18±0.06	2.13±0.03	2.52±0.07
Residual	2.78±0.01	4.05±0.02	1.97±0.04	2.45±0.08
Organic acids (g/L)				
Citric acid	0.11±0.03	0.09±0.04	0.08±0.01	0.10±0.02
Malic acid	4.22±0.08	4.12±0.06	4.25±0.03	3.80±0.04
Lactic acid	0.31±0.04	0.31±0.07	0.48±0.07	0.53±0.03
Acetic acid	0.37±0.05	0.25±0.03	0.95±0.08	0.91±0.03
Colour analysis				
L*	47.31±0.11	55.41±0.08	49.19±0.18	41.28±0.05
a*	-2.19±0.07	-2.03±0.17	-2.42±0.1	-0.67±0.12
b*	4.92±0.14	10.63±0.14	7.58±0.13	9.94±0.09
Hue	66.00±0.18	79.18±0.11	72.29±0.12	86.14±0.17
Chroma	5.38±0.05	10.82±0.1	7.95±0.16	9.96±0.13

Data were reported as mean ± standard deviation of 3 replications. a: expressed as malic acid; TTA: Total titratable acidity; BI: Browning index; ABV: Alcohol by volume; SG: Specific gravity; CO_2: Carbon dioxide; SO_2: Sulphur dioxide; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8**: Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

In various studies pH values of ciders were determined between 3.5-3.7, total sugars, 3.4-4.2 g/L; total titratable acidity 2.23-7.6 g/L (as malic acid), alcoholic strength, 2.50-12.30 %; specific gravity, 0.9962-1.0404; carbon dioxide, 0.05-0.245 %; sulphur dioxide, 26-228 mg/L; volatile acid, 1.1-13.0 g/L; total sugars, 1.53.4-101.6 g/L; residual sugars, 2.6-82.3 g/L, colour L*, 51.66; a*, -1.56; b*, 15.77; (Canbaş et al., 2000; Rita et al., 2011; Torrea et al., 2011; Li et al., 2015; Lobo et al., 2018).

4.3.1. Total titratable acidity

The total acid-related well to the perception of acid flavour, while the pH relates better to various aspects of fermentation biochemistry. If the total acid is too high, the pH will be low enough to safeguard against contamination but the final cider will be unacceptably sharp to the palate and may never be pleasant to drink (Andrew and Lea, 1997).

In this study, the amount of total titratable acidity is shown in Table 4.6. The amount of the samples were determined between 6.65-7.01 g/L (as malic acid) at the end of the fermentation. The lowest and highest amount of total titratable acidity was determined in C7 and C3 cider.

In other studies, the amount of total titratable acidity was determined between 2.23 and 7.6 g/L (Torrea et al., 2011; Braga et al., 2013; Qin et al., 2018).

Table 4.6. The amount of total titratable acidity in the experimental ciders

Cider	TTA[a], g/L
C1*	6.87±0.02
C2*	6.90±0.01
C3**	7.01±0.02
C4**	6.99±0.01
C5*	6.86±0.04
C6*	6.75±0.02
C7**	6.65±0.03
C8**	6.67±0.01

Data were reported as mean ± standard deviation of 3 replications. a: expressed as malic acid; C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

4.3.2. Tannins

Tannins describe as basic flavour compounds in cider. Since improving the tannin content in the cider needed for optimal success, the tannins or polyphenols of apples were largely implicated in cider quality (Sanoner, 1999). The level of tannins occurred more astringency-taste in the ciders. Bitterness is expressed as taste, whereas astringency is represented by mouth feel (Mitchell 2006).

The amount of tannins was determined from 0.046 to 0.378 g/L. The highest and lowest content of tannins was determined in C2 and C6 cider (Table 4.7).

Zhao et al., (2014) in their study reported that tannins was found 0.259 g/L.

Table 4.7. Amount of tannins in ciders

Cider	Tannins, g/L
C1*	0.249±0.005
C2*	0.378±0.004
C3**	0.137±0.004
C4**	0.249±0.006
C5*	0.086±0.004
C6*	0.046±0.001
C7**	0.085±0.002
C8**	0.054±0.003

Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

4.3.3. Alcohol strength

Alcohol strength of the experimental ciders are shown in Table 4.8. The ethanol content of the ciders ranged from 8.13 to 8.75 % (v/v). In various studies, alcohol strength of cider were determined between 4.5% (v/v) and 10.9% (v/v) (Anton et al., 2014; Peng et al., 2015; Qin et al., 2018).

Table 4.8. Alcoholic strength of ciders.

Cider	ABV (%)
C1*	8.75±0.01
C2*	8.55±0.03
C3**	8.53±0.01
C4**	8.49±0.02
C5*	8.31±0.02
C6*	8.13±0.03
C7**	8.37±0.01
C8**	8.19±0.03

Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

4.3.4. Carbon dioxide (CO_2)

Content of carbon dioxide (CO_2) in experimental ciders are shown in Table 4.9. Sugar is converted to ethanol and carbon dioxide (CO_2) and other compounds (Donalies et al., 2008). Carbon dioxide were determined between 0.05 % and 0.196 % (w/w). The lowest and highest carbon dioxide of the cider was determined in C5 cider and C4 cider.

Alberti, et al., (2011) in their study reported that carbon dioxide were determined between 0.03 g/mg and 0.07 g/mg.

Table 4.9. Content of carbon dioxide (CO_2) in experimental ciders

Cider	CO_2 %(w/w)
C1*	0.145±0.002
C2*	0.121±0.001
C3**	0.138±0.001
C4**	0.196±0.003
C5*	0.05±0.005
C6*	0.06±0.001
C7**	0.143±0.004
C8**	0.102±0.003

Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

4.3.5. Sulphur dioxide (SO_2)

According to Figure 3.1, Sulphur dioxide were added into apple juice, end of fermentation and before bottling. Once sulphur dioxide dissolves in the juice, it converts into a pH-dependent mixture of bisulphite, sulphite ions, and molecular sulphur dioxide. The "unbound" sulphur dioxide provides the antimicrobial environment in the juice, while the bisulphite and sulphite ions contribute to flavour. The quantity of sulphur dioxide needed to inhibit microbial activity is directed related to the pH of the juice; lower pH means less should be added, while higher pH juice requires more. Common cider producers add sulphur dioxide directly after pressing and juicing but before fermentation. However, in some cases, it can be added afterwards to act as an antioxidant or stabilizer. This prevents the finished cider from releasing hydrogen peroxide or aldehydes that produce “off” odours and flavours (Jarvis, 2014).

Total of SO_2 were determined as 30.84 mg/L of C1, 36.18 mg/L of C2, 38.43 mg/L of C3, 37.92 mg/L of C4, 42.68 mg/L of C5, 36.33 mg/L of C6, 38.2 mg/L of C7, 37.3 mg/L of C8 cider (Table 4.10).

In point of results, the highest and lowest of SO_2 were determined in C5 and C1 cider.

In other study reported that total SO_2 was identified between 73 mg/L and 140 mg/L (Cambaş et al., 2000; Bozoğlu et al., 2015).

Table 4.10. The amount of sulphur dioxide (SO_2) in ciders.

Cider	SO_2 (mg/L)
C1*	30.84±0.04
C2*	36.48±0.08
C3**	38.43±0.07
C4**	37.92±0.01
C5*	42.68±0.05
C6*	36.33±0.02
C7**	38.2±0.09
C8**	37.3±0.06

Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

4.3.6. Volatile acidity

The primary volatile acidity is an acetic acid, which is a result of alcoholic fermentation and, it is also related to the smell and taste of vinegar. The amount of volatile acid in the experimental ciders was determined between 0.22 g/L and 0.74

g/L (Table 4.11). The lowest and highest volatile acid of the cider was determined in C5 cider and C7 cider.

In other studies, the volatile acidity was determined between 0.04 g/L and 1.83 g/L (Jarvis et al., 2000, Hernandez-orte et al., 2006, Alberti, et al., 2011, Sun et al., 2012, Anton et al., 2013, Braga et al., 2013).

Table 4.11. The amount of volatile acidity in ciders.

Cider	Volatile acidity (g/L)
C1*	0.55±0.05
C2*	0.26±0.08
C3**	0.31±0.02
C4**	0.53±0.05
C5*	0.22±0.07
C6*	0.27±0.04
C7**	0.74±0.03
C8**	0.62±0.09

Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

4.3.7. Total and residual sugars

The fermentation completed all the way, the cider had no perceivable residual sugar and dry. This means that the cider was not taste sweet, and might show more bitterness or acidity (Le quéré, 2006).

Total sugars of ciders were determined between 1.59 g/L and 5.38 g/L. The residual sugars ranged from 1.49 g/L to 5.29 g/L (Table 4.12).

In this view, the highest content of total and residual sugars ranged from 5.38 g/L to 5.29 g/L in C2 cider. The lowest of total and residual sugars ranged from 1.59 g/L and 1.49 g/L in C3 cider. The amount of total and residual sugars in ciders are given in Table 4.12.

Residual sugars were determined between 3.4 g/L and 3.9 g/L (Peng et al., 2015). Qin et al., (2018) in their study reported that residual sugars were ranged from 8.87 g/L to 87.44 g/L.

Table 4.12. The amount of total and residual sugars in ciders

Cider	Total sugars (g/L)	Residual sugars (g/L)
C1*	4.52±0.02	4.50±0.01
C2*	5.38±0.01	5.29±0.04
C3**	1.59±0.03	1.49±0.02
C4**	2.92±0.03	2.69±0.03
C5*	2.92±0.02	2.78±0.01
C6*	2.13±0.01	1.97±0.01
C7**	4.18±0.01	4.05±0.02
C8**	2.52±0.04	2.45±0.02

Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

4.3.8. Organic acids

The organic acids in cider perform a vital role in both the cider-making process and in the final flavour of a finished cider. They are present in both apples and cider and add a sour taste and a pungent odour to these respective substances (LEA, 1978). Acids also serve as a preservative in the cider since microorganisms grow less in lower pH conditions and contribute to the fermentation process (Rajashekhara et al., 1998). Malic acid provides to the tart and sour flavours

obtained in cider, and ranges from 4.5 to 7.5 g of malic acid per litre of cider is preferred (Ackermann et al., 1992). Lactic acid is also usually determined in cider, and it is mainly grown from malolactic fermentation, a process that converts malic acid into lactic acid. This process carries out the flavour of the cider while reducing a lot of the acidity and producing CO_2 as well (Coton et al., 2010). Citric acid can be used to add taste after fermentation, but these acids are not typically found in high concentration in apples naturally (Beech, 1993).

The organic acids were determined by HPLC (UV/Vis detector (SPD-20A) monitored at 210 nm. The amount of organic acids in ciders are shown in Table 4.13. The amount of malic acid in the experimental ciders was ranged from 2.96 g/L to 4.25 g/L. The lowest and highest value of organic acids were determined in C2 cider and C6 cider. The amount of lactic acid was determined between 0.23 g/L and 0.59 g/L. The amount of acetic acid was determined between 0.01 g/L and 0.95 g/L.

The amount of citric acid was not detected in C1, C2, C3 and C4. But C5, C6, C7 and C8 were determined between 0.08-0.11 g/L.

Zhang et al., (2008) and Qin et al., (2018) in their study was determined as malic acid 0.02-5.80 g/L, lactic acid 0.15-3.39 g/L, and acetic acid 0.05-0.1 g/L.

Table 4.13. The amount of organic acids in ciders

Cider	Organic acids (g/L)			
	Citric acid	Malic acid	Lactic acid	Acetic acid
C1*	-	3.27±0.02	0.59±0.01	0.02±0.01
C2*	-	2.96±0.01	0.44±0.03	0.03±0.01
C3**	-	3.91±0.02	0.23±0.003	0.02±0.02
C4**	-	3.98±0.0	0.43±0.02	0.01±0.02
C5*	0.11±0.01	4.22±0.02	0.31±0.01	0.37±0.01
C6*	0.08±0.0	4.25±0.02	0.48±0.02	0.95±0.03
C7**	0.09±0.02	4.12±0.01	0.31±0.01	0.25±0.0
C8**	0.10±0.01	3.80±0.03	0.53±0.01	0.91±0.02

Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

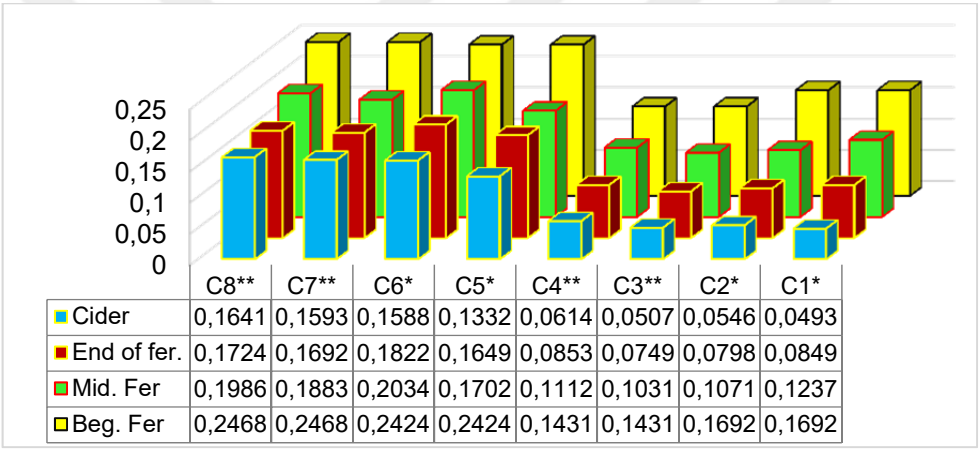
Zhang et al., (2008) reported that as high content of malic acid led to the sour flavour of cider. Acetic acid was the principal volatile acid in alcoholic drinks, also an essential constituent to the final product of cider, for it is recent of ethyl acetate. The amount of acetic acid would be described as the gum of cider, which is an essential criterion of cider quality.

Citric acid can be formed at the beginning of fermentation by *S. cerevisiae* and later taken into the cell and catabolized it. Lactic acid could be formed by reduction of pyruvic acid and transformation of malic acid (Whiting, 1976).

4.3.9. Browning index

The Maillard reaction happened between alpha-amino groups and residual sugars, was the most significant cause of browning in apple juice. The cause of browning index described as in the manufacture of fruit juice during processing. This reaction improved good sensory characteristics of juice, colour, aroma and flavour (Umano et al., 1995).

The result of browning indices are shown in Figure 4.7. The values of browning indices of the samples were determined between 0.1431-0.2468 at the beginning of alcoholic fermentation. The lowest amounts were found in C3 and C4, which were 0.1431. The highest amounts were found in C7 cider and C8 cider, which were 0.2468. During alcoholic fermentation, all browning indices values were slightly decreased and then they varied from 0.0749 to 0.1822 at the end of fermentation. After bottling, all ciders browning indices were dropped to less than 0.01±0.03. According to the results of analysis of variance of BI ($p \leq 0.005$).



	C8**	C7**	C6*	C5*	C4**	C3**	C2*	C1*
Cider	0,1641	0,1593	0,1588	0,1332	0,0614	0,0507	0,0546	0,0493
End of fer.	0,1724	0,1692	0,1822	0,1649	0,0853	0,0749	0,0798	0,0849
Mid. Fer	0,1986	0,1883	0,2034	0,1702	0,1112	0,1031	0,1071	0,1237
Beg. Fer	0,2468	0,2468	0,2424	0,2424	0,1431	0,1431	0,1692	0,1692

Results are the means of three repetitions. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

Figure 4.7. The change in BI during fermentations and ciders

4.3.10. Colour analysis

The colour of the samples was measured with the Colour Quest XE HunterLab colour measuring device and evaluated according to the international L* a* b* system (Hunter Colour System) during the alcoholic fermentation and

apple ciders. The L* axes runs from top to bottom. It is maximum for 100, which would be a perfect reflecting diffuser. The maximum for L* would be zero, which would be black. The positive a* is red, negative a* is green. Positive b* is yellow, negative b* is blue. The sample of ciders were calculated as Chroma ($(\sqrt{a*^2 + b^{*2}}$) and Hue ($\tan^{-1}\frac{b*}{a*}$). A diagram of HunterLab L*, a*, b* values were given in Figure 4.8 (Hunter, 1975).

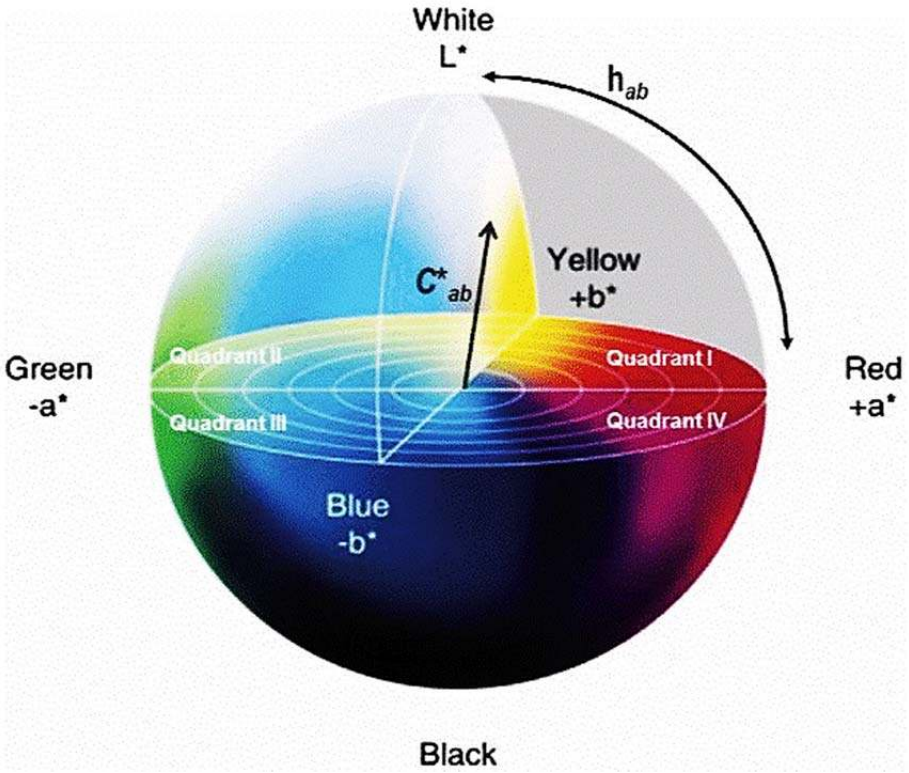


Figure 4.8. A diagram of HunterLab L*, a*, b*, H and C values

Table 4.14. L*, a*, b*, Hue and Chroma values in experimental ciders

Cider	L*	a*	b*	Hue	Chroma
C1*	59.61±0.01	-0.25±0.01	4.62±0.02	86.90±0.01	4.63±0.02
C2*	75.41±0.02	-1.09±0.02	4.48±0.01	76.32±0.02	4.61±0.01
C3**	51.74±0.01	-0.29±0.01	4.25±0.01	86.09±0.01	4.26±0.03
C4**	44.66±0.01	-0.18±0.01	3.28±0.03	86.85±0.01	3.29±0.01
C5*	47.31±0.02	-2.19±0.03	4.92±0.01	76.00±0.02	5.38±0.01
C6*	49.19±0.01	-2.42±0.01	7.58±0.01	72.29±0.01	7.95±0.02
C7**	55.41±0.01	-2.03±0.02	10.63±0.0	79.18±0.01	10.82±0.01
C8**	41.28±0.01	-0.67±0.01	9.94±0.01	86.14±0.0	9.96±0.01

Data were reported as mean ± standard deviation of 3 replications. C1*: Spontaneous fermentation in fresh apple juice; C2*: Inoculated with commercial yeast in fresh apple juice; C3**: Inoculated with commercial yeast in fresh apple juice; C4**: Spontaneous fermentation in fresh apple juice; C5*: Spontaneous fermentation in apple juice concentrate; C6*: Inoculated with commercial yeast in apple juice concentrate; C7**: Spontaneous fermentation in apple juice concentrate; C8** : Inoculated with commercial yeast in concentrated juice; *- no supplementation of DAP; **- with supplementation of DAP;

In point of view from Table 4.14 shows that differences between L*, a*, b*, Hue and Chroma values of ciders produced by different raw materials (fresh apple juice and apple juice concentrate) were observed.

The lowest and highest L* values were measured as 41.28 in C8 and 75.41 in C2 cider. The lowest and highest a* values were measured as -2.42 in C6 and -0.25 in C1 cider. As specified in the Hunter colour system, if a* is positive, the colour would be defined as red and b* is negative, the colour would be green. As seen from Table 4.12, a* values were measured in the green zone in all ciders.

The highest b* values were measured as 10.63 in C7. The lowest values were measured as 3.28 in C4 cider. If b* values are positive, the colour would be yellow and b* values are negative, the colour would be blue.

In this study, b* values were positive in yellow colour in green all ciders.

The highest Hue value was measured as 86.90 in C1 cider. The lowest Hue value was measured as 72.29 in C7 cider.

The Chroma value gives the density of the colour, as well as the highest and lowest values were measured as 10.82 in C7 cider and 3.29 in C4 cider.

4.4. Volatile aroma compounds of experimental ciders

The 8 experimental ciders were identified 55 aroma compounds, including 13 of higher alcohols, 13 of ethyl esters, 5 of acetate esters, 9 of volatile acids, 3 of six-carbon alcohol, 5 of phenolic compounds, 4 of lactones and 3 of carbonyl compounds. Total aroma compounds are shown in Table 4.15 and 4.16.

The total amount of aroma compounds were identified as 85.18 mg/L in C1, 89.14 mg/L in C2, 72.8 mg/L in C3, 74.65 mg/L C4, 75.26 mg/L in C5, 66.92 mg/L C6, 62.4 mg/L C7 and 70.3mg/L in C8 cider.

Table 4.15. The total amount of aroma compounds in ciders

Compounds (µg/L)	Cider			
	C1†	C2†	C3‡	C4‡
Higher alcohols	66908.7±0.04	70249±0.02	57219.6±0.01	62153.2±0.05
Ethyl esters	5526.8±0.01	8031.6±0.04	3274.9±0.05	2552.7±0.01
Acetates esters	1338±0.06	1145.3±0.01	1511.1±0.01	1359.2±0.06
Volatile acids	6596.4±0.01	7448.7±0.03	7011.9±0.03	5266.3±0.08
Six carbon alcohol	2970.2±0.05	463.8±0.08	2194.3±0.04	2170.2±0.03
Phenolic compounds	993.1±0.07	1131.7±0.04	1127.2±0.04	688±0.01
Lactones	626.3±0.02	352.3±0.01	264.8±0.07	288.7±0.05
Carbonyl compounds	218±0.01	309.1±0.02	191±0.01	171.2±0.04
TOTAL	**85177.5**	**89131.5**	**72794.8**	**74649.5**

Data were reported as mean ± standard deviation of 3 replications. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; †- no supplementation of DAP; ‡- with supplementation of DAP;

Table 4.16. The total amount of aroma compounds in ciders

Compounds (µg/L)	Cider			
	C5†	C6†	C7‡	C8‡
Higher alcohols	52837.4±0.01	49145.3±0.08	38770.7±0.03	54273.1±0.06
Ethyl esters	5692.7±0.06	3531.9±0.02	6121.3±0.05	3238.2±0.02
Acetates esters	2020.8±0.03	1981.4±0.02	3133.4±0.08	1832±0.03
Volatile acids	12074±0.04	9528.8±0.04	12433.5±0.01	8454.8±0.03
Six carbon alcohol	584±0.02	1228.6±0.05	338.6±0.03	906.2±0.01
Phenolic compounds	1440.6±0.01	807.6±0.01	912.4±0.04	750.8±0.02
Lactones	491.2±0.04	315.6±0.03	605±0.04	315±0.03
Carbonyl compounds	115.2±0.03	374.4±0.07	82.2±0.03	467±0.01
TOTAL	**75255.9**	**66913.6**	**62397.1**	**70237.1**

Data were reported as mean ± standard deviation of 3 replications. C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡: Inoculated with commercial yeast in concentrated juice; †- no supplementation of DAP; ‡- with supplementation of DAP;

4.4.1. Higher alcohols

Higher alcohols, which is also fusel alcohols that have to contain more than two carbons (ethanol), and thus have higher molecular weight and higher boiling point. Higher alcohols are formed in small amounts by yeast metabolism during alcoholic fermentation. Volatile flavour compounds are produced both from sugar catabolism and from amino acids decarboxylation and deamination (Ehrlich mechanism) (Ebeler, 2001; Jackson, 2000). Also, these are described as an intense and pungent smell, which plays a significant role in wine bouquet (Satora et al., 2016).

Ciders were identified as higher alcohols, and their Kovats index values, the amount of the difference between the lower areas and aroma compounds of statistical significance is given in Table 4.17 and Table 4.18.

As shown in table 4.17 and 4.18, a total of 13 higher alcohols content was 66.91 mg/L in C1, 70.25 mg/L in C2, 57.22 mg/L in C3, 62.15 mg/L in C4, 52.84

mg/L in C5, 49.15 mg/L in C6, 38.77 mg/L in C7 and 54.28 mg/L in C8 ciders were identified. Statistically significant differences as 1-propanol, isobutyl alcohol, 1-butanol, 1-pentanol, 2-methyl-3-pentanol, furfural alcohol, benzyl alcohol and 2-phenyl ethanol were found in ciders ($p<0.05$).

The highest amount of the higher alcohols were found as isoamyl alcohol, then 2, 3-butanediol and 2-phenylethanol. The lowest and highest amount of isoamyl alcohol was determined in C7 (20.03 mg/L) cider and C2 (45.56 mg/L) cider. The amount of 2-phenylethanol was identified as 17.36 mg/L in C1, 17.8 mg/L in C2, 16.26 mg/L in C3, 19.6 mg/L in C4, 17.69 mg/L in C5, 12.05 mg/L in C6, 15.87 mg/L in C7 and 16.94 mg/L in C8 cider.

Picinelli et al., (2000) reported that 1-propanol was exhibited with a comparatively high level in all of the samples and was a spontaneous fermentation product in ciders. The positive impact of this compound on the sensory evaluation of foam properties.

2-phenylethanol is like as honey, floral and rose odour to ciders, which is created from L-phenylalanine via metabolic conversions under anaerobic conditions (Sun et al., 2013). 2-phenethylethanol can be also determined in apple juice and grew faster fermentation rates (Ye et al., 2014).

Higher alcohols were determined as 131.0-161.63 mg/L of 1-propanol, 147.63-386.23 mg/L of isobutyl alcohol and 161.16-278.23mg/L of isoamyl alcohol in the apple wines (Satora et al., 2016).

Qin et al., (2018) in their study was determined in the 14 ciders, which as 16 higher alcohols, including 1-propanol, 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol, 1-hexanol and 2-phenethylethanol.

Seguinot et al., (2018) reported that propanol, isoamyl alcohol and isobutanol was determined as 10.65-24.17 mg/L, 189.31-199.33 mg/L and 19.57-27.69 mg/L.

Table 4.17. Higher alcohols identified in ciders

Higher alcohols (µg/L)	LRI	ID	Cider				S
			1†	2†	3‡	4‡	
1-Propanol	971	B	313.7±19	280.4±8	224.2±19	235.5±11	*
Isobutyl alcohol	1029	A,B	3482.1±238	2978.4±221	1433.4±48	2575.1±201	*
1-Butanol	1079	A,B	1236.5±3	1972.8±53	1110.6±25	1323.0±2	*
Isoamyl alcohol	1215	A,B	41462.8±887	45564.7±1385	36826.4±539	36733.4±536	ns
1-pentanol	1265	A,B	46.1±9	90.7±8	35.1±5	58.6±3	*
2-Methyl-3-pentanol	1274	B	220.0±65	22.1±1	50.6±4	47.3±8	*
Heptanol	1328	A,B	16.5±7	10.0±4	24.0±5	31.7±2	ns
2,3-Butanediol	1479	A,B	204.6±12	85.8±1	154.0±13	216.4±31	ns
1-Octanol	1535	A,B	133.4±37	nd	87.6±27.2	nd	ns
Furfural alcohol	1596	A,B	257.7±21	405.4±63	94.0±8	241.6±18	*
Methionol	1684	A,B	1133.8±129	1031.5±16	903.2±27	1145.8±17	ns
Benzyl alcohol	1818	B	855.6±69	11.2±4	17.1±1	18.5±3	*
2-Phenyl ethanol	1924	A,B	17359.6±189	17796.0±242	16259.4±163	19526.3±339	*
TOTAL			**66908.7**	**70249**	**57219.6**	**62153.2**	

Results are the means of three repetitions. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; nd, not-detected; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

Table 4.18. Higher alcohols identified in ciders

Higher alcohols (µg/L)	LRI	ID	Cider				S
			5†	6†	7‡	8‡	
1-Propanol	971	B	313.2±15	359.4±12	247.2±16	240.0±24	*
Isobutyl alcohol	1029	A,B	3696.3±237	3905.7±285	2051.9±69	1206.9±117	*
1-Butanol	1079	A,B	87.1±5	91.7±8	114.1±17	44.8±4	*
Isoamyl alcohol	1215	A,B	38450.9±872	29811.5±745	20033.9±1739	32739.4±928	ns
1-pentanol	1265	A,B	nd	nd	57.7±5	31.7±4	*
2-Methyl-3-pentanol	1274	B	nd	265.1±14	43.6±7	nd	*
Heptanol	1328	A,B	nd	nd	nd	nd	ns
2,3-Butanediol	1479	A,B	175.0±27	1493.5±253	98.0±3	2229.6±162	ns
1-Octanol	1535	A,B	nd	nd	nd	nd	ns
Furfural alcohol	1596	A,B	80.5±11	48.3±5	61.7±5	43.7±2	*
Methionol	1684	A,B	539.4±8	1101.0±121	186.6±6	780.7±13	ns
Benzyl alcohol	1818	B	16.0±3	20.8±6	8.2±2	16.0±3	*
2-Phenyl ethanol	1924	A,B	17684.7±247	12048.3±593	15867.8±63	16940.3±278	*
TOTAL			**52837.4**	**49145.3**	**38770.7**	**54273.1**	

Results are the means of three repetitions. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; nd, not-detected; S, Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

4.4.2. Esters

Esters are mainly significant to wine flavour and are regularly secondary aromas, which are arisen from the fermentation process, and seldom tertiary aromas resulting from ageing, where alcohol acid rearrangements can occur (Clarke R.J. and Bakker, J., 2004).

Esters are actually ethyl esters of organic acids, including ethyl acetate, ethyl lactate and diethyl succinate, ethyl esters of aliphatic fatty acids, including ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and acetates of higher alcohols, including isoamyl, benzyl and phenylethyl acetates (Jackson, 2000; Zamora, 2009).

A total of 18 ester compounds were identified in the experimental ciders, in which 13 were determined as ethyl esters of fatty acids and 5 as acetates of higher alcohols. The maximum amount of esters were identified in C7 cider.

The highest and lowest amount of ethyl hexanoate was identified in the C2 and C4 cider.

Ciders were identified as esters, and their Kovats index values, the amount of the difference between the lower areas and aroma compounds of statistical significance is given in Table 4.19 and Table 20.

As seen from Table 4.19 and Table 20, ethyl esters were negatively affected by DAP addition ciders. Esters were identified as 6.8 mg/L in C1, 9.2 mg/L in C2, 4.8 mg/L in C3, 3.9 mg/L in C4, 7.7 mg/L in C5, 5.5 mg/L in C6, 9.3 mg/L in C7, 5.07 mg/L in C8 ciders.

Peng et al., (2015) reported that ethyl acetate was one of the most significant esters affecting flavour in ciders, give a sweet and fruity odour to the general wine aroma at a fitting concentration. However, a high concentration of ethyl acetate could impart a like glue odour to ciders (Braga et al., 2013).

Zhao et al., (2015) reported that ethyl hexanoate, ethyl octanoate and decanoate were also predominated and given an apple, fruity, and sweet aroma in ciders.

9 key aroma compounds were determined as ethyl acetate, isobutyl acetate, isopentylacetate, ethyl octanoate, ethyl 4-hydroxybutanoate, isobutylalcohol, isoamyl acetate, methionol, and 2-phenylethanol in the apple wine (Peng et al., 2015).

7 esters were also significantly determined as isoamyl acetate, hexyl acetate, ethyl butyrate, ethyl acetate, ethyl hexanoate, ethyl lactate and ethyl octanoate in cider (Anton et al., 2014; Peng et al., 2016).

The highest amount of ester was identified isoamyl acetate, and then ethyl 4-hydroxybutanoate and ethyl octanoate.

The highest amount of isoamyl acetate was identified by 2417.8 μg/L of C7, 1404.5 μg/L of C5, 1364.1 μg/L of C6, 1271.4 μg/L of C8, 479.0 μg/L of C1, 393.8 μg/L of C3, 281.9 μg/L of C4 and 203.8 μg/L of C2 cider.

Esters were determined as 205-876 μg/L of ethyl propanoate, 401-8430 μg/L of ethyl butanoate 325-4558 μg/L of ethyl hexanoate, 12.8-6017 μg/L of ethyl lactate, 42.8-5646 μg/L of ethyl octanoate, 50.8-8370 μg/L of ethyl decanoate, 18.4-385 μg/L of ethyl benzoate, 28.2-210 μg/L of diethyl succinate, 5.2-8.6 μg/L of ethyl phenylacetate, 22.8-5190 μg/L of butyl acetate, 560-12646 μg/L of isoamyl acetate, 16.8-3785 μg/L of hexyl acetate, 3.3-62.4 μg/L of (z)-3-hexenyl acetate, 7.9-395 μg/L of 2-phenethyl acetate in the 14 ciders (Qin et al., 2018).

Seguinot et al., (2018) reported that isoamyl acetate, isobutyl acetate, ethyl acetate, ethyl hexanoate and ethyl octanoate was determined as 2.69-4.09 mg/L, 0.22-0.24 mg/L, 36.80-49.12 mg/L, 1.94-2.17 mg/L and 2.62-3.25 mg/L.

Table 4.19. Ethyl and acetate esters identified in apple cider

Ethyl esters (µg/L)	LRI	ID	Cider				S
			1†	2†	3‡	4‡	
Ethyl propanoate	954	B	51.3±2	42.9±1	38.2±4	24.5±1	ns
Ethyl butanoate	1006	B	239.5±14	280.4±11	302.5±23	235.5±19	*
Ethyl hexanoate	1232	A,B	373.0±59	5109.1±73	514.7±67	61.6±18	*
Ethyl lactate	1343	A,B	306.8±31	369.1±42	264.3±25	526.0±36	*
Ethyl octanoate	1402	A,B	388.1±11	448.9±13	527.3±19	376.6±15	*
Ethyl decanoate	1524	A,B	nd	nd	nd	nd	*
Ethyl benzoate	1601	A,B	47.1±4	nd	41.9±3	34.3±7	ns
Diethyl succinate	1618	A,B	124.6±32	70.7±26	477.1±89	91.2±20	*
Ethyl phenylacetate	1704	A,B	838.5±183	28.2±4	306.9±47	33.2±5	ns
Ethyl 4-hydroxy-butanoate	1782	A,B	2308.1±242	1024.5±174	407.8±94	974.9±127	*
Ethyl dodecanoate	1823	A,B	98.5±3	142.7±13	101.8±8	36.1±7	ns
Ethyl hexadecanoate	2184	A,B	276.1±39	437.5±31	225.1±48	125.7±25	*
Ethyl octadecanoate	2437	A,B	75.2±6	77.6±9	67.3±5	33.1±2	*
TOTAL			**5526.8**	**8031.6**	**3274.9**	**2552.7**	

Results are the means of three repetitions. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; nd, not-detected; S, Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

Table 4.19. (Continued)

Acetate esters (µg/L)	LRI	ID	1†	2†	3‡	4‡	S
Butyl acetate	982	B	220.9±14	340.3±39	485.8±26	333.4±36	ns
Isoamyl acetate	1054	A,B	479.0±34	203.8±28	393.8±47	281.9±31	*
Hexyl acetate	1245	A,B	99.1±6	280.7±18	183.9±12	317.7±15	ns
(Z)-3-Hexenyl acetate	1270	B	27.9±2	23.1±1	42.0±5	44.4±6	ns
2-Phenethyl acetate	1796	A,B	511.1±48	297.4±38	405.6±72	381.8±51	*
Total			**1338**	**1145.3**	**1511.1**	**1359.2**	
SUM TOTAL			**6864.8**	**9176.9**	**4786**	**3911.9**	

Results are the means of three repetitions. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, With supplementation of DAP;

Table 4.20. Ethyl and acetate esters identified in ciders

Ethyl esters (µg/L)	LRI	ID	Cider				S
			5†	6†	7‡	8‡	
Ethyl propanoate	954	B	41.8±3	43.4±2	72.9±1	35.1±1	ns
Ethyl butanoate	1006	B	329.7±16	163.7±9	408.3±28	321.8±41	*
Ethyl hexanoate	1232	A,B	632.4±54	556.4±77	500.7±69	354.6±58	*
Ethyl lactate	1343	A,B	545.9±34	205.9±27	526.9±23	194.2±21	*
Ethyl octanoate	1402	A,B	1067.1±17	667.2±21	974.7±12	496.7±26	*
Ethyl decanoate	1524	A,B	633.7±23	480.3±31	479.4±67	712.7±25	*
Ethyl benzoate	1601	A,B	30.3±2	26.6±4	69.3±8	31.6±4	ns
Diethyl succinate	1618	A,B	56.3±14	87.2±18	139.4±41	124.4±37	*
Ethyl phenylacetate	1704	A,B	53.4±14	251.7±22	142.0±48	67.4±17	ns
Ethyl 4-hydroxy-butanoate	1782	A,B	1915.9±195	524.2±136	2354.9±205	571.3±129	*
Ethyl dodecanoate	1823	A,B	61.4±2	117.5±25	51.3±3	85.3±9	ns
Ethyl hexa-decanoate	2184	A,B	251.3±27	328.3±19	317.9±21	179.3±43	*
Ethyl octa-decanoate	2437	A,B	73.5±4	79.5±8	83.6±11	63.8±3	*
Total			**5692.7**	**3531.9**	**6121.3**	**3238.2**	*

Results are the means of three repetitions. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; nd, not-detected; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

Table 4.20. (Continued)

Acetate esters (µg/L)	LRI	ID	5†	6†	7‡	8‡	S
Butyl acetate	982	B	127.3±21	147.9±18	376.2±17	238.4±39	ns
Isoamyl acetate	1054	A,B	1404.5±140	1364.1±104	2417.8±185	1271.4±95	*
Hexyl acetate	1245	A,B	42.4±6	58.3±7	31.9±3	23.8±3	ns
(Z)-3-Hexenyl acetate	1270	B	48.3±8	41.3±7	45.8±2	22.4±4	ns
2-Phenethyl acetate	1796	A,B	398.3±33	369.8±82	261.7±29	276.0±41	*
Total			**2020.8**	**1981.4**	**3133.4**	**1832**	
SUM TOTAL			**7713.5**	**5513.3**	**9254.7**	**5070.2**	

Results are the means of three repetitions. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

4.4.3. Volatile acids

Fatty acids synthesized by the yeast and bacteria during the alcoholic fermentation. They are short-chain fatty acids. Such as propanoic, butanoic, hexanoic, octanoic and decanoic acids as well as long chain fatty acids, including oleic and linoleic acids (Ribéreau-Gayon, Glories et al., 2006a).

Fatty acids occurred from the apples and their components were dependent on the harvest time, apple cultivars and alcoholic fermentation (Anton et al., 2014).

Qin et al., (2018) reported that ethanoic acid was assembled by oxidation of acetaldehyde during alcoholic and malolactic fermentation. It was given acidic and vinegar flavour to cider.

The experimental ciders were identified as volatile acids, and their Kovats index values, the amount of the difference between the lower areas and aroma compounds of statistical significance is given in Table 4.21 and 4.22.

A total of 9 volatile compounds was determined in the experimental ciders. As seen from table 4.21 and 4.22, volatile acids were identified as 6.6 mg/L in C1, 7.5 mg/L in C2, 7 mg/L in C3, 5.3 mg/L in C4, 12 mg/L in C5, 9.5 mg/L in C6, 12.4 mg/L in C7, 8.4 mg/L in C8 cider.

The maximum and minimum amount of volatile acids were determined in C7 and C1 cider.

As seen from Table 4.21 and 4.22, a statistically significant difference was found between the amounts of ethanoic, propanoic, isobutanoic, hexanoic and octanoic acids in the experimental ciders.

The highest peak area of acetic acid was determined in C8 (1126.8 µg/L) cider and C7 (1119.5 µg/L) cider. The highest amount of hexanoic acid was determined in C5 (2401.0 µg/L) and C7 (2086.2 µg/L) cider.

Ugliano et al., (2010) reported that hexanoic and octanoic acid was determined between 1379-2007 µg/L and 834-848 µg/L in wines.

Antón-Díaz et al., (2016) in their study was determined between 2.2 mg/L and 13.5 mg/L in ciders, which were hexanoic, octanoic and decanoic acids.

Qin et al., (2018) in their study reported that ethanoic acid, isobutanoic acid and hexanoic acid was determined between 127.8-880 μg/L, 9.5-34.6 μg/L and 1.4-750 μg/L in the 14 ciders.

Table 4.21. Volatile acids identified in cider

Volatile acids (µg/L)	LRI	ID	Cider				S
			1†	2†	3‡	4‡	
Ethanoic acid	1364	B	374.0±139	359.0±165	428.5±173	507.1±176	*
Propanoic acid	1461	A,B	26.0±4	nd	21.0±6	12.2±2	*
Isobutanoic acid	1489	A,B	114.5±5	215.8±9	116.8±4	106.9±2	*
Butanoic acid	1572	B	218.7±16	346.1±2	276.5±13	234.9±5	ns
Hexanoic acid	1802	A,B	1576.2±32	1834.8±138	1853.6±162	1169.7±126	*
Octanoic acid	2009	A,B	2918.7±168	2893.2±73	3202.4±39	2400.3±87	*
Decanoic acid	2235	A,B	1055.0±93	1254.5±53	949.8±47	694.9±15	ns
9-Decenoic acid	2326	A,B	243.3±34	445.8±174	49.4±2	89.9±12	ns
Dodecanoic acid	2473	A,B	70.0±31	99.5±8	113.9±21	50.4±3	ns
Total			**6596.4**	**7448.7**	**7011.9**	**5266.3**	

Results are the means of three repetitions. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; nd, not-detected; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

Table 4.22. Volatile acids identified in ciders

Volatile acids (µg/L)	LRI	ID	Cider				S
			5†	6†	7‡	8‡	
Ethanoic acid	1364	B	539.1±196	660.7±128	1119.5±95	1126.8±262	*
Propanoic acid	1461	A,B	nd	20.4±1	18.2±3	22.8±2	*
Isobutanoic acid	1489	A,B	99.1±6	148.8±15	160.4±3	190.2±13	*
Butanoic acid	1572	B	141.5±17	131.1±5	135.2±5	113.1±4	ns
Hexanoic acid	1802	A,B	2401.0±119	1427.3±19	2086.2±147	1272.2±152	*
Octanoic acid	2009	A,B	4999.2±218	4111.8±78	5702.0±385	3389.1±87	*
Decanoic acid	2235	A,B	2923.8±3	2271.6±36	2183.4±28	1939.2±78	ns
9-Decenoic acid	2326	A,B	829.5±3	439.7±33	855.5±32	255.4±16	ns
Dodecanoic acid	2473	A,B	140.8±4	317.4±17	173.1±8	146.0±35	ns
Total			**12074**	**9528.8**	**12433.5**	**8454.8**	

Results are the means of three repetitions. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; nd, not-detected; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

4.4.4. Six-carbon alcohols

Six-carbon alcohols are hexanols and hexenols from plant tissues, which are given the herbaceous smells in wines (Ribereau-Gayon. P. et al., 2006).

The six-carbon alcohols supply vegetative and herbaceous distinctions to the wines. When their concentrations are over than odour threshold values, it is a negative effect on the wine quality. Although the six-carbon alcohols are most significant for apple juice, their concentrations reduce during alcoholic fermentation (Aung et al., 2015).

The experimental ciders were identified as six-carbon alcohols and their Kovats index values, the amount of the difference between the lower areas and aroma compounds of statistical significance is given in Table 4.23.

In our experimental ciders were identified following six-carbon alcohols: 2-hexanol, 1-hexanol, cis-3-hexenol.

As seen from Table 4.22, the level of six-carbon alcohols were identified as 2.9 mg/L in C1, 0.4 mg/L in C2, 2.2 mg/L in C3, 2.1 mg/L in C4, 0.5 mg/L in C5, 1.2 mg/L in C6, 0.3 mg/L in C7 and 0.9 mg/L in C8 ciders.

The maximum and minimum amount of six-carbon alcohols were determined in C3 and C7 ciders.

The highest amount of cis-3-hexenol was determined in C1 (39.9 μg/L) cider, the lowest was in C7 (21.7 μg/L) cider.

The highest amount of 1-hexanol was also found in C1 (2897.9 μg/L) cider, and lowest was in C7 (265.9 μg/L) cider.

Lobo et al., (2016) in their study reported that cis-3-hexenol was determined between 68 μg/L and 505 μg/L in Spanish ciders.

Qin et al., (2018) in their study reported that 1-Hexanol, cis-3-Hexenol and 2-Hexanol was determined between 31.7-5277 μg/L, 0.8-143 μg/L and 107-1021 μg/L in the 14 ciders.

Table 4.23. Six carbon alcohols identified in ciders

C6 alcohols (µg/L)	LRI	ID	Cider 1†	2†	3‡	4‡	S
2-Hexanol	1253	B	32.4±3	24.7±4	37.7±2	35.5±2	*
1-Hexanol	1349	A,B	2897.9±159	407.3±19	2130.0±58	2095.0±37	*
cis-3-Hexenol	1362	A,B	39.9±3	31.8±3	26.6±1	39.7±1	*
Total			**2970.2**	**463.8**	**2194.3**	**2170.2**	

C6 alcohols (µg/L)	LRI	ID	Cider 5†	6†	7‡	8‡	S
2-Hexanol	1253	B	43.0±1	42.9±1	51.0±2	40.5±2	*
1-Hexanol	1349	A,B	512.7±37	1149.4±138	265.9±78	827.3±152	*
cis-3-Hexenol	1362	A,B	28.3±2	36.3±2	21.7±1	38.4±3	*
Total			**584**	**1228.6**	**338.6**	**906.2**	

Results are the means of three repetitions. C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

4.4.5. Phenolic compounds

Phenolic compounds are not present in apple must. These are produced by enzymatic decarboxylation by the yeast during fermentation and grow in ageing. There are vinyl and ethyl phenols (Ribéreau-Gayon, et al., 2000).

The experimental ciders were identified as phenolic compounds and their Kovats index values, the amount of the difference between the lower areas and aroma compounds of statistical significance is given in Table 4.24 and 4.25.

In our experimental ciders were identified following phenolic compounds: 4-allylguaiacol, 2-methoxy-4-vinylphenol, 4-allylphenol, 4-vinylphenol and 2,6-dimethoxy phenol.

As seen from 4.23 and 4.24, total content of phenolic compounds was determined as 0.99 mg/L in C1, 1.13 mg/L in C2, 1.12 mg/L in C3, 0.6 mg/L in C4, 1.4 mg/L in C5, 0.8 mg/L in C6, 0.91 mg/L in C7 and 0.7 mg/L in C8 cider.

Statistically significant differences as 4-allylguaiacol, 2-methoxy-4-vinylphenol, 4-allylphenol and 2, 6-dimethoxy phenol were found in ciders ($p<0.05$).

The highest amount of 4-allylguaiacol was determined in C5 (996.4 µg/L) cider and then C1 (351.2 µg/L) cider. The lowest amount of 4-allylguaiacol was determined in C8 (139.2 µg/L) cider and then C7 (231.7 µg/L) cider.

The highest amount of 2-methoxy-4-vinylphenol was identified in C7 (437 µg/L) cider and then C3 (385.4 µg/L) cider. The lowest amount of 2-methoxy-4-vinylphenol was identified in C4 (173.8 µg/L) cider and then C7 (203.4 µg/L) cider.

The highest amount of 4-allylphenol and 2, 6-dimethoxy phenol was found in C1 (244.3 µg/L) cider and C2 (254.6 µg/L) cider. The lowest amount of 4-allylphenol and 2, 6-dimethoxy phenol was found in C7 (59.4 µg/L) and C6 (37.4 µg/L) cider.

Anton-Diaz et al., (2016) reported that 4-vinylphenol varied between 1557.0 µg/L and 1745.0 µg/L in ciders.

Zihan Qin et al., (2018) reported that phenolic compounds varied between 0.1 mg/L and 0.2 mg/L in the 14 ciders.

Lorenzini et al., (2019) in their study reported that 2-methoxy-4-vinylphenol was determined between 4.5 µg/L - 86.7 µg/L.

Table 4.24. Phenolic compounds identified in ciders

Phenolic compounds (µg/L)	LRI	ID	Cider				S
			1†	2†	3‡	4‡	
4-Allylguaiacol	2295	A,B	351.2±0.6	281.1±0.9	318.6±0.4	235.3±0.5	*
2-Methoxy-4-vinylphenol	2307	A,B	203.4±0.3	211.6±0.2	385.4±0.6	173.8±0.9	*
4-allylphenol	2314	A,B	244.3±4	230.1±8	99.2±5	111.1±9	*
4-vinylphenol	2485	A,B	134.8±7	154.3±3	259.1±6	121.9±4	ns
2,6-dimethoxy phenol	2538	B	59.4±6	254.6±8	64.9±9	45.9±3	*
TOTAL			**993.1**	**1131.7**	**1127.2**	**688**	

Results are the means of three repetitions. C1†: Spontanously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontanously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; not-detected; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

Table 4.25. Phenolic compounds identified in ciders

Phenolic compounds (µg/L)	LRI	ID	Cider				S
			5†	6†	7‡	8‡	
4-Allylguaiacol	2295	A,B	996.4±0.8	251.6±0.4	231.7±0.9	139.2±0.6	*
2-Methoxy-4-vinylphenol	2307	A,B	283±0.1	298.9±0.4	437±0.6	254.4±0.3	*
4-allylphenol	2314	A,B	33.5±9	121.4±3	59.4±2	129.1±8	*
4-vinylphenol	2485	A,B	83.9±5	98.3±9	142.9±2	174.3±7	ns
2,6-dimethoxy phenol	2538	B	43.8±9	37.4±5	41.4±8	53.8±6	*
TOTAL			**1440.6**	**807.6**	**912.4**	**750.8**	

Results are the means of three repetitions. C1†: Spontanously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontanously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; not-detected; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

4.4.6. Lactones

Lactones contribute to the wine aroma during fermentation. The simpler lactones like γ-butyrolactone, which is according to the Ehrlich reaction, the proceeds from the lactonization of γ-hydroxybutyric acid, an uncertain molecule assembled by deamination and decarboxylation of glutamic acid (Ribéreau-Gayon, Glories, et al., 2006).

A total of 4 lactones were determined in the experimental ciders. The experimental ciders were identified as lactones and their Kovats index values, the amount of the difference between the lower areas and aroma compounds of statistical significance is given in Table 4.26.

As seen from Table 4.26, the amount of lactones were determined as 0.63 mg/L in C1, 0.35 mg/L in C2, 0.26 mg/L in C3, 0.29 mg/L in C4, 0.49 mg/L in C5, 0.32 mg/L in C6, 0.61 mg/L in C7 and 0.32 mg/L in C8 ciders. The maximum amount of lactone was determined in C1 cider and then followed by C7 cider.

Statistically significant differences as γ-butyrolactone, pantolactone, δ-octalactone were found in ciders ($p<0.05$).

The highest amount of γ-butyrolactone was determined in C1 (522.5 μg/L) cider and then C7 (499.8 μg/L) cider. The lowest amount of γ-butyrolactone was determined in C3 (147.0 μg/L) cider and then C8 (188.7 μg/L) cider.

The highest amount of pantolactone was identified in C7 (54.5 μg/L) cider and then C1 (46.5 μg/L) cider. The lowest amount of pantolactone was identified in C6 (24.0 μg/L) cider and then C3 (27.3 μg/L) cider.

The highest amount of δ-octalactone was determined in C3 (53.1 μg/L) cider and C1 (44.7 μg/L) cider. The lowest amount of δ-octalactone was determined in C2 (18.4 μg/L) and C4 (26.1 μg/L) cider.

Anton-Diaz et al., (2016) reported that γ-butyrolactone ranged from 762.0 μg/L to 801.0 μg/L in ciders. Zihan Qin et al., (2018) in their study, amount of γ-butyrolactone was identified between 0.03 mg/L and 0.1 mg/L in the 14 ciders.

Table 4.26. Lactones identified in ciders

Lactones (µg/L)	LRI	ID	Cider				S
			1†	2†	3‡	4‡	
γ-Butyrolactone	1552	A,B	522.5±2.5	257.1±0.9	147.0±6.4	198.6±2.3	*
Pantolactone	1968	A,B	46.5±1.9	35.0±1.8	27.3±1.1	37.7±6.8	*
δ-Octalactone	1980	B	44.7±3.3	18.4±2.6	53.1±0.7	26.1±5.4	*
Mevalolactone	2493	B	nd	nd	nd	nd	ns
TOTAL			**626.3**	**352.3**	**264.8**	**288.7**	

Lactones (µg/L)	LRI	ID	Cider				S
			5†	6†	7‡	8‡	
γ-Butyrolactone	1552	A,B	389.8±2.2	217.4±6.1	499.8±0.9	188.7±3.1	*
Pantolactone	1968	A,B	44.1±4.5	24.0±1.9	54.5±4.1	28.3±2.5	*
δ-Octalactone	1980	B	nd	nd	nd	nd	ns
Mevalolactone	2493	B	25.7±2.1	61.2±4.7	24.9±3.5	49.1±1.1	ns
TOTAL			**491.2**	**315.6**	**605.0**	**315.0**	

Results are the means of three repetitions. C1†: Spontanously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontanously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; nd, not-detected; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

4.4.7. Carbonyl compounds

Carbonyl compounds contain various aldehydes and ketones. Aldehydes make some a nut-smell after oxidized wines. If it contains high concentrations in wines, it can be the offensive aroma (butter smell). There can happen with ageing and under conditions of heavy oxidation. This compound is very reactive and the capability to connect with sulphur dioxide (Ribéreau-Gayon, Glories, et al., 2006).

In the experimental ciders were identified as 3 carbonyl compounds, including acetoin, 4-hydroxy-4-methyl-2-pentanone and 3-hydroxy-4-phenyl-2-butanone (Table 4.27).

The experimental ciders were determined as carbonyl compounds and their Kovats index values, the amount of the difference between the lower areas and aroma compounds of statistical significance is given in Table 4.27.

As seen from Table 4.27, the carbonyl compounds were identified as 0.22 mg/L in C1, 0.31 mg/L in C2, 0.19 mg/L in C3, 0.17 mg/L in C4, 0.12 mg/L in C5, 0.37 mg/L in C6, 0.08 mg/L in C7 and 0.47 mg/L in C8 cider.

The maximum and minimum amounts of carbonyl compounds were determined in C8 and C7 ciders.

Statistically significant differences as 4-hydroxy-4-methyl-2-pentanone and 3-hydroxy-4-phenyl-2-butanonewere found in ciders ($p<0.05$).

The highest amount of 4-hydroxy-4-methyl-2-pentanone was determined in C8 (192.1 μg/L) cider and then C6 (163.1 μg/L) cider. The lowest amount of 4-hydroxy-4-methyl-2-pentanone was determined in C7 (29.5 μg/L) cider and then C5 (30.7 μg/L) cider.

The highest amount of 3-hydroxy-4-phenyl-2-butanonewere was identified in C8 (107.6 μg/L) cider and then C6 (94.7 μg/L) cider. The lowest amount of 3-hydroxy-4-phenyl-2-butanonewere was identified in C7 (14.3 μg/L) cider and then C4 (39.7 μg/L) cider.

Satora et al., (2016) reported that carbonyl compounds ranged from 17.60 μg/L to 52.90 mg/L in the apple wine.

Qin et al., (2018) in their study, the amount of acetoin was identified between 0.02 mg/L and 0.12 mg/L in the 14 ciders.

Table 4.27. Carbonyl compounds in ciders

Carbonyl compounds (µg/L)	LRI	ID	Cider 1†	2†	3‡	4‡	S
Acetoin	1224	A,B	102.8±3.8	189.3±3.8	90.4±3.8	86.3±3.8	ns
4-Hydroxy-4-methyl-2-pentanone	1318	B	61.5±3.8	72.7±3.8	50.4±3.8	45.2±3.8	*
3-Hydroxy-4-phenyl-2-butanone	2338	B	53.7±3.8	47.1±3.8	50.2±3.8	39.7±3.8	*
TOTAL			**218**	**309.1**	**191**	**171.2**	

Carbonyl compounds (µg/L)	LRI	ID	Cider 5†	6†	7‡	8‡	S
Acetoin	1224	A,B	42.4±2.1	116.6±3.5	38.4±3.1	167.3±7.8	ns
4-Hydroxy-4-methyl-2-pentanone	1318	B	30.7±7.2	163.1±1.8	29.5±4.9	192.1±9.6	*
3-Hydroxy-4-phenyl-2-butanone	2338	B	42.1±9.7	94.7±7.2	14.3±7.8	107.6±5.3	*
TOTAL			**115.2**	**374.4**	**82.2**	**467**	

Results are the means of three repetitions. C1†: Spontanously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontanously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; Linear retention indices (LRI) calculated on DB-Wax capillary GC column; ± SD. ID, Identification. A, GC retention and MS data in agreement with that of pure compound available in the lab; B, GC retention and MS data in agreement with spectra found in the library; nd, not-detected; S: Significance; *, significant $p<0.05$; ns, not significant; †, No supplementation of DAP; ‡, with supplementation of DAP;

4.5. Sensory analysis

In the present study, sensory analysis of the ciders were studied by using a 10 points test for flavour and aroma profile (Fig.3.12 and Fig.3.13).

Panellists detected the significant differences among the ciders in the flavour profile characteristics of colour, odour, taste, sweetness, sourness, bitterness, astringent and general acceptability, and their results are given in Table 4.28. Also, these results are shown in Figure 4.9 on the radar display for better visualization.

As seen from Figure 4.9, the C1, C2, C3 and C4 ciders were taken the almost similar colour score (2.50-3.10), and this indicated that fermentation was performed by fresh apple juice, which was not significant effects on the colour of ciders. C5, C6, C7 and C8 ciders were became more colour score (5.49-7.63), which was like a pale gold colour.

Odour is an important quality criterion of cider. Considering this point, the highest score of apple cider was 6.81 in C1, and then C7 cider.

C7 cider was taken the highest score in terms of taste with 6.10, and then C1 with 4.73. C5 cider was taken the highest score in terms of sweetness with 3.40. C3 cider was taken the highest score in terms of sourness with 6.16. C1 cider was taken the highest score in terms of bitterness and astringency with 3.54 and 3.26. C7 cider was taken the highest score in terms of general acceptability with 7.17.

Panellists detected the significant differences among the ciders in the aroma profile characteristics of fruity, floral, spicy, caramelize, herbaceous, vegetative, chemical and piquant, and their results are given in Table 4.29. Also, these results are shown in Figure 4.10 on the radar display for better visualization.

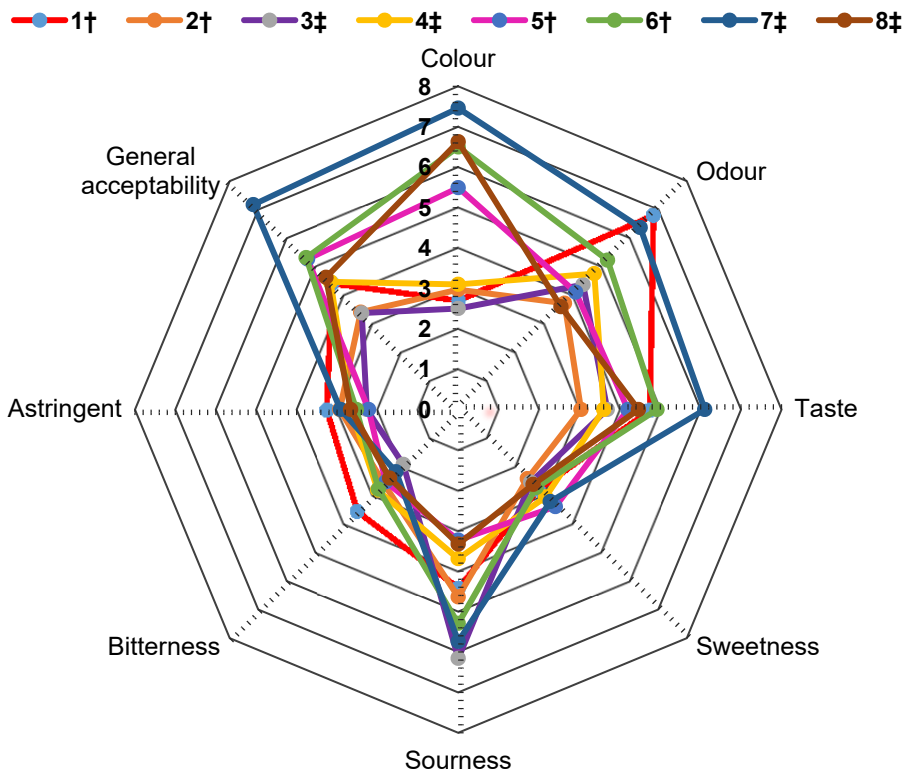
Fruity attributes is an important quality criterion of ciders. Considering this point, the highest score of apple cider was 7.38 in C1, and then C7 apple cider. C1 cider was taken the highest score in terms of floral with 4.21, and then C7 with 3.26. C1 cider was taken the highest score in terms of spicy with 2.56. C7 cider

was taken the highest score in terms of caramelizing with 3.01. C6 cider was taken the highest score in terms of herbaceous with 2.58. C8 cider was taken the highest score in terms of vegetative with 3.22. C2 cider was taken the highest score in terms of chemical with 3.66. C2 cider was taken the highest score in terms of piquant with 3.63.

Table 4.28. The result of flavour profile analysis in ciders

Characteristic	Cider							
	1†	**2†**	**3‡**	**4‡**	**5†**	**6†**	**7‡**	**8‡**
Colour	2.71	2.97	2.50	3.10	5.49	6.50	7.46	6.63
Odour	6.81	3.72	4.37	4.76	4.11	5.23	6.37	3.60
Taste	4.73	3.04	3.69	3.60	4.20	4.92	6.10	4.46
Sweetness	2.78	2.42	2.54	3.06	3.40	2.73	3.22	2.62
Sourness	4.40	4.64	6.16	3.69	3.23	5.29	5.74	3.33
Bitterness	3.54	2.60	1.92	2.82	2.53	2.79	2.18	2.40
Astringent	3.26	2.93	2.26	2.66	2.22	2.52	2.91	2.66
General acceptability	**4.43**	**3.41**	**3.38**	**4.47**	**5.27**	**5.32**	**7.17**	**4.63**

C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; †, No supplementation of DAP; ‡, with supplementation of DAP;



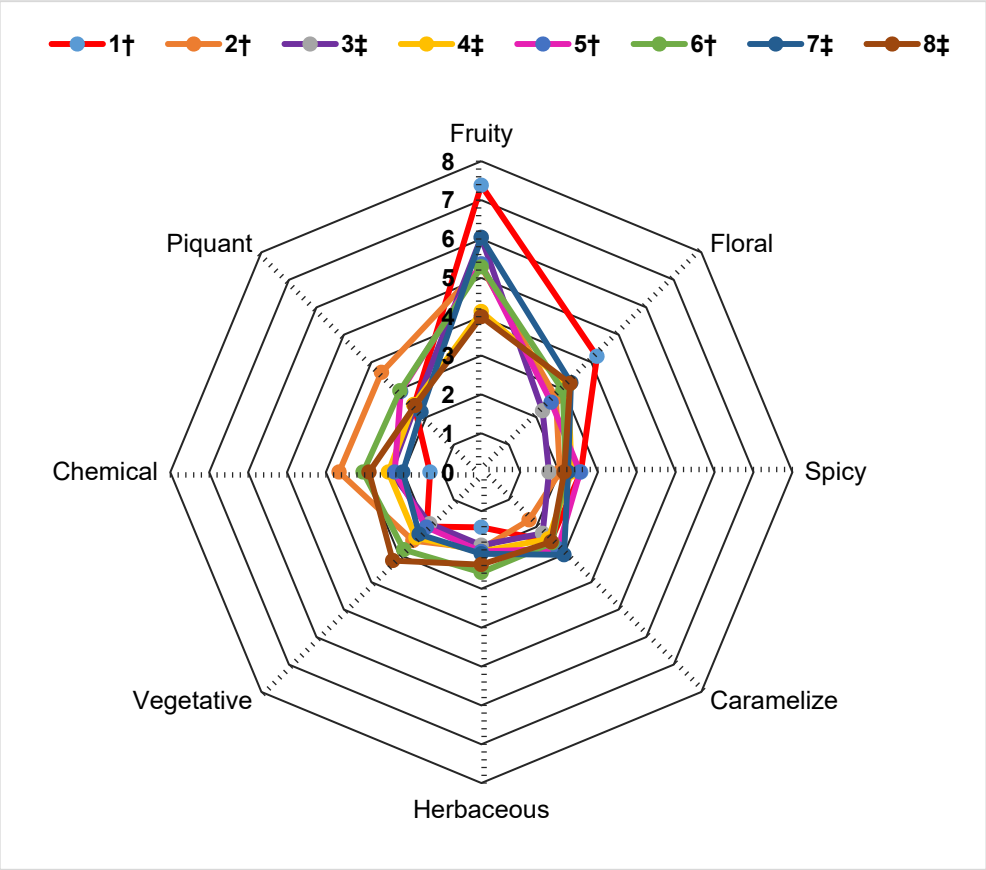
C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; †, No supplementation of DAP; ‡, with supplementation of DAP;
Figure 4.9. A radar diagram of flavour profile analysis in ciders

As seen from Figure 4.9, all cider's scores were closed to each other except odour, colour, taste and sourness criteria.

Table 4.29. The result of aroma profile analysis in ciders

Characteristic	Cider							
	1†	2†	3‡	4‡	5†	6†	7‡	8‡
Fruity	7.38	5.29	6.04	4.13	5.36	5.28	6.04	4.00
Floral	4.21	2.71	2.23	3.16	2.54	2.97	3.26	3.23
Spicy	2.56	2.02	1.74	2.32	2.54	2.17	2.23	2.13
Caramelize	2.52	1.74	2.23	2.43	2.79	2.57	3.01	2.54
Herbaceous	1.42	1.97	1.88	2.04	2.04	2.58	2.10	2.38
Vegetative	1.98	2.49	1.88	2.38	2.00	2.82	2.26	3.22
Chemical	1.32	3.66	2.33	2.39	2.23	3.04	2.02	2.89
Piquant	2.44	3.63	2.40	2.46	2.93	2.96	2.19	2.42

C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; †, No supplementation of DAP; ‡, with supplementation of DAP;



C1†: Spontaneously fermented in fresh apple juice; C2†: Inoculated with commercial yeast in fresh apple juice; C3‡: Inoculated with commercial yeast in fresh apple juice; C4‡: Spontaneously fermented in fresh apple juice; C5†: Spontaneously fermented in apple juice concentrate; C6†: Inoculated with commercial yeast in apple juice concentrate; C7‡: Spontaneously fermented in apple juice concentrate; C8‡ : Inoculated with commercial yeast in concentrated juice; †, No supplementation of DAP; ‡, with supplementation of DAP;
Figure 4.10. A radar diagram of aroma profile analysis in ciders

As seen from Figure 4.10, the cider's scores were almost similar to each other except fruity and floral criteria.

5. CONCLUSION

In the present study, the effects of commercial yeast usage, nitrogen addition (100 ppm) and usage of concentrated apple juice on cider quality were investigated. Experiments were carried out by spontaneously and inoculated with commercial dry yeast. Alcoholic fermentation performed at 18°C. For this purpose, the 8 different experimental ciders were tested. All analyses were carried out three repetitions. These experiments were performed in C1, C2, C3, C4, C5, C6, C7, and C8 cider. Fermentations (C1 to C4 ciders) were done by fresh apple juice and other fermentations were done by apple juice concentrate.

Specific gravity and pH was daily monitored during alcoholic fermentation. Also, enumeration of yeast population was done. In this point of view in the following results:

- ✓ The SG of apple musts was 1.065 at the beginning. Nitrogen addition led to increase for the utilisation of sugar. SG dropped faster in nitrogen added musts compared to others. C3 and C8 fermentation was completed on day 16. The SG was slightly decreased during alcoholic fermentation. The SG was reached to 0.9872-0.9884 at the end of fermentation.
- ✓ Amount of sugar in fresh and concentrated apple musts was 155.36 g/L and 154.1 g/L at the beginning. Total sugars varied between 1.59 g/L and 5.38 g/L at the end of fermentation. The all fermentations were completed between 16 to 25 days. The growth of fermentation was depended on the supplementation of DAP.
- ✓ The pH values of the samples were determined 3.48 at the beginning of alcoholic fermentations. The pH values were slightly decreased and ranged from 3.12-3.27 at the end of the fermentation.
- ✓ Fermentation was completed to the different supplementation of DAP apple must by the commercial yeast and spontaneous fermentation. It can

be said that fermentation (with supplementation of DAP 100 ppm) has a positive effect on fermentations (spontaneously fermented and inoculated with commercial yeast). They were also affected the fermentation times and final products. The inoculation with supplementation of DAP fermentations was quickly proceeded and completed between 16 and 17 days.

Results of the experimental ciders:

- ✓ The pH levels of ciders varied between 3.12 and 3.27. The lowest and highest pH level was measured in C3 and C6 cider. Considering that the highest total acidity value can be explained by the lowering of the pH in these experiments.
- ✓ Total titratable acidity of ciders varied between 6.65-7.01 g/L (as malic acid). The lowest and highest amount of total titratable acidity was determined in C7 and C3 cider. The content of total titratable acidity in all trials was above the minimum 6 g/L limit specified in North American semi-sweet to sweet sparkling cider.
- ✓ Browning indices values were measured between 0.0493 and 0.1641 after bottling. It was dropped to less than 0.02 after fermentation. The lowest and highest amounts were determined in C1 and C8 cider.
- ✓ The amount of tannins was determined from 0.046 to 0.378 g/L. The highest and lowest content of tannins was determined in C2 and C6 cider.
- ✓ The content of ethanol in ciders ranged from 8.13 to 8.75% (v/v). This alcohol strength was average amount in cider products.
- ✓ The amount of carbon dioxide varied between 0.05 and 0.196 % (w/w). The lowest and highest carbon dioxide of the cider was determined in C4 and C5 cider.

- ✓ The amount of sulphur dioxide of ciders ranged from 30.84 mg/L to 42.68 mg/L. These amounts were measured in lower than other commercial cider products.
- ✓ The amount of volatile acidity in the experimental ciders varied between 0.22 g/L and 0.74 g/L, which was the average amount.
- ✓ L* value means brightness and -a* value means greenness. These values are the basic colour of cider. The highest -a* value was determined as C6 cider. In this case, negative a* value of all samples were in the green zone. In addition, these values were depended on using different raw materials (fresh apple juice and apple juice concentrate).
- ✓ The lowest and highest total of sugars were determined in C3 (1.59 g/L) and C2 (5.38 g/L) cider. According to the results obtained, these values were significantly lower than other studies. The minimum and maximum numbers of organic acids were determined in C2 (3.43 g/L) and C6 (5.76 g/L). In point of views, these results were average level.
- ✓ The 8 experimental ciders were identified 55 aroma compounds, including 13 of higher alcohols, 13 of ethyl esters, 5 of acetate esters, 9 of volatile acids, 3 of six carbon alcohols, 5 of phenolic compounds, 4 of lactones and 3 of carbonyl compounds. The amount of aroma compounds were identified as 85.18 mg/L in C1, 89.14 mg/L in C2, 72.8 mg/L in C3, 74.65 mg/L in C4, 75.26 mg/L in C5, 66.92 mg/L in C6, 62.4 mg/L in C7 and 70.3mg/L in C8 cider.
- ✓ The highest amount of the higher alcohols were determined in C2 (45.56 mg/L) cider as isoamyl alcohol, then 2, 3-butanediol and 2-phenylethanol. The lowest amount of isoamyl alcohol was determined in C7 (20.03 mg/L) cider. The highest and lowest amount of ethyl hexanoate was identified in the C2 and C4 cider.
- ✓ A total of 18 ester compounds were identified in the experimental ciders. The highest and lowest amount of ethyl hexanoate was identified in the C2

and C4 cider. Ethyl esters could be negatively affected by DAP addition ciders.

- ✓ A total of 9 volatile acids were determined between 5.3 mg/L and 12.4 mg/L. The maximum and minimum numbers of volatile acids were determined in C7 and C1 cider. The maximum and minimum amount of volatile acids were determined in C3 and C7 ciders. The highest amount of cis-3-hexenol was determined in C1 (39.9 μg/L) cider, the lowest was in C7 (21.7 μg/L) cider.
- ✓ The value of six-carbon alcohols ranged from 0.3 mg/L to 2.9 mg/L in ciders. The six carbon alcohols (2-hexanol, 1-hexanol, cis-3-hexenol) were identified in our experimental ciders. The amount of six-carbon alcohols were identified as 2.9 mg/L in C1, 0.4 mg/L in C2, 2.2 mg/L in C3, 2.1 mg/L in C4, 0.5 mg/L in C5, 1.2 mg/L in C6, 0.3 mg/L in C7 and 0.9 mg/L in C8 ciders. The maximum and minimum amount of six carbon alcohols was determined in C3 and C7 cider. The lowest and highest amount of 1-hexanol was also found in C7 (265.9 μg/L) and C1 (2897.9 μg/L) cider. The lowest and highest amount of cis-3-hexenol was determined in C7 (21.7 μg/L) and C1 (39.9 μg/L) cider.
- ✓ A total of 5 phenolic compounds was determined between 0.7 mg/L and 1.4 mg/L. The lowest and highest amount of 4-allylguaiacol was determined in C8 (139.2 μg/L), C7 (231.7 μg/L) cider and C5 (996.4 μg/L) and C1 (351.2 μg/L) cider. The lowest and highest amount of 2-methoxy-4-vinylphenol was identified in C4 (173.8±0.9 μg/L), C7 (203.4 μg/L) cider and C7 (437 μg/L), C3 (385.4 μg/L) cider. The lowest and highest amount of 4-allylphenol and 2, 6-dimethoxy phenol was found in C7 (59.4 μg/L), C6 (37.4 μg/L) cider and C1 (244.3 μg/L), C2 (254.6 μg/L) cider.
- ✓ The value of lactones ranged from 0.26 mg/L to 0.63 mg/L. The maximum amount of lactone was determined in C1 cider, and then followed by C7

cider. The lowest and highest amount of lactones were determined in C6, C8 and C7 cider.

- ✓ The amount of carbonyl compounds ranged from 0.08 mg/L to 0.47 mg/L. The maximum and minimum amount of carbonyl compounds were determined in C8 and C7 ciders.
- ✓ A flavour profile analysis of sensory data were evaluated to the most approved sample by the panellists was C7 cider. C7 cider was received the highest score in terms of general acceptability with 7.17.
- ✓ An aroma profile analysis of sensory data were evaluated to the highest score in term of fruity with 7.38 in C1, and then C7 cider.

In point of results, C7 cider was preferred to other experimental ciders.

As a result of the analysis, with supplementation of DAP fermentations, the raw material was positively affected after the fermentation process was accelerated.

REFERENCES

ACKERMANN, J., FISCHER, M., AMADO, R., 1992. Changes in sugars, acids, and amino acids during ripening and storage of apples (cv. Glockenapfel). Journal of Agricultural and Food Chemistry. 40 (7): 1131–1134.

ALBERTI, A., VIEIRA, R.G., DRILLEAU, J.F., WOSIACKI, G., and NOGUEIRA, A. 2011. Apple wine processing with different nitrogen contents, Braz. Arch. Biol. Technol. 54, 551–558.

ALBERTI, A., BRAGA, C.M., JASTER, H., and NOGUEIRA, A. 2014. Dissolved oxygen content in apple must: Technological implications in cider processing, J. Inst. Brew. 120, 65–70.

ANDREW, G.H., LEA and DRILLEAU, J.F., 2003. Cider making;

ANSANEY-GALEOTE, V., BLONDIN, B., DEQUIN, S., SABLAYROLLES, J. M., 2001. Stress effect of ethanol on fermentation kinetics by stationary phase cells of *Saccaromyces cerevisiae*. Biotechnology Letters, 677–681.

ANTON, M.J., VALLES, B.S., HEVIA, G.A., & LOBO, P.A., 2014. Aromatic profile of ciders by chemical quantitative, gas chromatography-ofactometry, and sensory analysis. Journal of Food Science, 79(1), S92–S99.

ANTON-DIAZ, M.J., VALLES, B.S., MANGAS-ALONSO, J.J., FERNANDEZ-GARCIA, O., 2016. Impact of different techniques involving contact with lees on the volatile composition of cider. Food Chemistry 190 (2016) 1116–1122.

ARDÖ, Y., 2006. Flavour formation by amino acid catabolism, Biotechnol. Adv. 24, 238–242.

ARTHEY, D., and ASHURDS, P.R, 'Fruit processing' 1996, 98;

AUNG, M.T., LEE, P.R., YU, B., LIU, and SH.Q., 2015. Cider fermentation with three *Williopsis saturnus* yeast strains and volatile changes. Annals of Microbiology, Vol 65, Issue 2, pp 921–928.

BAILEY, J.E., and OLLIS, D.F., 1986. Biochemical Engineering Fundamentals, second ed. Mc-Graw Hill Higher Education.

BARBOSA, C., FALCO, V., MENDES-FAIA, A., and MENDES-FERREIRA, A., 2009. Nitrogen addition influences formation of aroma compounds, volatile acidity and ethanol in nitrogen deficient media fermented by *Saccharomyces cerevisiae* wine strains, Journal of Bioscience and Bioengineering VOL. 108 No. 2, 99–104, 2009

BARBOSA, C., MENDES-FAIA, A., and MENDES-FERREIRA, A., 2012. The nitrogen source impacts major volatile compounds release by *Saccharomyces cerevisiae* during alcoholic fermentation, Int. J. Food Microbiol. 160, 87–93.

BEECH, F.W., 1993. Yeasts in Cider Making. In The Yeasts 2nd Vol 5. 'Yeast Technology'. Rose, A. H. and Harrison, J. S. Academic Press, London. pp 169 - 213.

BELL, S.J., HENSCHKE, A.P. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape and Wine Research 11, 242–295.

BELTRAN, G., NOVO, M., GUILLAMON, J.M., MAS, A., and ROSES, N., 2008. Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. Int J Food Microbiol 121:169-77.

BELY, M., SALMON, J.M., and BARRE, P., 1994. Assimilable nitrogen addition and hexose transport activity during enological fermentations. J. Inst. Brew. 100, 279-282.

BERRY, D.R., 1995. Alcoholic beverage fermentation, in Fermented Beverage Products (Lea AGH, Piggot JR. Eds), Blackie Academic & Professional, Glasgow.

BIZEAU, C., LE QUERE, J.M., MICHEL, A., and DRILLEAU, J.F., 1992 Possibilities for modelling cider fermentation Industries Agricoles et Alimentaires 109. 7-9.

BOZOĞLU, D., ERTUNC, S., AKAY, B., BURSALI, N.R., and VURAL, N., 2015. The effect of temperature, pH and SO_2 on ethanol concentration and sugar consumption rate in apple wine process. J. Chem. Soc. Pak., Vol. 37, No. 03.

BOYLSTON, T.D., WANG, H., REITMEIER, C.A., and GLATZ, B.A., 2003. Effects of processing treatment and sorbate addition on the flavour characteristics of apple cider. J Agric. Food Chem. 51:1924–31.

BRAGA, C.M., FERREIRA, A.A., and MARQUES, Z.K., 2013. Classification of juices and fermented beverages made from unripe, ripe and senescent apples based on the aromatic profile using chemo metrics. Food Chemistry 141 (2013) 967–974

BROWN, S., BADENES, M.L., and BYRNE, D.H., 2012. Fruit breeding. Handbook of plant breeding (pp. 329e367). New York: Springer.

CAMPBELL, I., 1988. Culture, storage, isolation and identification of yeasts. In: Campbell I and FG Priest (Eds), Yeast-A Practical Approach. Chapman and Hall, London, pp. 1-11.

CANBAŞ, A., CABAROĞLU, T., and ESKİÜÇTEPE, T., 2000. A research on the production of low, normal and high alcoholic apple wines from starking and Golden Delicious cultivars. Ç.Ü.Z.F Dergisi, 15(2):71-78

CLARK, R.J., and BAKKER. J., 2014. Wine chemistry.

COHEN, E., BIRK, Y., MANNHEIM, C.H., and SAGUY, I.S., 1998. A rapid method to monitor quality of apple juice during thermal processing. Lebensm.-Wiss. u.-Technol., 31, 612–616

COLOMBIE, S., LATRILLE, E., and SABLAYROLLES, J.M., 2007. Online estimation of assimilable nitrogen by electrical conductivity measurement during alcoholic fermentation in enological conditions. J. Biosci. Bioeng. 103 (3), 229-235.

COMPENDIUM OF INTERNATIONAL METHODS OF WINE AND MUST ANALYSIS. 2016. Volume 1, 2.

COTON, M., ROMANO, A., SPANO, G., ZIEGLER, K., VETRANA, C., DESMARAIS, C., LONVAUD-FUNEL, A., LUCAS, P., and COTON, E., 2010. "Occurrence of biogenic amine-forming lactic acid bacteria in wine and cider". Food Microbiology. 27 (8): 1078–1085.

COULTER, A., GODDEN, P., and PRETORIUS, I., 2004. Succinic acid, how it is formed, what is its effect on titratable acidity, and what factors influence its concentration in wine. https://www.researchgate.net/publication/284696694

CRAMER, A.C., VLASSIDES, S., and BLOCK, D.E., 2002. Kinetic model for nitrogen-limited wine fermentations. Biotechnology and Bioengineering 77, 49–60.

CRUZ, S.H., CILLI, E.M., and ERNANDES, J.R., 2002. Structural complexity of the nitrogen source and influence on the yeast growth and fermentation. J. Inst. Brew. 108, 54-61.

DONALIES, E.B., NGUYEN T.T., STAHL, U., and NEVOIGT, E., 2008. Improvement of Saccharomyces Yeast Strains Used in Brewing, Wine Making and Baking, Adv. Bio-chem. Eng. Bio-technol., 111, 67.

DRILLEAU, J.F., 1990. Fermentation of cider. Rev. Pomme à cidre, 21, 20-22.

DRILLEAU, J.F., 1992. Pommes et cidres materières premières du Calvados. In R. Cantagrel Élaboration et connaisance des spiritueux (pp. 193–200). Paris: Lavoisier-Tec & Doc.

EWART, A., 1995. White Wines. In Fermented Beverage Production Lea, A .G. H. and Piggott J. R. Blackie, Glasgow. pp 97-120

FAIRBAIRN, S., MCKINNON, A., MUSARURWA, H.T., FERREIRA, A.C., and BAUER. F.F., 2017. The impact of single amino acids on growth and volatile aroma production by *saccharomyces cerevisiae* strains. Microbiol. 8:2554.

FLEET, G.H, and HEARD, G.M., 1993. Yeasts growth during fermentation. In: Fleet, G.M. (Ed), Wine Microbiology and Biotechnology. Harwood Academic Publishers, Chur, Switzerland, pp. 27-54.

GAMERO, A., HERNÁNDEZ-ORTE, P., QUEROL, A., and FERREIRA, V., 2011. Effect of aromatic precursor addition to wine fermentations carried out with different Saccharomyces species and their hybrids, Int. J. Food Microbiol. 147, 33–44.

GARDE-CERDÁN, T., and ANCIN-AZPILICUETA, C., 2008. Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. Food Sci. Technol. Int., 41 (3), 501-510.

GÖKMEN, V., and ACAR, J., 1999. Simultaneous determination of 5-hydroxymethylfurfural and patulin in apple juice by reversed-phase liquid chromatography. 847(1-2):69-74.

GUICHARD. H., POUPARD, P., LEGOAHEC, L., MILLETA, M., BAUDUIN, R., LE QUEREB, J.M., 2019. *Brettanomyces anomalus*, a double drawback for cider aroma. LWT - Food Science and Technology 102 (2019) 214–222

GUTIÉRREZ, A., CHIVA, R., SANCHO, M., BELTRAN, G., ARROYO-LÓPEZ. F.N., and GUILLAMON, J.M., 2012. Nitrogen requirements of commercial wine yeast strains during fermentation of a synthetic grape must. Food Microbiology 31 (2012) 25e32

HERNANDEZ-ORTE, P., BELY, M., CACHO, J., and FERREIRA, V., 2006. Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different *Saccharomyces cerevisiae* strains during fermentation in controlled synthetic media. Australian Journal of Grape and Wine Research 12, 150–160

HERRERO, M., GARCIA, L.A., and DIAZ, M., 2003. The Effect of SO_2 on the Production of Ethanol, Acetaldehyde, Organic Acids, and Flavour Volatiles during Industrial Cider Fermentation, J. Agric. Food Chem., 51 , 3455.

HERRERO, M., GARCIA, A.L., and DIAZ, M., 2006. Volatile Compounds in Cider: Inoculation Time and Fermentation Temperature Effects, J. Inst. Brev., 112, 210.

HUNTER, L* a* b* colour scale. 2008. Technical services department hunter associates laboratory.

JARVIS, B., 2014. Cider (Cyder; Hard Cider). Encyclopedia of Food Microbiology. pp. vol 1 pp 437–443.

JALVIS, B., and LEA, A.G, 2000. Sulphate binding in ciders. Int. Food Sci. Technol. 35(1), 113-127.

JOVANA, J., BOJANA, M., ANAMARIJA I.S., IVAN, L.J., MILOVANOVIĆ, P.T., and JASNA, S.J., 2009. Determination of 5-hydroxymethylfurfural IN apple juice. Food Processing, Quality and Safety 1-2(2009) 35-39

JULIEN, A., ROUSTAN, J.L., DULAU, L., and SABLAYROLLES, J.M., 2000. Comparison of Nitrogen and Oxygen Demands of Enological Yeasts: Technological Consequences Am. J. Enol. Viticult. 51 (3), 215-222.

JULIEN, A., TRIOLI, G., and DULAU, L., 2001. Alcoholic fermentation control: the yeast growth factors and arrest prevention. Rev. Enologues., 101, 25-29.

KELKAR, S., and DOLAN, K., 2012. Modelling the effects of initial nitrogen content and temperature on fermentation kinetics of hard cider. Journal of Food Engineering 109, 588–596

KEMSAWASD, V., VIANA, T., ARDÖ, Y., and ARNEBORG, L., 2015. Influence of nitrogen sources on growth and fermentation performance of different wine yeast species during alcoholic fermentation. Apple Microbiol. Bio-technol. (2015) 99:10191–10207.

LE QUÉRÉ, J.M., HUSSON, F., RENARD, C.G.C., and PRIMAULT, J., 2006. French cider characterization by sensory, technological and chemical evaluations. LWT - Food Science and Technology. 39 (9): 1033–1044.

LEA, A., 1978. The Phenolics of Ciders: Bitterness and Astringency. Journal of the Science of Food and Agriculture. 29 (5): 478–483.

LEA, A.G.H., and TIMBERLAKE, C.F., 1978. Phenolics of ciders the effect of processing. J. Sci. Food Agric. 29 484-492

LEA, A.G.H., 1984. Colour and Tannin in English Cider Apples. Flüssiges Obst. 51 356 - 361

LEA, A.G.H., 1991. The Use of Enzymes in Beverage Production. In Enzymes in Food Processing Tucker, G. A. and Woods, L. F. J. Blackie, Glasgow. pp 194-220

LEA, A.G.H., 1994. Apple Juice. In Production and Packaging of Non-Carbonated Fruit juices and Fruit Beverages, 2nd. Ashurst, P. R. Blackie, Glasgow.

LEA, A.G.H., and DRILLEAU, J.F., 2003. Cidermaking. In: Fermented Beverage Production, ed. A. G. H. Lea, J. R. Piggot. Blackie Academic and Professional, London. pp. 59-87

LEE, J.H., KANG TH., and UM, B.H., 2013. Evaluation of physicochemical properties and fermenting qualities of apple wines added with medicinal herbs. Food Sci Biotech 22:1039-46.

LEQUERE, J.M., and DRILLEAU, J.F., 1998. Microbiology and technology of cider. Rev. Enologues., 88, 17-20.

LIU, SH.Q., AUNG, A.T., LEE, P.R, and YU, B., 2016. Yeast and volatile evolution in cider co-fermentation with *Saccharomyces cerevisiae* and *Williopsis saturnus.* Ann Microbiol (2016) 66:307–315.

LOBO, A.P., ANTÓN-DÍAZ, M.J., MANGAS ALONSO J.J., SUÁREZ VALLES, B. 2016. Characterization of Spanish ciders by means of chemical and olfactometric profiles and chemo-metrics. Food Chemistry 213 (2016) 505–513.

LOBO, A.P., ANTÓN-DÍAZ, M.J., PANDO BEDRIÑANA, R., FERNÁNDEZ GARCÍA, O., HORTAL-GARCÍA, R., SUÁREZ VALLES, B. 2018. Chemical, olfactometric and sensory description of single-variety cider apple juices obtained by cryo-extraction. LWT - Food Science and Technology 90 (2018) 193–200.

LORENZINI, M., SIMONATO, B., SLAGHENAUFI, D., UGLIANO, M., and ZAPPAROLI, G., 2019. Assessment of yeasts for apple juice fermentation and production of cider volatile compounds. LWT - Food Science and Technology 99 (2019) 224–230.

MA, SH., NEILSON, A., LAHNE, G., PECK, G., O'KEEFE, S., HURLEY, E.K., SANDBROOK, A., and STEWART, A., 2018. Juice clarification with pectinase reduces yeast assimilable nitrogen in apple juice without affecting the polyphenol composition in cider. Vol. 83, Iss. 11, 2018, Journal of Food Science.

MADRERA, R.R., Lobo, A.P., and Alonso, J.J.M., 2010. Effect of cider maturation on the chemical and sensory characteristics of fresh cider spirits. Food Research International 43 (2010) 70–78.

MADRERA, R.R., HEVIA, A.G., GARCIA, N.P., and VALLES, B.S., 2017. Evolution of aroma compounds in sparkling ciders. LWT - Food Science and Technology 41 (2008) 2064e2069.

MALHERBE, S., FROMION, V., HILGERT, N., and SABLAYROLLES, J.M., 2004. Modelling the effects of assimilable nitrogen and temperature on fermentation kinetics in enological conditions. Biotechnology and Bioengineering 86, 261-272.

MELIHA, A., JESS, K., and PAULSON, G., 2015. Global Agricultural Information Network.

MENDES-FERREIRA, A., MENDES-FAIA, A., and LEAO, C., 2014. Growth and fermentation patterns of *Saccharomyces cerevisiae* under different ammonium concentrations and its implications in winemaking industry. Journal of Applied Microbiology, 97, 540–545.

MITCHELL, P., 2006. Cider and Perry: Sensory evaluation. Course manual. Per shore, UK: Mitchell F&D Limited.

MOLINA, A.M., SWIEGERS, J.H., VARELA, C., PRETORIUS, I.S., and AGOSIN, E., 2017. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. Apple Micro-biol. Bio-technol. (2007) 77:675–687.

NOBILE, D., D'AMATO, M. A., ALTIERI, D., CORBO, C., and SINIGAGLIA, M., 2003. Modeling the yeast growth-cycle in a model wine system. Journal of Food Science 68, 2080–2085.

NOGUEIRA, A., LEQUÉRÉ, J.M., and BULDIN, R., 2003. Oxygène et stabilité des cidres. Rev. Pomme à cidre, 5, 16 - 17.

NURGEL, C., ERTEN, H., CANBAS, A., CABAROĞLU, T., and SELLI, S., 2002. Influence of *Saccharomyces cerevisiae* strains on fermentation and flavour compounds of white wines made from cv. Emir grown in Central Anatolia, Turkey. Journal of Industrial Microbiology & Biotechnology 29, 28 –33

PENG, B., LI, F., CUI, L., and GUO, Y., 2015. Effects of fermentation temperature on key aroma compounds and sensory properties of apple wine. Journal of Food Science. Vol. 80, Nr. 12,

PENG, B., GE, N.A., CUI, L., and ZHAO, H., 2016. Monitoring of alcohol strength and titratable acidity of apple wine during fermentation using near-infrared spectroscopy. Food Science and Technology 66 (2016) 86e92

PİCİNELLİ, A., SUÁREZ, B., MORENO, J., RODRÍGUEZ, R., CASO-GARCÍA, L. M., and MANGAS, J.J., 2000. Chemical characterization of Austrian cider. Journal of Agricultural and Food Chemistry, 48(9), 3997–4002.

PINO, JA. QUERIS, O. 2011. Analysis of volatile compounds of mango wine. Food Chem 125:1141-6.

QIN, Z., MIKAEL, A., WENDER, L.P., and BREDIE, J., 2018. Flavor profiling of apple ciders from the UK and Scandinavian region. Food Research International 105 (2018) 713–723

RAJASHEKHARA, E., SURESH, E. R., and ETHIRAJ, S., 1998. Thermal Death Rate of Ascospores of Neosartorya fischeri ATCC 200957 in the Presence of Organic Acids and Preservatives in Fruit Juices. Journal of Food Protection. 61 (10): 1358–1362.

RIBÉREAU-GAYON, P., DUBOURDIEU, D., DONÉCHE, B., and LONVAUD, A. 2000. The Microbiology of Wine and Vinifications, Handbook of Enology Volume 1, John Wiley and Sons, Ltd., West Sussex, 71-73.

RIBÉREAU-GAYON, P., GLOIRES, Y., MAUJEAN, A., and DUBOURDIEU, D., 2006. Handbook of Enology Volume 2: The Chemistry of Wine and Stabilization and Treatments, John Wiley and Sons, Ltd., England.

RITA, R., ZANDA, K., DAINA, K., and DALIJA, S. 2011. Compositions of aroma compounds in fermented apple juice: Effect of apple variety, fermentation temperature and inoculated yeast 546 concentration, Procedia. Food Sci. 1, 1709–1716.

ROLLERO, S., BLOEM, A., CAMARASA, C., SANCHEZ, I., ORTIZ-JULIEN, A., SABLAYROLLES, J.M., DEQUIN, S., and MOURET. J.R., 2015. Combined effects of nutrients and temperature on the production of fermentative aromas by *Saccharomyces cerevisiae* during wine fermentation. Apple Micro-biol. Bio-technol. (2015) 99:2291–2304.

ROLLERO, S., MOURET, J.R., SANCHEZ, I., CAMARASA, C., ORTIZ-JULIEN, A., SABLAYROLLES, J.M., and DEQUIN, S., 2016. Key role of lipid management in nitrogen and aroma metabolism in an evolved wine yeast strain. Micro-bio. Cell Fact (2016) 15:32.

ROWLES, K., 2010. Processed Apple Product Marketing Analysis: Hard Cider and Apple Wine, Department of Agricultural Resource and Managerial Economics, Cornell University, Ithaca, New York, p. 212.

RUSSELL, JAMES, B., DIEZ-GONZALEZ., and FRANCISCO., 1997. The Effects of Fermentation Acids on Bacterial Growth. Advances in Microbial Physiology. 39. pp. 205–234.

SAERENS, M.G., DELVAUX, F., VERSTREPEN, K.J, VAN DIJCK, P., THEVELEIN, J.M., and DELVAUX, F.R., 2008. Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. Applied and environmental microbiology. vol. 74, 2008.

SANTOS, C.M., GDE, A.P., BRAGA, C.M., ROSSI, M.J., NINOW, J., SANTOS, T.P., WOSIACKI, G., JORGE, R.M., and NOGUEIRA, A., 2015. Apple Amino acid Profile and Yeast Strains in the Formation of Fusel Alcohols and Esters in Cider Production. J Food Sci. 2015 Jun;80(6):C1170-7. doi: 10.1111/1750-3841.12879.

SALMON, J.M., and BARRE, P., 1998. Improvement of nitrogen assimilation and fermentation kinetics under enological conditions by depression of alternative nitrogen-assimilatory pathways in an industrial *Saccharomyces cerevisiae* strain. Ap. Environ. Micro-bio., 64.

SANONER, P., 1999. "Polyphenol Profiles of French Cider Apple Varieties". Journal of Agricultural and Food Chemistry. 47 (12): 4847–4853.

SATORA, P., SROKA, P., DUDA-CHODAK, A., TARKO, T., and TUSZYNSKI, T., 2008. The profile of volatile compounds and polyphenols in wines produced from dessert varieties of apples. Food Chem 111:513-9.

SATORA, P., CIOCH, M., TARKO, T., and WOŁKOWICZ, J., 2016. Killer strains of *Saccharomyces*: Application for apple wine production. Journal of the Institute of Brewing, 122(3).

SCOTT, J.A., and SWAFFIELD, C.H., 1998. Observations on the influence of temperature, dissolved oxygen and juice source on stored alcoholic cider development. Food bio-technol. 12(1), 13-26

SCHOLTEN, G., 1992. What would RSK values for cider be like? Flüssiges Obst. 59 466-471

SEGUINOT, P., ROLLERO, S., SANCHEZ, I., SABLAYROLLES, J.M., ORTIZ-JULIEN, A., CAMARASA, C., and MOURET. J.R. 2018. Impact of the timing and the nature of nitrogen additions on the production kinetics of fermentative aromas by *Saccharomyces cerevisiae* during winemaking fermentation in synthetic media. Food Microbiology 76 (2018) 29e39.

SU, A.Y., ORIGONE, A.C., RODRÍGUEZ, M.E., QUEROLA, A., GUILLAMÓNA, J.M., and LOPES, C.A., 2019. Fermentative behaviour and competition capacity of cryotolerant *Saccharomyces* species in different nitrogen conditions. International Journal of Food Microbiology 291 (2019) 111–120.

SUN, S.Y., CHE, C.Y., SUN, T.F., LV, Z.Z., HE, S.X., GU, H.N., and GAO, Y., 2013. Evaluation of sequential inoculation of *Saccharomyces cerevisiae* and *Oenococcus oeni* strains on the chemical and aromatic profiles of cherry wines. Food Chemistry, 138(4), 2233–2241.

SWIEGERS, J.H., BARTOWSKY, E.J., HENSCHKE, P.A., and PRETORIUS, I.S., 2005. Yeast and bacterial modulation of wine aroma and flavour. Australian Journal of Grape and Wine Research 11, 139–173.

TORREA, D., VARELA, C., UGLIANO, M., ANCIN-AZPILICUETA, C., FRANCIS, I.L., and HENSCHKE, P.A., 2011. Comparison of inorganic and organic nitrogen supplementation of grape juice–Effect on volatile composition and aroma profile of a Chardonnay wine fermented with *Saccharomyces cerevisiae* yeast. Food Chemistry 127 (2011) 1072–108

UGLIANO, M., TRAVIS, B., FRANCIS, I.L., and HENSCHKE, P.A., 2010. Volatile composition and sensory properties of Shiraz wines as affected by nitrogen supplementation and yeast species: rationalizing nitrogen modulation of wine aroma. J. Agric. Food Chem., Vol. 58, No. 23.

UMANO, K., HAGI, Y., NAKAHARA, K., SHYOJI, A., and SHIBAMOTO, T., 1995. Volatile chemicals formed in the headspace of a heated glucose/ l-cycteine Maillard model system. Journal of Agricultural and Food Chemistry, 43, 2212–2218.

U.S. Department of Agriculture, National Agricultural Statistics Service, Sampling Frame Standards for Coverage and Maintenance, Policy and Standards Memorandum, 14, (2008).

U.S., 2008. Apple Association.

VALLESA, B.S., BEDRINANAA, R.P., TASCONA, N.F., SIMONB, A.Q., and MADRERA. R.R., 2007. Yeast species associated with the spontaneous fermentation of cider. Food Microbiology 24. 25–31

VARELA, C., 2016. The impact of non-*Saccharomyces* yeasts in the production of alcoholic beverages. Apple Micro-biol. Bio-technol. (2016) 100:9861–9874.

VILLIERE, A., ARVISENET, G., BAUDUIN, R., LE QUERE, J.M., and SEROT, T., 2015. Influence of cider-making process parameters on the odourant volatile composition of hard ciders. J Inst Brew 121:95–105

VILANOVA, M., UGLIAN, M., VARELA, C., SIEBERT, T., PRETORIUS, I.S., and HENSCHK, P.A., 2007. Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by *Saccharomyces cerevisiae* wine yeasts. Apple Micro-biol. Bio-technol. (2007) 77:145–157.

WILKINS, C.K., 1990. Analysis of indole and skatole in porcine gut contents. International Journal of Food science & Technology. 25, 313.

WHITING, G.C., 1976. Organic acid metabolism of yeasts during fermentation of alcoholic beverages-a review. Journal International Brew 82:84–92

XU, Y., FAN, W., QIAN, M.C., 2007. Characterization of Aroma Compounds in Apple Cider Using Solvent-Assisted Flavour Evaporation and Headspace Solid-Phase Micro extraction. J. Agric. Food Chem., Vol. 55, No. 8.

YE, M., YUE, T., YUAN, Y., 2014. Changes in the profile of volatile compounds and amino acids during cider fermentation using dessert variety of apples. Euro Food Researching Technology (2014) 239:67–77.

YILMAZTEKIN, M., CABAROGLU, T., and ERTEN, H., 2013. Effects of fermentation temperature and aeration on production of natural isoamyl acetate by *Williopsis saturnus* var. *saturnus.* Bio-Med Res. Int. 1:1-6.

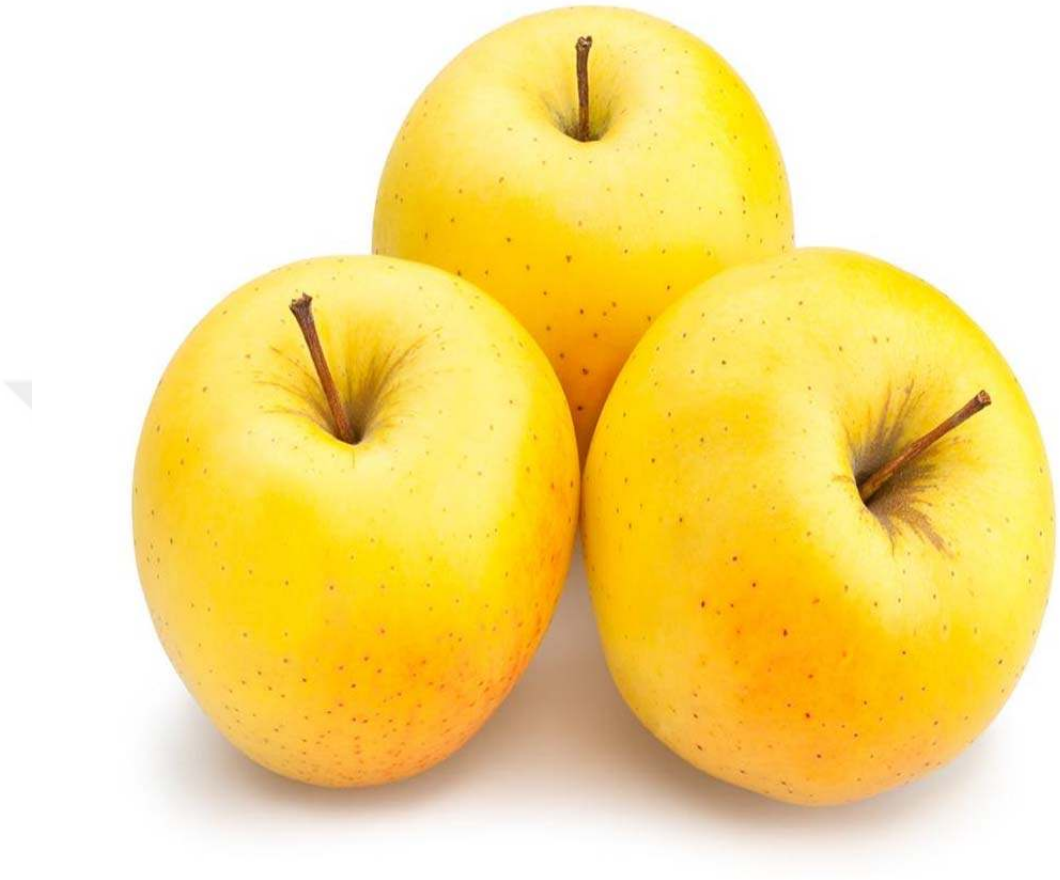
ZHANG, H., ZHOU, F., J.I.B., ROB, M., NOUT, J., FANG, Q., and YANG, Z., 2008. Determination of organic acids evolution during apple cider fermentation using an improved HPLC analysis method.

ZHAO, H., ZHOU, F., DZIUGAN, P., YAO, Y., ZHANG, J., LV, Z., and ZHANG, B., 2014. Development of Organic Acids and Volatile Compounds in Cider during Malolactic Fermentation. Czech J. Food Sci. Vol. 32, No. 1: 69–76

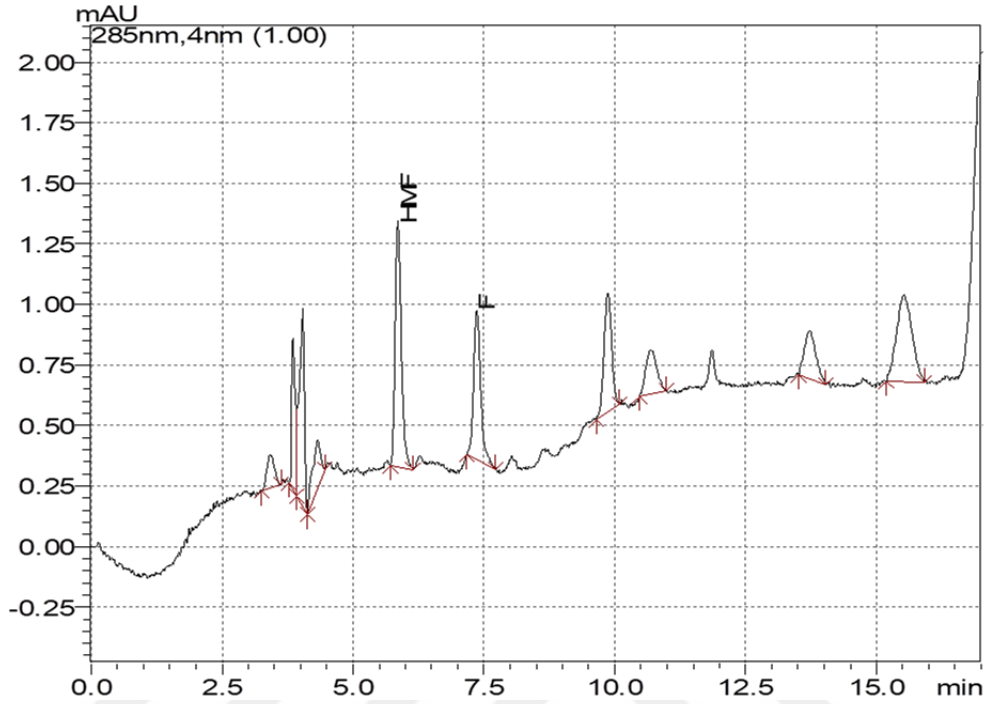
CURRICULUM VITAE

Naidandorj DAMDIN was born in 1984. After being graduated from high school, he enrolled in Food Preparation Technology & Service Department of Food Engineering & Biotechnology School, Mongolian University of Science & Technology, He started his Master of Science education in graduated from Mongolian University of Science & Technology as a Food Technology & Hygiene in Sep. 2010. He has been working as a lecture at department of Livestock Product & Monitoring and Inspection, School of Animal Science & Biotechnology, Mongolian University of Life Science since 2012. He graduated from PhD degree of Food Engineering, Institute of Natural and Applied Sciences, Faculty of Agriculture, Department of Food Engineering, Çukurova University in 2019.

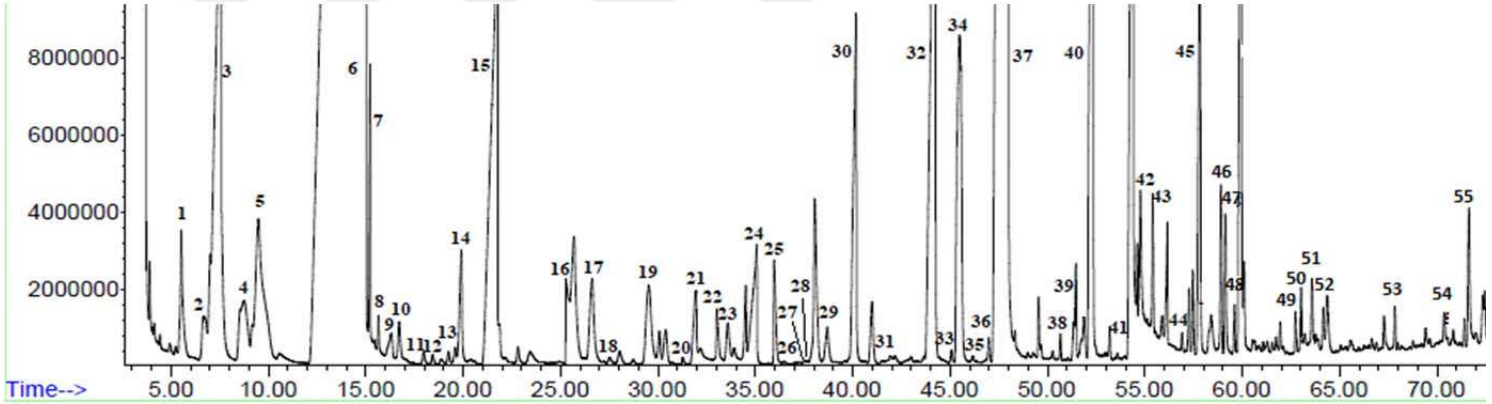
APPENDICES



Appendix 1. Golden Delicious (*Malus domestica*) apple

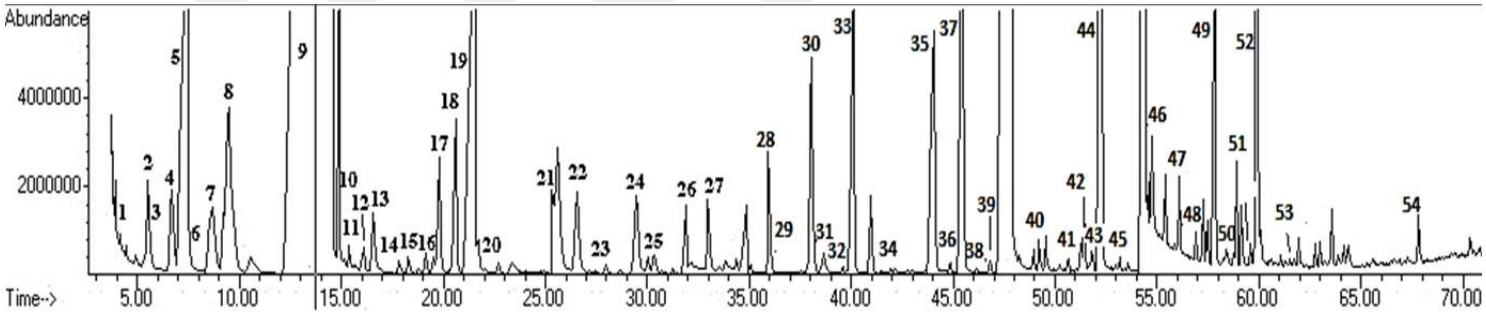


Appendix 2. Chromatogram image of the HMF and F of fresh apple juice



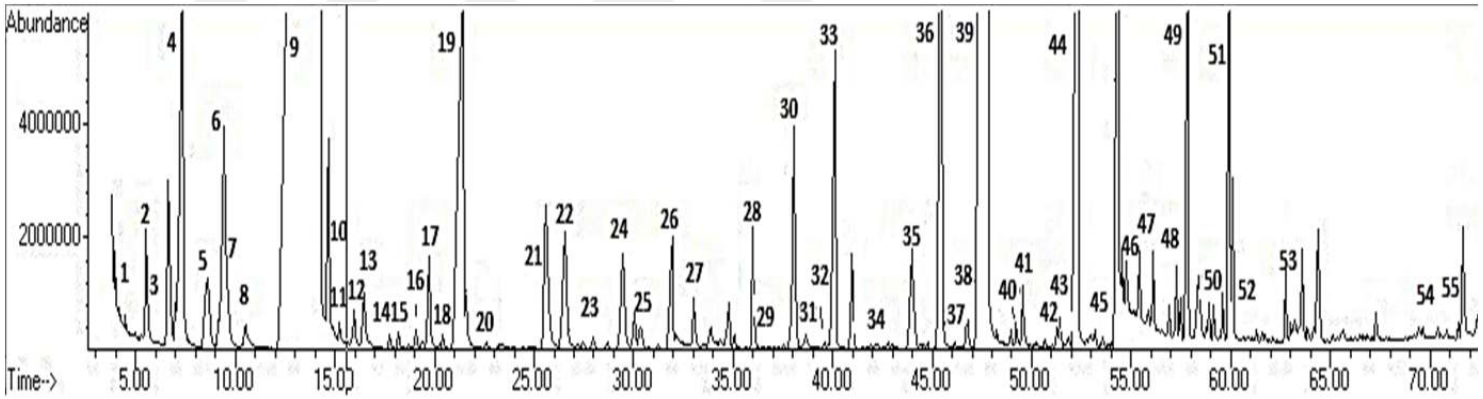
1. 1-Propanol; 2. Butyl acetate; 3. Isobutyl alcohol; 4. Isoamyl acetate; 5. 1-Butanol; 6. Isoamyl alcohol; 7. Ethyl hexanoate; 8. 1-pentanol; 9. Acetoin; 10. Hexyl acetate; 11. 2-Hexanol; 12. (Z)-3-Hexenyl acetate; 13. 3-Methyl-1-pentanol; 14. Ethyl lactate; 15. 1-Hexanol; 16. cis-3-Hexenol; 17. Acetic acid; 19. Heptanol; 20. 4-Nonanol (internal standart); 21. Propanoic acid; 22. 2,3-Butanediol; 23. Isobutanoic acid; 24. 1-Octanol; 25. γ-Butyrolactone; 26. Butanoic acid; 27. 1-Phenylethanone; 28. Furfuralcohol; 30. Diethyl succinate; 31. Methionol; 32. Ethyl phenylacetate; 33. Ethyl 4-hydroxybutanoate; 34. 2-Phenethyl acetate; 35. Hexanoic acid; 36. Benzyl alcohol; 37. Ethyl dodecanoate; 38. 2-Phenylethanol; 39. Pantolactone; 40. γ-Octalactone; 41. Octanoic acid; 42. Ethyl butanoate; 43. 4-Allylguaiacol; 44. 2-Methoxy-4-vinylphenol; 45. 3-hydroxy-4-phenyl-2-butanone; 46. Decanoic acid; 47. Ethyl hexadecanoate; 48. 4-allylphenol; 49. 9-Decenoic acid; 50. γ-Butyrolactone ; 51. 4-vinylphenol; 52. Dodecanoic acid; 53.Ethyl octadecanoate; 55. Pentadecanoic acid;

Appendix 3. GC-MS chromatogram image of volatile compounds of C1 cider



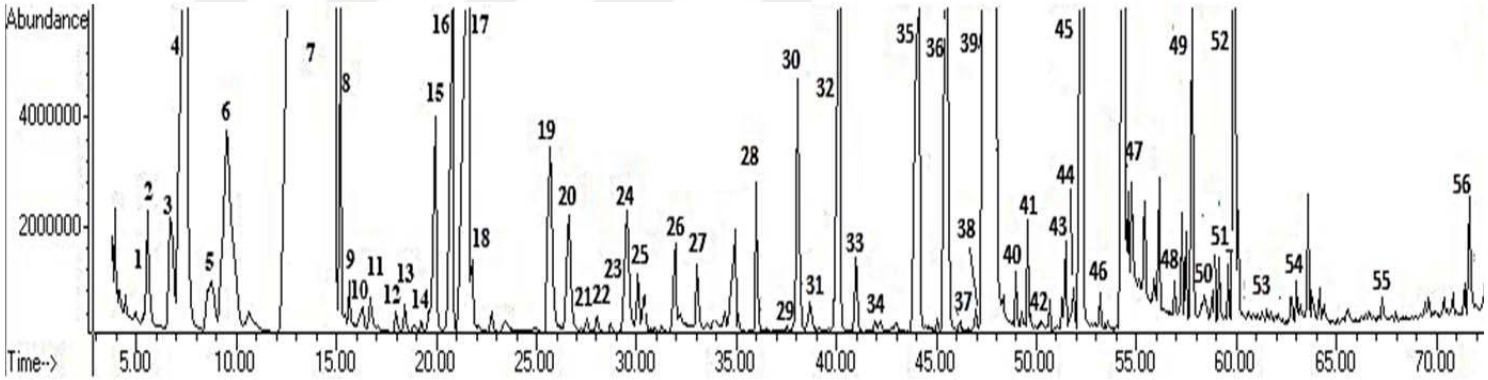
1. Ethyl propanoate; 2. 1-Propanol; 3. Ethyl butanoate; 4. Butyl acetate; 5. Isobutyl alcohol; 6. Isoamyl acetate; 7. 1-Hexanol; 8. 1-Butanol; 9. Isoamyl alcohol; 10. Ethyl hexanoate; 11. 1-pentanol; 12. Acetoin; 13. Hexyl acetate; 14. 2-Hexanol; 15. (Z)-3-Hexenyl acetate; 16. 2-Methyl-3-pentanol; 17. Acetonic acid; 18. Ethyl lactate; 19. 1-Hexanol; 20. Cis-3-Hexenol; 21. Acetic acid; 22. Ethyl octanoate; 23. Heptanol; 24. 4-Nonanol (internal standart); 25. 2,3-Butanediol; 26. Isobutanoic acid; 27. 2-Pentanol; 28. Butanoic acid; 29. 1-Phenylethanone; 30. Furfuralcohol; 31. Isopentanoic acid; 32. Diethyl succinate; 33. Methionol; 34. Ethyl phenylacetate; 34. Ethyl 4-hydroxybutanoate; 35. 1-Hexanoic acid; 36. Benzyl alcohol; 37. 1-Heptanoic acid; 38. 2-Phenylethanol; 39. 2-hexenoic acid; 40. Pantolactone; 41. Succinic acid; 42. 2-Phenethyl acetate; 43. Octanoic acid; 44. Ethyl butanoate; 45. 4-Allylguaiacol; 46. 2-Methoxy-4-vinylphenol; 47. 3-hydroxy-4-phenyl-2-butanone; 48. Decanoic acid; 49. Ethyl hexadecanoate; 50. 4-allylphenol; 51. 9-Decenoic acid; 52. γ-Butyrolactone; 53. Dodecanoic acid; 54. Ethyl octadecanoate;

Appendix 4. GC-MS chromatogram image of volatile compounds of C2 cider



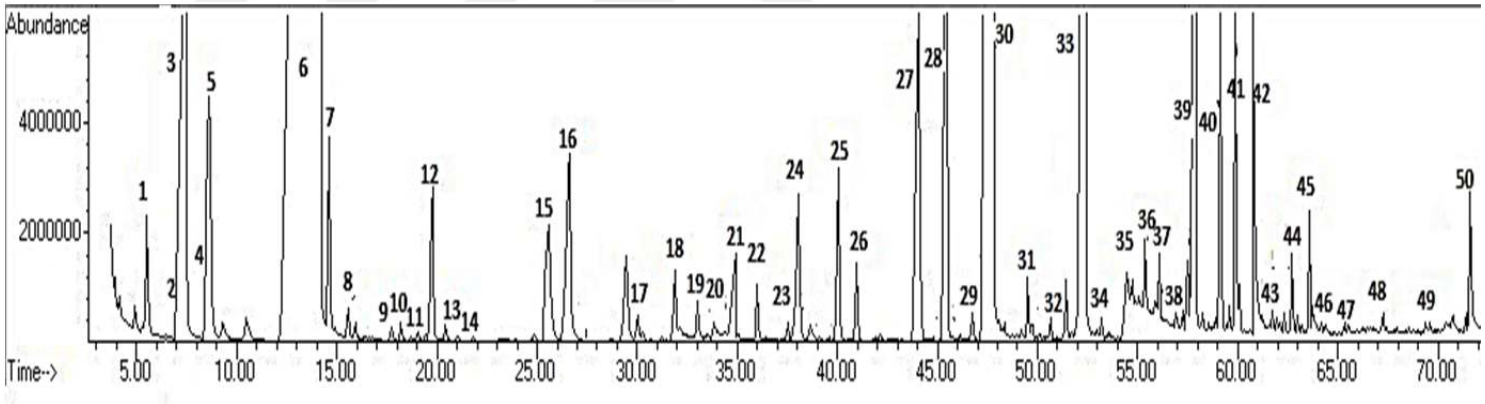
1. Ethyl propanoate; 2. 1-Propanol; 3. Ethyl butanoate; 4. Butyl acetate; 5. Isobutyl alcohol; 6. Isoamyl acetate; 7. 1-Hexanol; 8. 1-Butanol; 9. Isoamyl alcohol; 10. Ethyl hexanoate; 11. 1-pentanol; 12. Acetoin; 13. Hexyl acetate; 14. 2-Hexanol; 15. (Z)-3-Hexenyl acetate; 16. 2-Methyl-3-pentanol; 17. Acetonic acid; 18. Ethyl lactate; 19. 1-Hexanol; 20. Cis-3-Hexenol; 21. Acetic acid; 22. Ethyl octanoate; 23. Heptanol; 24. 4-Nonanol (internal standart); 25. 2,3-Butanediol; 26. Isobutanoic acid; 27. 2-Pentanol; 28. Butanoic acid; 29. 1-Phenylethanone; 30. Furfuralcohol; 31. Isopentanoic acid; 32. Diethyl succinate; 33. Methionol; 34. Ethyl phenylacetate; 35. Ethyl 4-hydroxybutanoate; 36. 1-Hexanoic acid; 37. Benzyl alcohol; 38. 1-Heptanoic acid; 39. 2-Phenylethanol; 40. 2-hexenoic acid; 41. Pantolactone; 42. Succinic acid; 43. 2-Phenethyl acetate; 44. Octanoic acid; 45. Ethyl butanoate; 46. 4-Allylguaiacol; 47. 2-Methoxy-4-vinylphenol; 48. 3-hydroxy-4-phenyl-2-butanone; 49. Decanoic acid; 50. Ethyl hexadecanoate; 51. 4-allylphenol; 52. 9-Decenoic acid; 53. γ-Butyrolactone; 54. Dodecanoic acid; 55. Hexadecanoic acid;

Appendix 5. GC-MS chromatogram image of volatile compounds of C3 cider



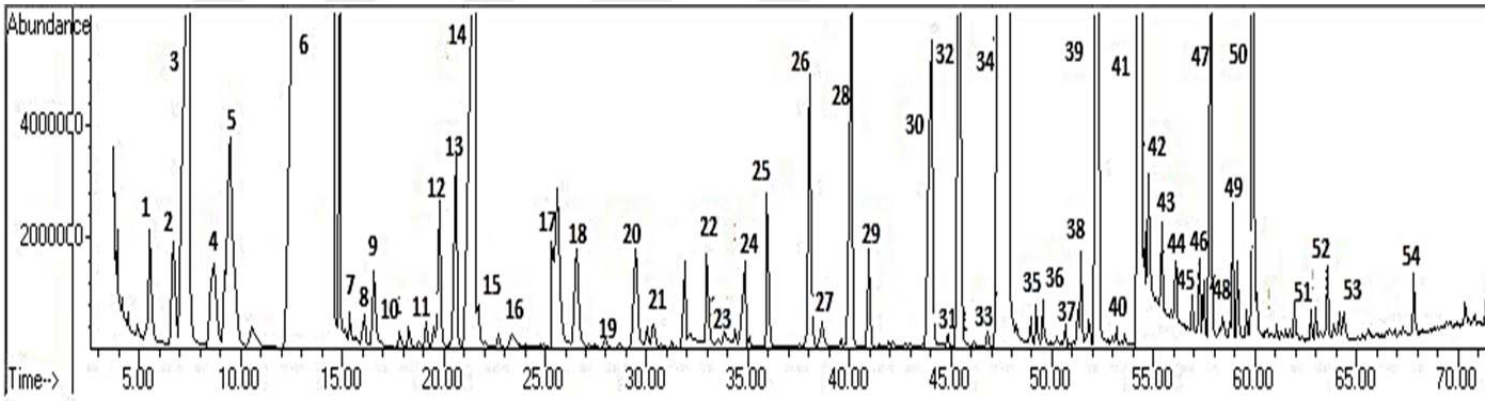
1. Ethyl propanoate; 2. 1-Propanol; 3. Ethyl butanoate; 4. Butyl acetate; 5. Isobutyl alcohol; 6. Isoamyl acetate; 7. 1-Hexanol; 8. 1-Butanol; 9. Isoamyl alcohol; 10. Ethyl hexanoate; 11. 1-pentanol; 12. Acetoin; 13. Hexyl acetate; 14. 2-Hexanol; 15. (Z)-3-Hexenyl acetate; 16. 2-Methyl-3-pentanol; 17. Acetonic acid; 18. Ethyl lactate; 19. 1-Hexanol; 20. Cis-3-Hexenol; 21. Acetic acid; 22. Ethyl octanoate; 23. Heptanol; 24. 4-Nonanol (internal standart); 25. 2,3-Butanediol; 26. Isobutanoic acid; 27. 2-Pentanol; 28. Butanoic acid; 39. 1-Phenylethanone; 30. Furfuralcohol; 31. Isopentanoic acid; 32. Diethyl succinate; 33. Methionol; 34. Ethyl phenylacetate; 35. Ethyl 4-hydroxybutanoate; 36. 1-Hexanoic acid; 37. Benzyl alcohol; 38. 1-Heptanoic acid; 39. 2-Phenylethanol; 40. 2-hexenoic acid; 41. Pantolactone; 42. Succinic acid; 43. 2-Phenethyl acetate; 44. Octanoic acid; 45. Ethyl butanoate; 46. 4-Allylguaiacol; 47. 2-Methoxy-4-vinylphenol; 48. 3-hydroxy-4-phenyl-2-butanone; 49. Decanoic acid; 50. Ethyl hexadecanoate; 51. 4-allylphenol; 52. 9-Decenoic acid; 53. γ-Butyrolactone; 54. Dodecanoic acid; 55. Ethyl octadecanoate; 56. Hexadecanoic acid;

Appendix 6. GC-MS chromatogram image of volatile compounds of C4 cider



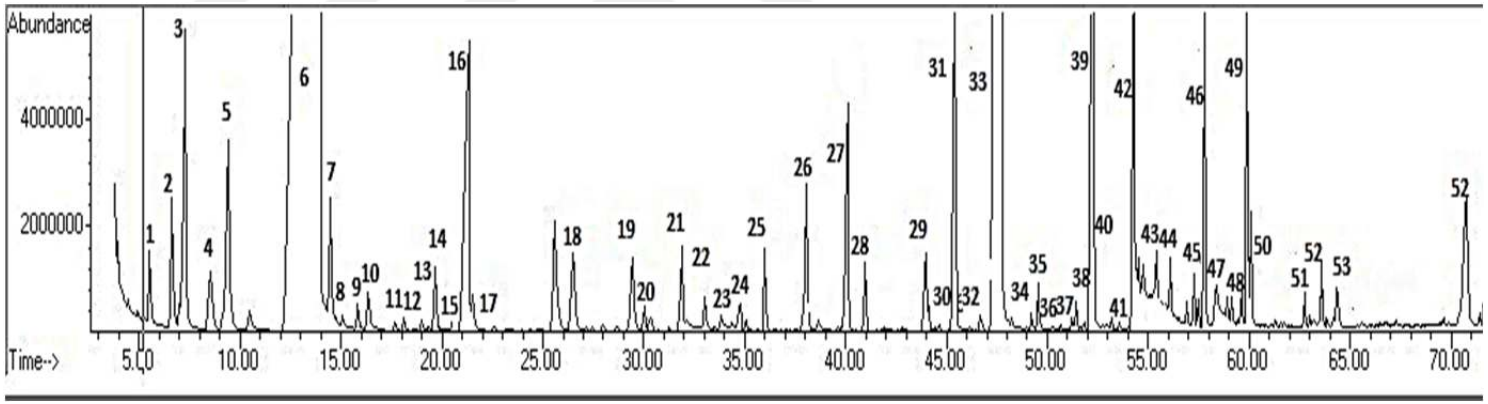
1. 1-Propanol; 2. Butyl acetate; 3. Isobutyl alcohol; 4. Isoamyl acetate; 5. 1-Butanol; 6. Isoamyl alcohol; 7. Ethyl hexanoate; 8. Acetoin; 9. Hexyl acetate; 10. 2-Hexanol; 11. (Z)-3-Hexenyl acetate; 12. 3-Methyl-1-pentanol; 13. Ethyl lactate; 14. 1-Hexanol; 15. cis-3-Hexenol; 16. Acetic acid; 17. Ethyl octanoate; 18. 4-Nonanol (internal standart); 19. 2,3-Butanediol; 20. Isobutanoic acid; 21. Butanoic acid; 22. 1-Phenylethanone; 23. Furfuralcohol; 24. Ethyl benzoate; 25. Diethyl succinate; 26. Methionol; 27. Ethyl phenylacetate; 28. Ethyl 4-hydroxybutanoate; 29. 2-Phenethyl acetate; 30. Hexanoic acid; 31. Benzyl alcohol; 32. Ethyl dodecanoate; 33. 2-Phenylethanol; 34. Pantolactone; 35. Octanoic acid; 36. Ethyl butanoate; 37. 4-Allylguaiacol; 38. 2-Methoxy-4-vinylphenol; 39. 3-hydroxy-4-phenyl-2-butanone; 40. Ethyl 2-hydroxy-3-phenylpropanoate; 41. Decanoic acid; 42. Ethyl hexadecanoate; 43. 4-allylphenol; 44. 9-Decenoic acid; 45. γ-Butyrolactone; 46. 4-vinylphenol; 47. Dodecanoic acid; 48.Ethyl octadecanoate; 49. Pentadecanoic acid; 50. Hexadecanoic acid;

Appendix 7. GC-MS chromatogram image of volatile compounds of C5 cider



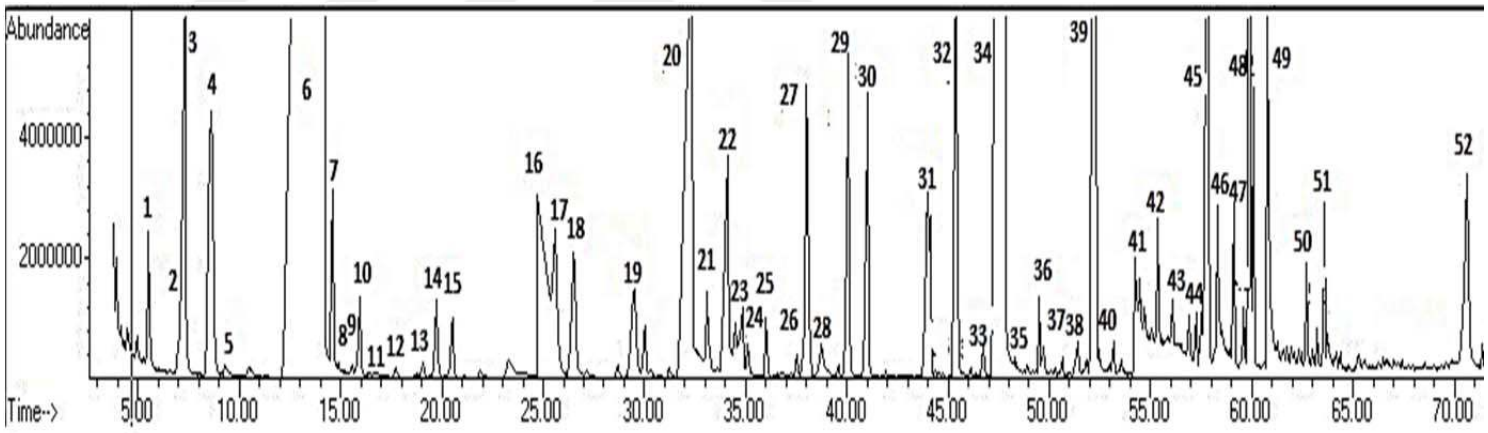
1. 1-Propanol; 2. Ethyl butanoate; 4. Butyl acetate; 5. Isobutyl alcohol; 6. Isoamyl acetate; 7. 1-Hexanol; 8. 1-Butanol; 9. Isoamyl alcohol; 10. Ethyl hexanoate; 11. Acetoin; 12. Hexyl acetate; 13. 2-Hexanol; 14. (Z)-3-Hexenyl acetate; 15. 2-Methyl-3-pentanol; 16. Acetonic acid; 17. Ethyl lactate; 18. 1-Hexanol; 19. Cis-3-Hexenol; 20. Acetic acid; 21. Ethyl octanoate; 22. 4-Nonanol (internal standart); 23. 2,3-Butanediol; 24. Isobutanoic acid; 25. 2-Pentanol; 26. Butanoic acid; 27. 1-Phenylethanone; 28. Furfuralcohol; 29. Isopentanoic acid; 30. Diethyl succinate; 31. Methionol; 32. Ethyl phenylacetate; 33. Ethyl 4-hydroxybutanoate; 34. 1-Hexanoic acid; 35. Benzyl alcohol; 36. 1-Heptanoic acid; 37. 2-Phenylethanol; 38. 2-hexenoic acid; 39. Pantolactone; 40. Succinic acid; 41. 2-Phenethyl acetate; 42. Octanoic acid; 43. Ethyl butanoate; 44. 4-Allylguaiacol; 45. 2-Methoxy-4-vinylphenol; 46. 3-hydroxy-4-phenyl-2-butanone; 47. Decanoic acid; 48. Ethyl hexadecanoate; 49. 4-allylphenol; 50. 9-Decenoic acid; 51. γ-Butyrolactone; 52. Dodecanoic acid; 53. Ethyl octadecanoate; 54. Hexadecanoic acid;

Appendix 8. GC-MS chromatogram image of volatile compounds of C6 cider



1. 1-Propanol; 2. Butyl acetate; 3. Isobutyl alcohol; 4. Isoamyl acetate; 5. 1-Butanol; 6. Isoamyl alcohol; 7. Ethyl hexanoate; 8. 1-pentanol; 9. Acetoin; 10. Hexyl acetate; 11. 2-Hexanol; 12. (Z)-3-Hexenyl acetate; 13. 3-Methyl-1-pentanol; 14. Ethyl lactate; 15. 1-Hexanol; 16. cis-3-Hexenol; 17. Acetic acid; 18. Ethyl octanoate; 19. 4-Nonanol (internal standart); 20. Propanoic acid; 21. 2,3-Butanediol; 22 Isobutanoic acid; 23. Butanoic acid; 24. 1-Phenylethanone; 25. Furfuralcohol; 26. Ethyl benzoate; 27. Diethyl succinate; 28. Methionol; 29. Ethyl phenylacetate; 30. Ethyl 4-hydroxybutanoate; 31. 2-Phenethyl acetate; 32. Hexanoic acid; 33. Benzyl alcohol; 34. Ethyl dodecanoate; 35. 2-Phenylethanol; 36. Pantolactone; 37. Octanoic acid; 38. Ethyl butanoate; 39. 4-Allylguaiacol; 40. 2-Methoxy-4-vinylphenol; 41. 3-hydroxy-4-phenyl-2-butanone; 42. Ethyl 2-hydroxy-3-phenylpropanoate; 43. Decanoic acid; 44. Ethyl hexadecanoate; 45. 4-allylphenol; 46. 9-Decenoic acid; 47. γ-Butyrolactone; 48. 4-vinylphenol; 49. Dodecanoic acid; 50.Ethyl octadecanoate; 51. Pentadecanoic acid; 52. Hexadecanoic acid;

Appendix 9. GC-MS chromatogram image of volatile compounds of C7 cider



1. 1-Propanol; 2. Butyl acetate; 3. Isobutyl alcohol; 4. Isoamyl acetate; 5. 1-Butanol; 6. Isoamyl alcohol; 7. Ethyl hexanoate; 8. 1-pentanol; 9. Acetoin; 10. Hexyl acetate; 11. 2-Hexanol; 12. (Z)-3-Hexenyl acetate; 13. 3-Methyl-1-pentanol; 14. Ethyl lactate; 15. 1-Hexanol; 16. cis-3-Hexenol; 17. Acetic acid; 18. Ethyl octanoate; 19. 4-Nonanol (internal standart); 20. Propanoic acid; 21. 2,3-Butanediol; 22. Isobutanoic acid; 23. Butanoic acid; 24. 1-Phenylethanone; 25. Furfuralcohol; 26. Ethyl benzoate; 27. Diethyl succinate; 28. Methionol; 29. Ethyl phenylacetate; 30. Ethyl 4-hydroxybutanoate; 31. 2-Phenethyl acetate; 32. Hexanoic acid; 33. Benzyl alcohol; 34. Ethyl dodecanoate; 35. 2-Phenylethanol; 36. Pantolactone; 37. Octanoic acid; 38. Ethyl butanoate; 39. 4-Allylguaiacol; 40. 2-Methoxy-4-vinylphenol; 41. 3-hydroxy-4-phenyl-2-butanone; 42. Ethyl 2-hydroxy-3-phenylpropanoate; 43. Decanoic acid; 44. Ethyl hexadecanoate; 45. 4-allylphenol; 46. 9-Decenoic acid; 47. γ-Butyrolactone; 48. 4-vinylphenol; 49. Dodecanoic acid; 50.Ethyl octadecanoate; 51. Pentadecanoic acid; 52. Hexadecanoic acid;

Appendix 10. GC-MS chromatogram image of volatile compounds of C8 cider