INVESTIGATION OF FORMATION OF α-DICARBONYL COMPOUNDS IN FRUIT BASED PRODUCTS

MEYVE BAZLI GIDALARDA α-DİKARBONİL BİLEŞİKLERİNİN OLUŞUMUNUN İNCELENMESİ

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ABSTRACT

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α-Dicarbonyl compounds and 5-hydroxymethyl-2-furfural (HMF) can be formed from sugars during the processing and storage of fruit products, due to their sugar-rich and acidic nature. Maillard reaction and caramelization are the reactions mainly responsible for the formation of these reactive intermediates in fruit products. α-Dicarbonyl compounds are the important intermediates for the flavor and browning development. On the other side, α-dicarbonyl compounds are significant precursors of toxic compounds such as advanced glycation end-products (AGE), furan, and acrylamide which are related to various degenerative and chronic diseases. During the formation of α-dicarbonyl compounds, the amino acid loss and vitamin degradation can also cause loss in the nutritional value of fruit products. Therefore, controlling these key intermediates during fruit processing and storage is crucial to maintain the quality and safety of fruit products.

In this framework, this disertation aims to investigate the chemistry of α-dicarbonyl compounds in fruit products in depth during their storage and processing. To achieve this aim, firstly, occurence of the α-dicarbonyl compounds in fruit based products was measured. Secondly, efforts were put in understanding their formation mechanism, and lastly, the

factors affecting their formation in fruit products were investigated. In addition, HMF has been considered as a quality marker in processed foods to date, however, in order to understand the importance of α-dicarbonyl compounds as quality and safety markers, HMF as well as α- dicarbonyl compounds was evaluated together.

In the beginning, the content of α-dicarbonyl compounds and HMF in a large number of different fruit products (n=184) such as dried fruits, fruit juices, fruit juice concentrates, fruit puree concentrates, and fruit purees was determined. Among the α-dicarbonyl compounds, 3-deoxyglucosone (3-DG), glucosone, 1-deoxyglucosone, 3-deoxypentosone, threosone, diacetyl, methylglyoxal, and glyoxal were monitored. This study reported for the first time that the main α-dicarbonyl compound was glucosone (ranging between not detectable – 25.7 mg/L) in fruit juices. The other fruit products with mid- and low-moisture conditions contained 3-DG as the dominant one. The highest concentrations of α-dicarbonyl compounds and HMF were mainly found in dried fruits at concerning levels. Thus, the concentration of 3-DG in dried fruits varied between 21.9 – 4117.0 mg/kg, while HMF was ranging from not detectable to 2400.9 mg/kg. In general, the concentrations of α-dicarbonyl compounds were significantly ($p < 0.05$) higher than the levels of HMF. The daily intake level of α-dicarbonyl compounds and HMF from fruit products was also calculated in order to make a risk assessment. The dietary intake calculations showed that fruit juice products also pose a risk with high exposure, despite fruit juices contained low concentrations of α-dicarbonyl compounds and HMF compare to dried fruits. This study revealed that it was essential to investigate α-dicarbonyl compounds together with HMF in detail during the storage and processing of fruit products.

During the storage of fruit products, the formation mechanism and the factors affecting the formation of α-dicarbonyl compounds was investigated in the following parts. In this regard, the changes in the concentrations of the reactants (sugars, amino acids) and the products (α-dicarbonyl compounds and HMF) were evaluated together with their formation mechanism by using multi-response kinetic modelling approach. This approach was applied to apple juices, orange juices, and peach nectars during the storage of 24 weeks at different temperatures. From the α-dicarbonyl compounds, glucosone, 3-DG, threosone, methylglyoxal and glyoxal were monitored during the storage. The main α-dicarbonyl compound was found as glucosone (ranging between 0.2 – 683.5 mg/L) in apple and orange juices during the storage, that was in accordance with the previous findings. In addition, HMF levels were found to be lower than the α-dicarbonyl compounds in stored fruit juice

samples. A striking result to emerge from the data was that free amino acids showed no significant ($p > 0.05$) changes during the storage. Thus, it was first hypothesized that the sugar decomposition pathway rather than the Maillard reaction route was responsible for the formation of α-dicarbonyl compounds and HMF in fruit juices during storage. The use of multi-response kinetic modelling approach provided a better understanding of the most possible pathway of sugar degradation reactions in fruit juices by performing model discrimination and estimating the reaction rate constants. Accordingly, the formation rates of α-dicarbonyl compounds in peach nectar (sucrose-added beverage) were lower than that in apple and orange juices (no added-sugar juices). Isomerization of glucose and fructose via 1,2-enolization was found as a kinetically important reaction step in stored juice samples. HMF was mainly formed from the dehydration through fructofuranosyl cation rather than the 3-DG dehydration. One kinetic model for three different fruit juices was established that makes it easier to understand the formation mechanism of α-dicarbonyl compounds and HMF in acidic, sugar-rich, aqueous food systems in general.

In the third part, how the factors (initial reactant concentration and pH) affect the formation of α-dicarbonyl compounds and HMF was investigated in fruit products with mid- and low-moisture content during storage. For this purpose, changes in the concentrations of reactants (sugar, amino acid) and products (α-dicarbonyl compounds and HMF) were monitored in fruit (apple, pomegranate) juice concentrates with different initial reactant concentration levels and in dried fruits (date, raisin, blueberry) with different pH levels during the storage of 20 weeks at 37 °C. Among the α-dicarbonyl compounds, glucosone, 3-DG, threosone, 3-deoxythreosone, 3,4-dideoxyglucosone-3-ene, diacetyl, methylglyoxal, and glyoxal were monitored in stored samples. Glucosone was the dominant one in 30 °Bx of fruit juice concentrates, similar to the previous findings. On the other hand, 3-deoxyglucosone was the major α-dicarbonyl compound in 50 °Bx and 70 °Bx of concentrates and in all dried fruits. HMF levels were also significantly lower than the concentrations of dominant α-dicarbonyl compounds during the storage, in support with the previous findings. The results also revealed that the decrease ratio of free amino acid concentration was increased from 34% to 77% when the initial reactant concentration increased from 30 °Bx to 70 °Bx in the fruit juice concentrates. Similarly, free amino acid loss was accelerated when the pH level changed from high-acidic (2.6) to neutral (6.6) in dried fruits, during the storage. With the increase in the loss of free amino acids, the concentrations of α-dicarbonyl compounds and HMF were increased in all fruit products. At the end of the storage, the level of 3-DG in

dried date with pH 6.6 was found as 7251 ± 896 mg/kg which has been the highest level of α-dicarbonyl compounds reported in the literature until now. To understand the role of Maillard reaction in fruit products during the storage, the amino acid adducts of α-dicarbonyl compounds and HMF were confirmed by using high-resolution mass spectrometry. To the results, generally high mass accuracy ($\Delta < 2$ ppm) of the confirmation of amino acid adducts of α-dicarbonyl compounds and HMF proved the contribution of Maillard reaction to non-enzymatic reactions in the fruit products. In the end, it was revealed that during storage of fruit products, sugar degradation reactions mainly contributed to the formation of α-dicarbonyl compounds and HMF in aqueous fruit products, whereas Maillard reaction play important role in non-enzymatic reactions in mid-/low-moisture fruit products.

In the last part, the effect of different processing stages of fruit juices on the formation of α-dicarbonyl compounds and HMF was investigated. For this purpose, changes in the concentrations of reactants (sugar, amino acid) and products (α-dicarbonyl compounds and HMF) were monitored in the samples of apple juice concentrate, orange juice, and peach puree concentrate collected from the critical process stages such as enzyme treatment, pasteurization, concentration during industrial processing. Among the α-dicarbonyl compounds, glucosone, 3-DG, 3-deoxypentosone, threosone, diacetyl, methylglyoxal, and glyoxal were monitored. The concentrations of sugars and free amino acids showed no significant ($p > 0.05$) changes during processing. The main α-dicarbonyl compound formed at each step of apple juice production was glucosone having a maximum concentration of 17.47 ± 0.16 mg/L at the end of the process. On the contrary, 3-deoxyglucosone was the dominant one present in orange juice and peach puree samples with a maximum concentration of 18.24 ± 0.86 mg/L and 29.71 ± 1.56 mg/kg, respectively. It was revealed that different production steps such as deaeration led to change in the formation of the main type of α-dicarbonyl compound in fruit products. This finding was the first reported in the literature. In addition, it was observed that continuous mild temperature conditions even below 100 °C can cause the accumulation of α-dicarbonyl compounds in aqueous fruit products. The presence of molecular oxygen, temperature, and the duration of the process were determined as the significant processing parameters affecting the formation of α-dicarbonyl compounds. Last but not least, the concentration of HMF was found to be quite lower than the level of α-dicarbonyl compounds in all samples during processing. This finding was in support with the previous findings obtained in this thesis study which showed the quite low or not detectable levels of HMF despite the high level of α-dicarbonyl

compounds in aqueous acidic fruits. Therefore, it is suggested that not only HMF but also α-dicarbonyl compounds should be considered in order to make a reliable evaluation of the quality and safety of processed fruit products.

Keywords: α-Dicarbonyl compounds, 5-hydroxymethyl-2-furfural, multiresponse kinetic modelling, fruit products.

ÖZET

MEYVE BAZLI ÜRÜNLERDE α-DİKARBONİL BİLEŞİKLERİNİN OLUŞUMUNUN İNCELENMESİ

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α-Dikarbonil bileşikleri ve 5-hidroksimetil-2-furfural (HMF) meyve ürünlerinin işleme ve depolamaları sırasında, meyvelerin şeker açısından zengin ve asidik yapıları nedeniyle şekerlerden kolayca oluşabilirler. Maillard reaksiyonu ve karamelizasyon, meyve ürünlerinde bu reaktif ara maddelerin oluşumundan esas olarak sorumlu olan reaksiyonlardır. α-Dikarbonil bileşikleri, aroma ve esmerleşme gelişiminde önemli ara bileşenlerdir. Diğer taraftan, α-dikarbonil bileşikleri, çeşitli dejeneratif ve kronik hastalıklarla bağlantılı olan ileri glikasyon ürünleri (AGE), furan, akrilamid gibi toksik bileşiklerin de önemli öncüleridir. α-Dikarbonil bileşiklerinin oluşumu sırasında amino asit kaybı ve vitamin degredasyonu meyve ürünlerinin besin değerinde kayıplara neden olur. Bu nedenle, meyve prosesi ve depolanması sırasında bu anahtar ara maddelerin kontrolü, kalite ve güvenlik sorunları için çok önemlidir.

Bu çerçevede, bu tez, meyve ürünlerinde depolama ve proses boyunca α-dikarbonil bileşiklerinin kimyasını derinlemesine araştırmayı amaçlamaktadır. Bu amaca ulaşmak için, öncelikle meyve bazlı ürünlerde α-dikarbonil bileşiklerinin seviyeleri belirlenmiştir. İkinci olarak, bunların oluşum mekanizmalarının anlaşılmasına odaklanılmış, ve son olarak meyve ürünlerinde bunların oluşumlarına etki eden faktörler araştırılmıştır. Ayrıca, HMF bugüne kadar işlenmiş gıdalarda bir kalite belirteci olarak kabul edilmiştir, ancak α-dikarbonil bileşiklerinin kalite ve güvenlik belirteçleri olarak önemini anlamak için HMF ile birlikte α-dikarbonil bileşikleri de değerlendirilmiştir.

Bu çalışmanın başlangıcında, kuru meyveler, meyve suları, meyve suyu konsantreleri, meyve püre konsantreleri ve meyve püreleri gibi çok sayıda farklı meyve ürününde (n=184) α-dikarbonil bileşikleri ve HMF içeriği araştırılmıştır. α-Dikarbonil bileşikleri arasından, 3-deoksiglukozon (3-DG), glukozon, 1-deoksiglukozon, 3-deoksipentozon, threozon, diasetil, metilglioksal ve glioksal izlenmiştir. Bu çalışma ilk kez, meyve sularında ana α-dikarbonil bileşiğinin glukozon (tespit edilemeyen ile 25.7 mg/L aralığında) olduğunu bildirmektedir. Orta ve düşük neme sahip diğer meyve ürünlerinde ise 3-DG'nun baskın olarak bulunmaktadır. α-Dikarbonil bileşiklerinin ve HMF'nin en yüksek konsantrasyonları ana olarak kuru meyvelerde endişe verici seviyelerde bulunmuştur. Kuru meyvelerdeki 3-DG konsantrasyonu 21.9 mg/kg ile 4117.0 mg/kg arasında değişirken, HMF tespit edilemeyen seviye ile 2400.9 mg/kg arasında değişmektedir. Genel olarak, α-dikarbonil bileşiklerinin konsantrasyonları HMF seviyelerinden önemli ölçüde ($p < 0.05$) yüksektir. Risk değerlendirmesi yapabilmek için, meyve ürünlerinden günlük α-dikarbonil bileşikleri ve HMF alım seviyeleri de hesaplanmıştır. Diyetle alım hesaplamaları, meyve sularının kuru meyvelere kıyasla daha düşük konsantrasyonda α-dikarbonil bileşikleri ve HMF içermesine rağmen, meyve suyu ürünlerinin de yüksek maruziyet ile risk oluşturduğunu göstermiştir. Bu çalışma, meyve ürünlerinin depolanması ve işlenmesi sırasında HMF ile birlikte α-dikarbonil bileşiklerinin daha ayrıntılı olarak araştırılmasının gerekli olduğunu ortaya çıkarmıştır.

Meyve ürünlerinin depolanması sırasında α-dikarbonil bileşiklerinin oluşum mekanizması ve oluşumuna etki eden faktörler sonraki bölümlerde incelenmiştir. Bu bağlamda, çok yanıtlı kinetik modelleme yaklaşımı kullanılarak reaktanların (şekerler, amino asitler) ve ürünlerin (α-dikarbonil bileşikleri ve HMF) konsantrasyonlarındaki değişimleri bunların oluşum mekanizmaları ile birlikte değerlendirilmiştir. Bu yaklaşım, farklı sıcaklıklarda, 24 haftalık süre ile depolanan elma suları, portakal suları ve şeftali nektarlarına uygulanmıştır. α-

Dikarbonil bileşiklerinden, glukozon, 3-DG, threozon, metilglioksal ve glioksal izlenmiştir. Önceki bulgularla uyumlu olarak, depolama sırasında elma ve portakal sularında ana dikarbonil, 0.2 mg/L ile 683.5 mg/L aralığında değişen miktarda glukozon olarak bulunmuştur. Bununla birlikte meyve suyu örneklerinde HMF düzeylerinin α-dikarbonil bileşiklere göre daha düşük olduğu tespit edilmiştir. Verilerden ortaya çıkan çarpıcı bir sonuç da serbest amino asitlerin depolama sırasında önemli ($p > 0.05$) bir değişiklik göstermemesidir. Bu nedenle, depolama sırasında meyve sularında α-dikarbonil bileşikleri ve HMF oluşumundan Maillard reaksiyonu yolundan ziyade şeker ayrışma yolunun sorumlu olduğu ilk olarak varsayılmıştır. Çok yanıtlı kinetik modelleme yaklaşımının kullanılması, model diskriminasyonu yaparak ve reaksiyon hızı sabitlerini tahmin ederek meyve sularında şeker bozunma reaksiyonlarının en olası yolunun daha iyi anlaşılmasını sağlamıştır. Buna göre, şeftali nektarındaki (sukroz katkılı içecek) α-dikarbonil bileşiklerinin oluşum hızları, elma ve portakal sularındakinden (ilave şekersiz meyve suları) daha düşüktür. Depolanan meyve suyu örneklerinde 1,2-enolizasyon yoluyla glukoz ve fruktozun izomerizasyonu kinetik olarak önemli bir reaksiyon adımı olarak bulunmuştur. HMF esas olarak 3-DG dehidrasyonundan ziyade fruktofuranozil katyonunun dehidrasyonu yoluyla oluşturulmuştur. Üç farklı meyve suyu için tek bir kinetik modelin oluşturulması, genel olarak asidik, şeker açısından zengin, sulu gıda sistemlerinde α-dikarbonil bileşiklerinin ve HMF'nin oluşum mekanizmasının anlaşılmasını kolaylaştırmaktadır.

Üçüncü kısımda, depolama sırasında orta ve düşük nem içeriğine sahip merve ürünlerinde α-dikarbonil bileşiklerinin ve HMF'nin oluşumuna başlangıç reaktan konsantrasyonu, pH gibi faktörlerin nasıl etki ettiği araştırılmıştır. Bu amaçla, farklı başlangıç reanktant konsantrasyon seviyelerine sahip meyve (elma, nar) suyu konsantreleri ve farklı pH seviyelerine sahip kuru meyvelerde (hurma, üzüm, yaban mersini), reaktanların (şeker, amino asit) ve ürünlerin (α-dikarbonil bileşikleri ve HMF) konsantrasyonlarındaki değişimler 37 °C'deki 20 haftalık depolama süresince izlenmiştir. Depolanan örneklerde α-dikarbonil bileşikleri arasında, glukozon, 3-DG, threozon, 3-deoksithreozon, 3,4-dideoksiglukozon-3-ene, diasetil, metilglioksal ve glioksal izlenmiştir. Önceki bulgulara benzer şekilde, 30 °Bx meyve suyu konsantrelerinde glukozon baskındı. Öte yandan, depolama süresince 50 °Bx ve 70 °Bx meyve suyu konsantrelerinde ve tüm kuru meyvelerde 3-deoksiglukozon baskın olandı. Depolama sırasında önceki bulguları destekler şekilde HMF seviyeleri de baskın α-dikarbonil bileşiklerinin konsantrasyonlarından önemli ölçüde daha düşüktü. Ayrıca, sonuçlar meyve suyu konsantrelerinde başlangıç reaktan

konsantrasyonu 30 °Bx'den 70/65 °Bx'e yükseldiğinde serbest amino asit konsantrasyonundaki azalış oranının % 34'den % 77'ye yükseldiğini ortaya koymuştur. Benzer şekilde, depolama sırasında kuru meyvelerde pH seviyesi yüksek-asidikten (2.6) nötre (6.6) değiştiğinde serbest amino asit kaybı artmıştır. Serbest amino asit kaybındaki artışla birlikte bütün meyve ürünlerinde α-dikarbonil bileşiklerinin ve HMF'nin konsantrasyonları artmıştır. Nispeten yüksek pH seviyesine (6.6) sahip kuru hurmadaki 3-DG'nun maksimum seviyesi 7251 ± 896 mg/kg olarak bulunmuştur ki bu şimdiye kadar literatürde bildirilen en yüksek α-dikarbonil bileşiği seviyesidir. Depolama sırasında meyve ürünlerinde Maillard reaksiyonunun rolünü anlamak için yüksek çözünürlüklü kütle spektrometrisi kullanılarak α-dikarbonil bileşiklerinin ve HMF'nin amino asit eklentileri doğrulandı. Sonuçlara göre, α-dikarbonil bileşiklerinin ve HMF'nin amino asit eklentilerinin genel olarak yüksek kütle doğruluğu ($\Delta < 2$ ppm) ile doğrulanması, meyve ürünlerindeki enzimatik olmayan reaksiyonlara Maillard reaksiyonunun katkısını kanıtlamıştır. Sonuçta, meyve ürünlerinin depolanması sırasında şeker degredasyon reaksiyonlarının sulu meyve ürünlerinde α-dikarbonil bileşiklerinin ve HMF'nin oluşumuna esas olarak katkıda bulunduğu saptanırken orta/düşük nemli meyve ürünlerinde ise Maillard reaksiyonunun enzimatik olmayan reaksiyonlarda önemli rol oynadığı ortaya çıkmıştır.

Son bölümde ise meyve sularının farklı proses aşamalarının α-dikarbonil bileşikleri ve HMF oluşumuna etkisi araştırılmıştır. Bu amaçla, endüstriyel üretim sırasında enzim muamelesi, pastörizasyon, konsantrasyon gibi kritik proses aşamalarından temin edilmiş elma suyu konsantresi, portakal suyu ve şeftali püresi örneklerinde reaktanların (şeker, amino asit) ve ürünlerin (α-dikarbonil bileşikleri ve HMF) konsantrasyonlarındaki değişimler izlenmiştir. α-Dikarbonil bileşikleri arasında, glukozon, 3-DG, 3-deoksipentozon, threozon, diasetil, metilglioksal ve glioksal izlenmiştir. Proses sırasında şekerlerin ve serbest amino asitlerin konsantrasyonları önemli ($p > 0.05$) bir değişim göstermemiştir. Proses sonunda 17.47 ± 0.16 mg/L maksimum konsantrasyonuna sahip glukozon, elma suyu prosesi sırasında tüm örnekleme noktalarında ana dikarbonil olmuştur. Buna karşılık, sırasıyla 18.24 ± 0.86 mg/L and 29.71 ± 1.56 mg/kg maksimum konsantrasyonları ile 3-DG, portakal suyu ve şeftali püresi örneklerinde baskın olmuştur. Bu, hava çıkarma gibi farklı proses türlerinin meyve ürünlerinde oluşan ana α-dikarbonil bileşiğin tipinde değişime sebep olduğunu göstermiştir. Bu bulgu literatürde ilk kez rapor edilmiştir. Bununla birlikte, proses sırasında α-dikarbonil bileşikleri konsantrasyonlarındaki artış, 100 °C'nin altındaki kesintisiz ılıman sıcaklık koşullarının bile sulu meyve ürünlerinde α-dikarbonil bileşiklerinin birikimine neden

olabileceğini ortaya koymuştur. Moleküler oksijenin varlığı, proses sıcaklığı ve proses süresi dikarbonil oluşumunu etkileyen dikkate değer proses parametreleri olarak belirlenmiştir. Son ama en az diğerleri kadar önemli olarak, proses sırasında tüm örneklerde HMF konsantrasyonu α-dikarbonil bileşiklerinin seviyesinden oldukça düşük bulunmuştur. Bu bulgu, sulu asidik meyvelerde yüksek α-dikarbonil bileşiikleri seviyelerine rağmen oldukça düşük veya saptanamayan düzeyde HMF seviyelerinin bulunduğu bu tez çalışmasındaki önceki bulguları desteklemektedir. Bu sebeple, işlenmiş meyve ürünlerinin kalite ve güvenliğinin güvenilir bir şekilde değerlendirilmesi için sadece HMF'nin değil aynı zamanda α-dikarbonil bileşiklerinin de birlikte değerlendirilmesi önerilmektedir.

Keywords: α-Dikarbonil bileşikler, 5-hidroksimetil-2-furfural, çok yanıtlı kinetik modelleme, meyve ürünleri.

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SYMBOLS AND ABBREVIATIONS

Symbols

a_w	Water activity
E_a	Activation energy
k	Reaction rate constant
n	Reaction rate order
T	Temperature
t	time

Abbreviations

AAP	American Academy of Pediatrics
AGEs	Advanced glycation end-products
AIJN	Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union
AL	Aluminum
ARP	Amadori Rearrangement Product
α-Dicarbonyl compounds	α-Dicarbonyl compounds
CEL	ε-(1-carboxyethyl)-lysine
CML	N-ε-carboxymethyl-lysine
DA	Diacetyl
DAD	Diode array detector
DDMP	2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-Pyran-4-One
DETAPAC	Diethylenetriaminepentaacetic acid
3-DF	3-deoxyfructose
1-DG	1-deoxyglucosone
2-DG	2-deoxyglucosone
3-DG	3-deoxyglucosone
3DG-H	Hydroimidazolone adducts of 3-deoxyglucosone
4-DG	2-deoxyglucosone
3-DGal	3-deoxygalactosone
1,4-DG	1,4-deoxyglucosone
3,4-DGE	3,4-dideoxyglucosone
DHAP	Dihydroxyacetonephosphate

DM	Degree of methoxylation
DNA	Deoxyribonucleic acid
3-DP	3-deoxypentosone
1-DT	1-deoxythreosone
3-DT	3-deoxythreosone
1,2-ED	1,2-enediol
2,3-ED	2,3-enediol
ESI	Electrospray Ionization
FAO	Food and Agriculture Organization of the United Nations
FFC	Fructofuranosyl cation
Fru	Fructose
G	Glucosone
G-H1	Hydroimidazolone adducts of glyoxal
Glu	Glucose
GO	Glyoxal
HFCS	High fructose corn syrup
HILIC	Hydrophilic interaction chromatography
HLB	Hydrophilic-lipophilic-balanced
HMF	5-Hydroxymethyl-2-furfural
HMFA	5-Hydroxymethyl-2-furoic acid
HPD	Highest posterior density
HPLC	High performance liquid chromatography
HRP	Heyns Rearrangement Product
HRMS	High resolution mass spectrometry
LD50	Lethal dose 50
LdB-AvE	Lobry de Bruyn-Alberda van Ekenstein transformation
LOD	Limit of detection
LOQ	Limit of quantitation
MG-H1	Hydroimidazolone adducts of methylglyoxal
MGO	Methylglyoxal
MRM	Multiple reaction monitoring
MS	Mass spectrometry
ND	Not detected
NOAEL	No adverse effect levels

$OH^{\cdot}$	Hydroxyl radical
OPD	*o*-phenylenediamine
P	Products
PE	Polyethylene
PET	Polyethylene terephthalate
PPO	Polyphenoloxidase
RAGE	Receptor for advanced glycation end products
ROS	Reactive oxygen species
SD	Standard deviation
SIM	Selected ion-monitoring mode
SMF	5-Sulphoxymethylfurfural
SPSS	Statistical package for the social sciences
Suc	Sucrose
Tr	Trace
UFLC	Ultra-fast liquid chromatography
UHT	Ultra high temperature
UPLC	Ultra-high pressure liquid chromatography
USDA	United States Department of Agriculture
WHO	World Health Organization

INTRODUCTION

The word “fruit” comes from the Latin word *fruor* which has a meaning of “I delight in”. In the human diet, fruits and fruit products play a significant role due to their content of many nutritional values such as vitamins, minerals, antioxidants, and phytochemical compounds. In addition, fruit products such as dried fruits, fruit purees, fruit concentrates are used as ingredients in several foods e.g. yogurts, baby foods, bakery products. Many fruit products have also advantages with their long shelf-lives exceeding 1 or 2 years. To extend the shelf-life of the fruit products, thermal processes such as concentration, pasteurization, and drying are widely used as the preservation methods. However, prolonged storage and thermal operations can cause complex chemical reactions which lead to the undesirable changes in the quality and safety of fruit products. The undesirable changes such as browning, off-flavors, toxic compounds and loss in nutritional quality can easily occur during processing and storage of fruit products, since the high sugar content as well as the acidic medium make the fruit products a complex reaction pool.

α-Dicarbonyl compounds and 5-hydroxymethylfurfural (HMF) are the compounds which might be responsible for the undesirable changes in fruit products. These compounds are the key intermediates mainly formed from sugars, during Maillard reaction and caramelization which occur simultaneously in various foods. α-Dicarbonyl compounds are known to be the important precursors of desired or undesired volatile aroma compounds depend upon the product type. In addition, α-dicarbonyl compounds and also HMF play a significant role in the formation of toxic compounds such as advanced glycation end-products (AGEs), furan and acrylamide which are linked to serious degenerative and chronic diseases [1]. From the viewpoint of quality and safety issues, investigation of α-dicarbonyl compounds in foods increasingly gets attention in recent years. Thus, the question is that what we know about the chemistry of α-dicarbonyl compounds in foods.

Until now, the formation mechanism of α-dicarbonyl compounds and HMF through Maillard reaction or caramelization has been tried to be clarified in many simple model systems such as glucose – glycine model systems [2-5]. Maillard reaction and/or caramelization have been investigated in a small number of food-like model systems in order to provide a better understanding of such complex reactions in complex real foods [6-8]. In recent years, a few studies have been reported the level of the most abundant α-dicarbonyl

compounds (3-DG, methylglyoxal and glyoxal) in commercially available real foods [9, 10]. In addition, the studies have been indicated the occurrence of some α-dicarbonyl compounds in several foods during processing such as roasting [11-16], baking [7, 17-20], frying [21, 22] and fermentation [23-26]. About last decade, the change in the concentration of α-dicarbonyl compounds was reported during the storage of some foods [16, 21, 27-33]. Although there have been studies reported the concentrations of α-dicarbonyl compounds in various foods during processing and storage, not many studies were available in the literature on the formation kinetics of these compounds. Only Berk, et al. [11] in 2021 and Tas and Gokmen [16] in 2019 have shown the formation of α-dicarbonyl compounds with a kinetic modeling approach in real foods, namely sesame during roasting and hazelnut during storage, respectively.

Since it is well known that Maillard reaction is accelerated in the alkaline, low-moisture, and high temperature conditions, foods prone to Maillard reaction have priority in the case of investigation of α-dicarbonyl compounds during processing or storage. However, in real food systems, chemical reactions such as Maillard reaction and caramelization occur simultaneously that makes clarification this reaction network very difficult. Contrary to Maillard reaction, caramelization favors high-acidic conditions in foods. The fruit products are highly suitable for the formation of α-dicarbonyl compounds and HMF via caramelization and/or Maillard reaction due to their acidic and sugar-rich environment. Nevertheless, the fate of α-dicarbonyl compounds in acidic, sugar-rich and real food systems such as fruit products during processing or storage is still remains lacking.

With all this in mind, **the main objective** of this thesis study is to investigate in depth the chemistry of α-dicarbonyl compounds in fruit products during storage and processing in many aspects: (i) occurrence, (ii) formation mechanism, and (iii) factors affecting the formation. Besides, α-dicarbonyl compounds and HMF was investigated together in order to make a reliable quality and safety evaluation, since HMF has been considered as a quality marker in various processed foods. In this regard, the research questions tried to be answered in this thesis study are given as:

- What is the **level of α-dicarbonyl compounds and HMF** in fruit products as high-acid and high-sugar real foods?
- Do α-dicarbonyl compounds in fruit products **pose a serious risk** in terms of dietary exposure?

- What is the effect of storage on the occurrence of α-dicarbonyl compounds and HMF in fruit products from **formation mechanism** point of view?
- What is **the role of parameters** affecting the formation of α-dicarbonyl compounds and HMF in different fruit products during storage?
- What is **the contribution of Maillard reaction** and caramelization to the fate of α-dicarbonyl compounds and HMF in fruit products during storage?
- What is the **effect of processing stages** on the fate of α-dicarbonyl compounds and HMF in fruit products?

Within this context, this thesis is divided into 5 chapters:

Chapter 1 gives general information about fruit products, α-dicarbonyl compounds and 5-hydroxymethylfurfural as well as multiresponse kinetic modeling of chemical reactions.

Chapter 2 reports the levels of α-dicarbonyl compounds and HMF in a large number of commercially available fruit products such as dried fruits, fruit juices, fruit purees, fruit puree concentrates, and fruit juice concentrates. In addition, the daily intake level calculation of α-dicarbonyl compounds and HMF provides an insight to make a risk assessment in fruit products concerning nutritional consequences.

Chapter 3 discusses the changes in the concentration of reactants, namely sugars and amino acids, and formation of α-dicarbonyl compounds and HMF during storage of fruit juices at different temperatures. It also describes the formation mechanism of these compounds by using multiresponse kinetic modeling approach.

Chapter 4 discusses the changes in the concentration of sugars, amino acids, α-dicarbonyl compounds and HMF during storage of fruit juice concentrates and dried fruits. Besides, the effect of parameters such as initial reactant concentrations and pH in the formation of α-dicarbonyl compounds and HMF is evaluated. This chapter also gives an insight to the role of Maillard reaction in fruit products during storage by means of high-resolution mass spectrometry analysis.

Chapter 5 describes the effect of processing on the changes in the concentration of sugars, amino acids, α-dicarbonyl compounds and HMF in different fruit products obtained from critical stages of industrial-scaled process.

CHAPTER 1

GENERAL INTRODUCTION

1.1. FRUIT BASED PRODUCTS

1.1.1. Overview

Fruits and fruit products have a significant role in a healthy diet due to the content of many valuable nutrients including essential vitamins such as vitamin A, B_6, C, E [34]. Fruit products are also the main nutritional sources for fruitarian, vegan and vegetarian persons together. Among fruit products, fruit juices also contribute to the daily liquid requirements in a healthy diet. Fruits together with vegetables are involved in the second widest part of the food pyramid which was introduced by United States Department of Agriculture (USDA) in 1992. In 2003, World Health Organization (WHO) declared a campaign named "5 a day" which recommends the consumption of at least two servings of fruits and three servings of vegetables in the forms of fresh, dried, frozen or canned per day [35]. More recently, Food and Agriculture Organization of the United Nations (FAO) has declared 2021 as the International Year of Fruits and Vegetables in order to raise awareness of the nutritional and health benefits of consuming fruits and vegetables [36]. Following those, consumers have shown an increasing interest in fruit and fruit products especially in developed countries such as North America and West Europe [37]. In support, numerous scientific studies have proved that consuming fruit products regularly in the diet helps preventing or fighting cardiovascular diseases, various types of cancer, type 2 diabetes, stroke and many other chronic diseases [38].

The unique composition of fruits makes them essential for the human diet as they contain nutritional compounds. The compositions of fruits depend on various conditions such as botanical variety, cultivation, climate, harvesting, processing, storage and transportation conditions. Fruits, as living complex organisms, have high water and sugar content, and in minor amount of vitamins, minerals, organic acids, phenolic compounds, nitrogen-containing compounds, color and aroma compounds. Most fresh fruits have the water contents greater than 85%. High water content of fruits lead to sugar hydrolysis via the acid catalysis [39]. Both digestible carbohydrates mainly in the form of sugars and starches, and indigestible carbohydrates largely in the form of cellulose and pectin (fibers) are found in fruits ranged between 10 to 25 % [39, 40]. Sucrose, glucose, and fructose are the major digestible sugars found in fruits which influence the sweetness of fruit. Fruits are the main sources of certain vitamins and minerals, e.g. vitamin C and provitamin A are largely present in the citrus fruits and yellow-orange fruits, respectively. Processing and long-term storage

cause the destruction of vitamins in particular the most-sensitive one, vitamin C. Potassium, calcium, magnesium and phosphorus are the major minerals found in fruits ranging in 0.03% to 0.6% [39]. Organic acids in minor amounts such as citric acid, malic acid, tartaric acid give the technological character of fruit products through the sugar/acid ratio. In addition, the relative quantities of organic acids affect the wide pH range of fresh fruits. Most fruits have acidic pH ranging between 2.5 to 5 except some fruits like date palm fruit with pH range of 5.5 - 7.0 [40]. Fruits contain various phenolic compounds typically ranging in 0.1 and 2 %. Nitrogen-containing compounds found in different combinations as proteins, amino acids, amides, amines or nitrates have a minor contribution to nutrient composition of fruits, frequently less than 1% [39]. The pigments including chlorophyll, carotenoids, flavonoids, melanoidins and caramels are responsible for the skin and tissue color of the fresh and processed fruits.

1.1.2. Processing and Storage of Fruit Products

The fruit sector plays an important role both in providing healthy foods to consumers and economic contribution to producers that the production has been doubled between 2000 and 2018 [36]. The world is producing more fruit but still not enough to meet the WHO's recommendation which encourages the consuming at least 2 servings of fruits especially in developing and undeveloped countries [36]. The difficulties to reach the target include the short shelf-life, specific storage requirements, transportation problems, region and climate dependence of fresh fruits. Therefore, fresh fruits have been started to process as juices, nectars, purees, marmalades, dried, frozen and canned fruits. Moreover, there is an increasing trend to use fruit products like dried fruits, fruit purees and concentrates as ingredients in several foods such as yogurts, baby foods, baking products, breakfast cereals instead of refined sugars after WHO calls on countries to reduce free sugars intake among adults and children [41]. From fruit products, juices are the most preferred one with increasing demand especially by the healthy food conscious consumer. After the discovery of pasteurization by Louis Pasteur in 1861, the company Welch was the first to preserve grape juice using heat treatment in 1869, followed by Müller-Thurgau in 1896 [34, 39]. Thus began the production of preserved fruit juices which could be stored for extended periods. Advances in process technology (aseptic technique), equipment design, product formulation (use of enzymes, clarifying agents) have now made it possible to produce various type of fruit juice in their characteristic flavor profile without using chemical preservatives.

Fruit juices are the extractable fluid contents of cells or tissues of fruits [42]. Fruit juice-based drinks can be divided into four main categories according to the processing technique: Juices, concentrates, nectars and purees. In general, the process design for juice production is applied considering the fruit types categorized as pomes, stone fruits, grape-like fruits and citrus fruit. Among juices, apple juice (clear and/or cloudy) and orange juice (cloudy and pulpy) are mostly consumed fruit juices in the world due to the largest volume of production [37]. In addition to juices, fruit nectars also represent a growing market segment since it is possible to regulate the acidity, flavor/aroma and other ingredients such as vitamins, sweeteners may also be added [42]. Production of fruit juices can be divided into four main stages: Front-end operation, juice extraction, juice clarification and refining, and juice pasteurization and concentration. General diagram for the production of these juice types is given in **Figure 1.1**.

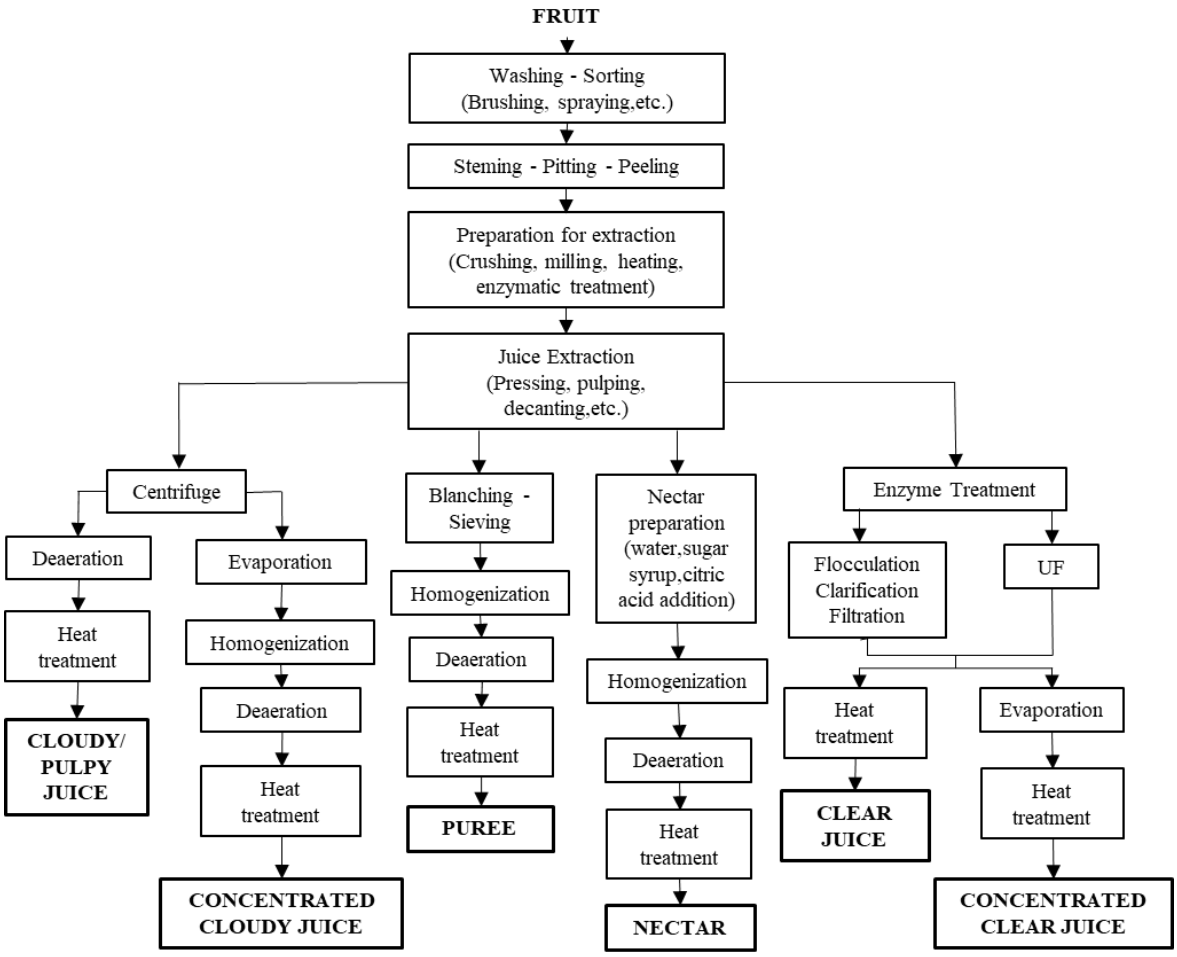


Figure 1.1. General diagram for fruit juice, concentrated fruit juice, fruit puree and fruit nectar production, adapted from [34, 39, 40].

Front-end operations include the preparation steps before juice extraction such as washing, sorting, stemming, destoning, peeling, crushing, milling, heat treatment, enzymatic maceration depending the fruit type. Additional preparation step such as heat treatment is crucial for citrus juice and pulpy nectar production in order to inactivate the enzymes causing pulp-serum separation [34]. The pre-heat treatment is generally performed at 80-85 °C for few minutes then cooled very quickly [40]. Apart from citrus juice and pulpy nectar like products, enzymatic maceration as another preparation step might be needed before pressing in particular for high-pectin containing fruits like apples in order to make the extraction easier and to increase the yield and it is generally performed at about 50 °C for 1 or 2 hours. The method of extraction and clarification differentiates depending the fruit types as given in **Figure 1.1.** [40]. Clarification process includes firstly enzymatic treatment then either flocculation with agents or ultrafiltration steps. For this purpose, enzyme mixtures including pectinases (pectinesterase, polygalacturanase, pectinlyase), amylase, hemicellulose, and arabanase are added to juice and the reaction takes place one or two hours at 45 – 50 °C [43]. After enzyme treatment, clarification step is carried out with the addition of different clarifying agents such as gelatin and bentonite performs followed by filtering or with only using ultrafiltration technique [34]. Use of clarifying agents, a traditional method, is carried out at 45 – 50 °C for about two hours. Alternatively, the ultrafiltration process is operated by using specific pore-size membranes selectively retain large molecules such as proteins, pectin fractures, starch [34]. Then a pasteurization process is necessary for the destruction of spoilage microorganisms and also inactivation of the pectic enzymes responsible for juice separation. The most common conditions for pasteurization are 85 °C for 15 – 30 s or 95 – 110 °C for a few seconds [44]. From this point on, the juice is ready to consume or available for processing into concentrate for extended shelf-life and improving transportation. The most frequently used concentration method is evaporation which includes controlled removal of water generally at 50 - 80 °C until the desired Brix value obtained.

Among fruit based products, dried fruits serve as a healthful snacks providing a concentrated form of fresh fruits prepared by different drying techniques. In addition to health promoting effect, drying of fruits lead to extend the shelf life of fruits by reducing the water content to a level so as to prevent the growth of microorganisms and moisture-mediated chemical reactions. Drying also brings benefits such as easily storage, low-cost transportation, reduced packaging costs by reducing the weight and volume of fruits [45]. Generally, drying is performed in combination with physical or chemical pretreatments such as thermal

blanching, ultrasonic waves, sulfuration, dipping in chemical solutions, etc. in order to enhance drying kinetics, reduce energy consumption and preserve the quality of products [46]. To-day, chemical pretreatments give place to thermal and/or non-thermal physical pretreatments since residual chemicals like alkali liquor, sulfur dioxide can cause food safety problems [46]. Among physical pretreatments, hot water blanching is the most popular one due to its simple and easy operation in addition to the advantages such as inactivation of enzymes, expelling intracellular air, enhancing permeability of the cell membrane, dissociating the wax layer, etc [46]. With the proper pretreatments, various advanced techniques such as conventional hot-air drying, solar drying, microwave drying, osmotic dehydration, explosion puffing, freeze-drying, oven drying and vacuum drying are used for drying fruits [47]. Fruits can be dried in their original form such as grapes, slices or cubes of apple, mango, etc. or in processed forms such as puree of peach, mango, papaya, etc. Different types of dryers should be selected for drying depend upon the physical form of the fruit such as whole, slices, granular, paste, leather, or powder [39, 40]. In addition to the physical form, other factors such as the sugar content, hygroscopicity and stickiness characteristics of fruits, presence of a skin, high temperature sensitivity affect the drying process [40]. For instance, high sugar content may lead to undesirable caramelization during drying due to the incorrect operating temperature [40]. On the other hand, the hygroscopicity and stickiness of the product may cause the problems such as deposits in dryers or caking during drying [40]. Therefore, all factors affecting the drying should be considered in selecting both the dryers and the process conditions.

Since unsuitable or pro-longed storage conditions cause undesirable changes in fresh fruits or fruit products, different types of operations mentioned above are performed to extend shelf-life and reduce the high-cost storage requirements. Fresh fruits generally have the potential storage life changing from few days to 6 or 8 weeks depending the temperature and the relative humidity [40]. On the other hand, processed fruits such as juices, dried fruits, purees, etc. can have a shelf life exceeding 1 year depending the processing and packaging type [48]. For example, the juices which have been subjected to a “light” pasteurization (typically a few seconds at around 90 – 92 °C) but not aseptic packaging have a shelf life of around 8 – 12 weeks under cold conditions (2 – 5 °C) [48]. On the other hand, the long-life juices packed aseptically in laminated cartons typically carry a shelf life of 6 to 12 months with no requirement of chilling [48]. Besides, drying of fruits provides storage for exceeding 1 year at room temperatures if the water activity is reduced to appropriate levels and the

packaging is selected proper [40]. To extend the shelf life of fruits and ensure product stability, novel and combined processing (non-thermal and thermal heat treatments) and packaging techniques are recently performed considering the microbiological, physical and chemical characteristics of the product.

1.1.3. Changes During Processing or Storage of Fruit Products

High temperature or the duration of the thermal process and unsuitable or prolonged storage conditions can promote reactions that could affect the overall quality of foods. The quality attributes in fruit products can be categorized as microbiological (pathogens, spoilage microorganisms), nutritional (vitamins, dietary minerals, antioxidants), organoleptic (appearance, color, flavor, texture), chemical (composition, deterioration) [49]. The main target of thermal treatment is to reduce or destroy microorganisms in order to extend the shelf-life without causing nutritional loss and taste/aroma deterioration.

The deteriorative reactions such as enzymatic and non-enzymatic browning resulted in undesirable consequences in fruit products. Browning of fruit products causes one of the main problems in fruit industry by both affecting the flavor and nutritional value and leading to the formation of undesirable compounds [39]. Undesired enzymatic browning can be easily inhibited by heat treatments or using additives during processing [30]. On the other hand, non-enzymatic reactions are more complex than enzymatic browning since simultaneous reactions take place and the large number of secondary reactions may occur [39]. Maillard reaction, caramelization, and ascorbic acid degradation reactions have been reported as the main non-enzymatic reactions responsible for the non-enzymatic browning [4, 50]. Maillard reaction is well known to be faster in high temperature long storage conditions, high pH and low water activity. In the case of fruit products especially fruit juices, the storage temperature gains attention since the media has acidic pH and high moisture content [51]. It has been reported that caramelization favors high temperature operations such as above 120 °C and extremely high acid conditions such as below pH 3.0 [4]. Considering the process temperatures and fruits acidic nature, caramelization seems one of the main factors responsible for the non-enzymatic browning in most fruit products. Sucrose, the one of the most abundant sugars in fruit products, can easily hydrolyze in an acid media under a rate corresponding to a first-order process during processing or storage [50]. Additionally, the reducing sugars (fructose and glucose) may increase at a rate determined as a result of sucrose hydrolysis [50]. The concentration of reactants,

temperature, and acid-catalyst concentration are the factors determine the rate of hydrolysis [39]. Following the hydrolysis of sucrose, the reducing sugars can degrade to form the undesirable compounds such as HMF, α-dicarbonyl compounds depending the process type and storage conditions [4]. Consequently, these relatively small chemical compounds can produce undesirable brown pigment of intense color in fruit products [39]. In addition, ascorbic acid degradation by oxidative or non-oxidative pathway has been defined as one of the major contributor of browning particularly in citrus fruit products [30, 52, 53]. Researchers have identified chemical markers to investigate the relationship between these non-enzymatic reactions and browning in various fruit products [29, 50, 54-56]. Accordingly, significant correlations have been found between color development and the chemical compounds (HMF and α-dicarbonyl compounds) formed during Maillard reaction, caramelization and/or ascorbic acid degradation. For example, it was reported that browning level of apple juice concentrates and citrus juice concentrates increased with the increase in the HMF concentration during storage [55, 57]. Similarly, significant changes in α-dicarbonyl compounds concentrations were reported and positively correlated with color formation during the storage of apple juice and orange juice [29, 30]. However, the simultaneous contributions of these non-enzymatic reactions make difficult to determine the exact factor leading the browning in fruit products. In addition, it should be noted here that these chemical markers (HMF and α-dicarbonyl compounds) not only cause the organoleptic loss but also lead to the formation of potential toxic compounds such as advanced glycation end-products (AGEs), furan, and acrylamide [5, 58]. Hence, these compounds have been related with some degenerative diseases such as diabetes, cataract, Alzheimer disease, tumor growth [1, 59]. Since fruit products are highly suitable for the mentioned non-enzymatic reactions due to their acidic natures and high reducing sugar content, investigation the levels and formation of HMF and α-dicarbonyl compounds under different conditions is of importance considering their aforementioned potential toxic effects in fruit products.

1.2.FOOD DERIVED α-DICARBONYL COMPOUNDS and 5-HYDROXYMETHYLFURFURAL

1.2.1. Reactions Affecting the Formation of α-Dicarbonyl Compounds and 5-Hydroxymethylfurfural

α-Dicarbonyl compounds and 5-hydroxymethylfurfural (HMF) are the intermediate products mostly derived from the reactions of caramelization, Maillard reaction, lipid peroxidation, ascorbic acid degradation during processing or storage of foods. In addition, microorganism metabolism in fermented food and beverages and the defense mechanism in plants against environmental stresses lead to the formation of α-dicarbonyl compounds and HMF [60]. The type of reaction causing α-dicarbonyl compounds and HMF formation largely depends on the composition of foods and processing conditions. In the case of fruit products, it has been reported that Maillard reaction, caramelization and ascorbic acid degradation are the reactions mainly responsible for the formation of them due to the acidic and high sugar nature of fruits [4, 50, 53]. The α-dicarbonyl compounds formed through degradation of ascorbic acid have been reported as glyoxal, methylglyoxal, diacetyl (DA), L-threosone, 3-deoxy-L-threosone and 3-deoxy-L-pentosone [52, 53, 61, 62]. Ascorbic acid degradation occurs both via oxidative and non-oxidative pathways [53]. Although ascorbic acid is very unstable under alkaline conditions (above pH 7.0), in the presence of oxygen, and under high temperature conditions (above 98°C) at low pH values (below pH 7.0), it has the maximum stability at pH 3.0 - 4.0 [53, 63]. In order to understand the ascorbic acid degradation in foods or food-like model systems, researchers have mainly focused the investigation of changes in browning degree depending the changes in the concentration of ascorbic acid [53, 64]. From the limited studies on the relationship between ascorbic acid and α-dicarbonyl compounds in foods, it can be said that ascorbic acid degradation contributes to the formation of α-dicarbonyl compounds and HMF mainly in citrus products, since the highest amount of ascorbic acid presents in the citrus fruits [65]. Nevertheless, the quantitative contribution of ascorbic acid to the formation of α-dicarbonyl compounds is very low when compare to other precursors contribution such as reducing sugars [30]. For example, a study based on isotope incorporation during storage of orange juice showed that the contribution of ascorbic acid to the formation of α-dicarbonyl compounds was found as the followings: glyoxal; 7%, methylglyoxal; 11% and 3-deoxyglucosone; 3%, whereas the contribution of reducing sugars was found as bellows: glyoxal; 99%, methylglyoxal; 87% and 3-deoxyglucosone; 93% [30]. Therefore, Maillard reaction and caramelization have a special interest in the formation of α-dicarbonyl compounds and HMF in fruit products which have high reducing

sugar content and acidic pH. Thus, the effect of Maillard reaction and caramelization on the formation of α-dicarbonyl compounds will be discussed in this thesis.

Maillard reaction was discovered by Louis-Camille Maillard in 1912 rather by chance while he was trying to synthesize peptides by heating amino acids with glucose that resulted in development of browning and flavor [66]. After over 100 years of the discovery, numerous researches have been performed in order to understand the complex reaction network both in foods and biological systems. In food science, primary studies focused mainly on the investigation of the development of both desirable and undesirable browning (e.g. in dried fruit and milk powder) accompanying with nutritional loss (e.g. lysine blockage) [67, 68]. Besides food studies, as it was recognized that the Maillard reaction also occurred at 37 °C, a possible significance of this reaction was started to investigate in physiological processes [69, 70]. Following that, an unknown variant of human hemoglobin, which was later designated as HbA_{1C}, was described in 1955 [71] and then in 1968, it was linked to diabetes mellitus for the first time [72]. What is interesting from this founding that the N-terminal valine residue of the β chain of this hemoglobin variant exists as an Amadori product which is formed from glucose and amino acids during Maillard reaction as already known in foods [73, 74]. By the discovery of HbA_{1C}, the Maillard reaction has been also of concern in view of diabetic complication and ageing in addition to food safety and protein chemistry issues.

The Maillard reaction which is named also amino-carbonyl reaction or non-enzymatic browning has been comprehensively studied on the basis of the reaction chemistry [67, 75-79]. In 1953, Hodge [68] proposed his famous scheme of the Maillard reaction pathways including the Amadori rearrangement with a key role in the reaction. This scheme is still the key reference for all Maillard scientists in order to understand the pathways. Hodge [68] divided the reaction into three main stages: early, intermediate and final stages (**Figure 1.2**).

The early stage of the Maillard reaction starts with the addition of a non-protonated amino compound to a carbonyl compound (reducing sugar) to form a carbinolamine. Then the carbinolamine compound dehydrates to form *N*-substituted glycosylamine (Schiff base, imine) which undergoes a rearrangement via the 1,2-eneaminols as a result of the functionality of hydroxyl group in the α-position. The rearrangement of rather instable Schiff bases results in the formation of Amadori Rearrangement Product (ARP, 1-amino-1-deoxy-ketose) if the sugar is an aldose or in the formation of Heyns Rearrangement Product (HRP, 2-amino-2-deoxy-aldose) if the sugar is a ketose. The early stage marker of the Maillard

reaction is known as furoyl derivatives of ARP with lysine which could be determined quantitatively by controlled acid hydrolysis conditions [80]. Content of furosine, N-ε-fructoselysine, is used as an indicator of heat treatment and quality of foods during storage, as well as the calculation of percentage of blocked lysine [81]. The intermediate stage of the Maillard reaction starts with the degradation of ARP depend upon the pH of the medium. The complex intermediate phase reactions can be reviewed in 2 parts as below and above pH 7.0. At pH 7.0 or below, ARP mainly undergoes 1,2-enolisation yielding 3-deoxyglucosone (3-DG) which is later degraded to furfural (if sugar is a pentose) and HMF (if sugar is a hexose). In the case of pH>7, ARP undergoes 2,3-enolization resulting the formation of 1-deoxyglucosone (1-DG) which forms reductones, and fission products like dicaetyl, acetol and pyruvaldehdyde [2]. In the presence of large amounts of amines, 3-DG might degrade to nitrogen-containing compounds rather than HMF or furfural.

Similarly, HMF might form aldimines and/or ketimines in the presence of amino compounds. The formation reaction of aldehydes and α-aminoketones through the reactions between α-dicarbonyl compounds and α-amino acids releasing carbon dioxide is known as Strecker degradation [77]. Strecker degradation is one of the pathways leading to acrylamide and furan formation with involvement of α-dicarbonyl compounds [82]. The intermediate stage involves various reactions such as cyclisations, dehydrations, retro-alsolisations, rearrangements, isomerisations leading to the formation of desirable (aroma compounds) and/or undesirable components (toxic compounds). In addition to those reactions in early stage in the Hodge scheme, Hayashi and Namki [83] indicated that *N*-glycosylamine plays an important role on the formation of glyoxal. Therefore, a group of reactions, which are known as Namiki pathway, resulted in the formation of α-dicarbonyl compounds from Schiff bases were later involved in the Maillard reaction network as shown in **Figure 1.2** [83]. In the final stage, the Maillard reaction results in the formation of heterogeneous brown nitrogenous polymers which are called melanoidins having high molecular weight. Melanoidins are the health-beneficial consequences of the Maillard reactions since they have antioxidative and anticarcinogenic activities by scavenging free oxygen and carbonyl radicals [84].

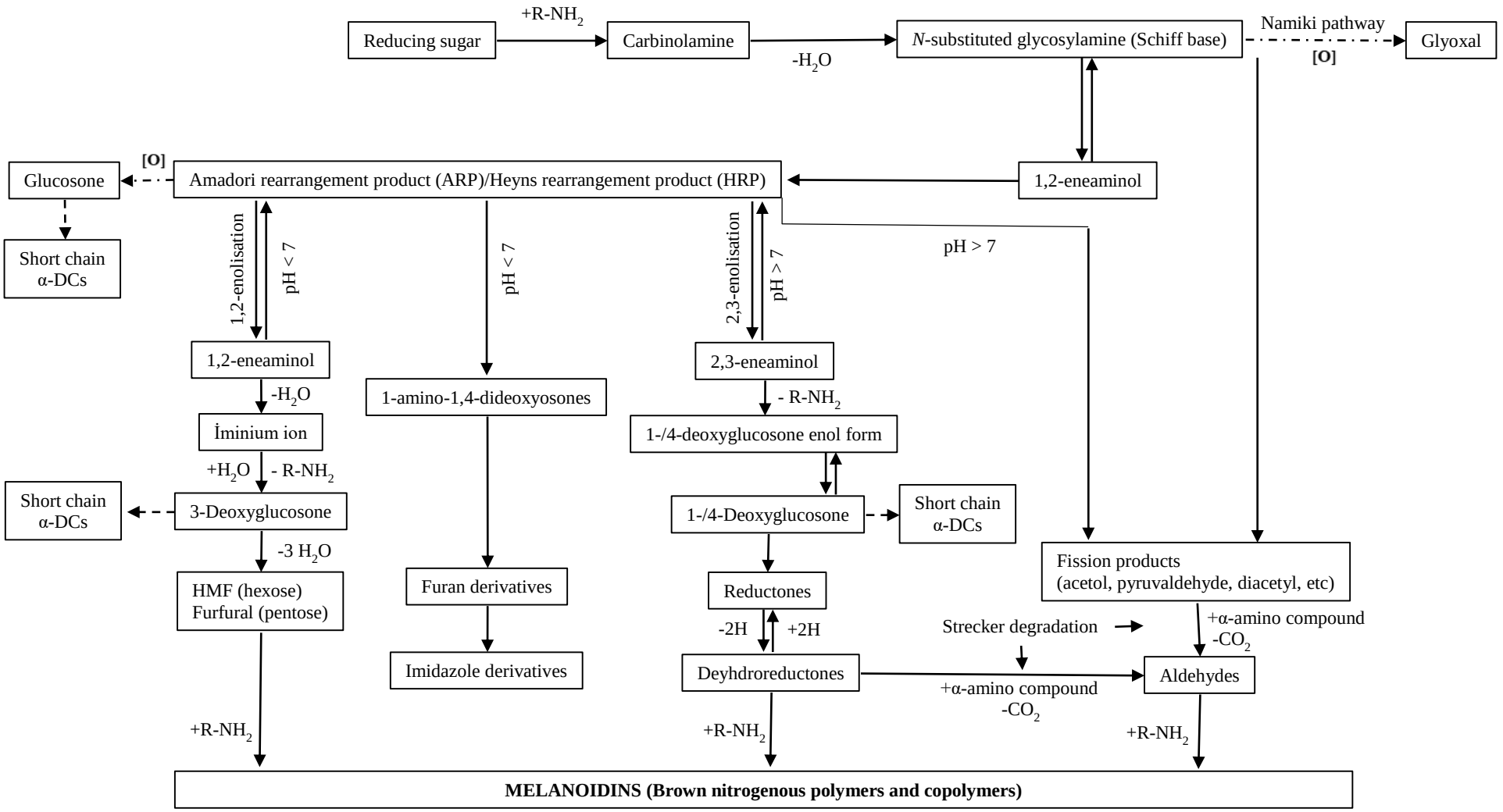


Figure 1.2. Simplified outline of the Maillard reaction in relation to α-dicarbonyl compounds (α-DCs) formation, adapted from [68, 83-85].

Caramelisation is also defined by Hodge [68] as “partial mechanisms” in a single browning reaction. In the presence of acidic and/or alkaline catalysts, dehydration and fragmentation reactions which are initiated with enediols occur through the degradation of reducing sugars (**Figure 1.3**). Sugars are very reactive in acyclic forms and the ring opening of the cyclic form of sugars lead to initiate the isomerization and epimerization reactions. Isomerization of aldose and ketose sugars occurs through 1,2-enolization reaction called the Lobry De Bruyn-Alberda Van Ekenstein transformation (LdB-AvE) [86]. In addition, glucose, fructose and mannose are found in equilibrium through 1,2-enediol intermediate in alkaline solutions. The LdB-AvE rearrangement also involves epimerization which results in the change of the configuration of C-2 in aldoses. Although epimerization of glucose to mannose (via 1,2-enediol) and fructose to psicose (via 2,3-enediol) also occurs, it is reported that these transformations are not as significant as the glucose-fructose interconversion [87]. In addition to hexoses, the LdB-AvE isomerization of the reducing end of the oligosaccharides such as maltose to maltulose and lactose to lactulose is also observed. The enolisation reaction is of particular importance since it initates dehydration or β-elimination, dicarboxylic cleaving, retro-aldol reaction and later, aldol condensation which produce heterocyclic and carbocyclic compounds. In alkali media, enolization is followed by fragmentation reactions and aldol condensation. Although the LdB-AvE rearrangement favors alkaline media, it can also occur in acidic media that is followed by β-elimination of water molecules. The presence of metal cations such as Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} in the media cause the increase in the rate of chain opening and that catalyzes the LdB-AvE transformation [86]. Additionally, in the presence of oxygen, enediols are catalyzed by transition metal ions such as Cu^{2+} [88]. The sugar degradation reactions lead to the formation of key intermediates such as 3-DG which contribute to the caramel flavor and color formation. The formation of caramelization compounds is influenced by the temperature and whether the medium is acidic or basic. Dehydration and cyclisation reactions dominate in thermally and/or acid-induced conditions whereas the cleavage of the carbon chain of the sugar is favorable under alkaline conditions. For example, derivatives of furan such as HMF can be observed by elimination of water molecules from sugars in an acidic media depending on the reaction conditions. The type CH_3-CH=CH-CH(OH)-C=O, a part of the structure of hydroxydimethylfuranone, is reported as a probably responsible for the characteristic caramel flavor [89]. On the other hand, the heterocyclic and carbocyclic compounds have different odor threshold values.

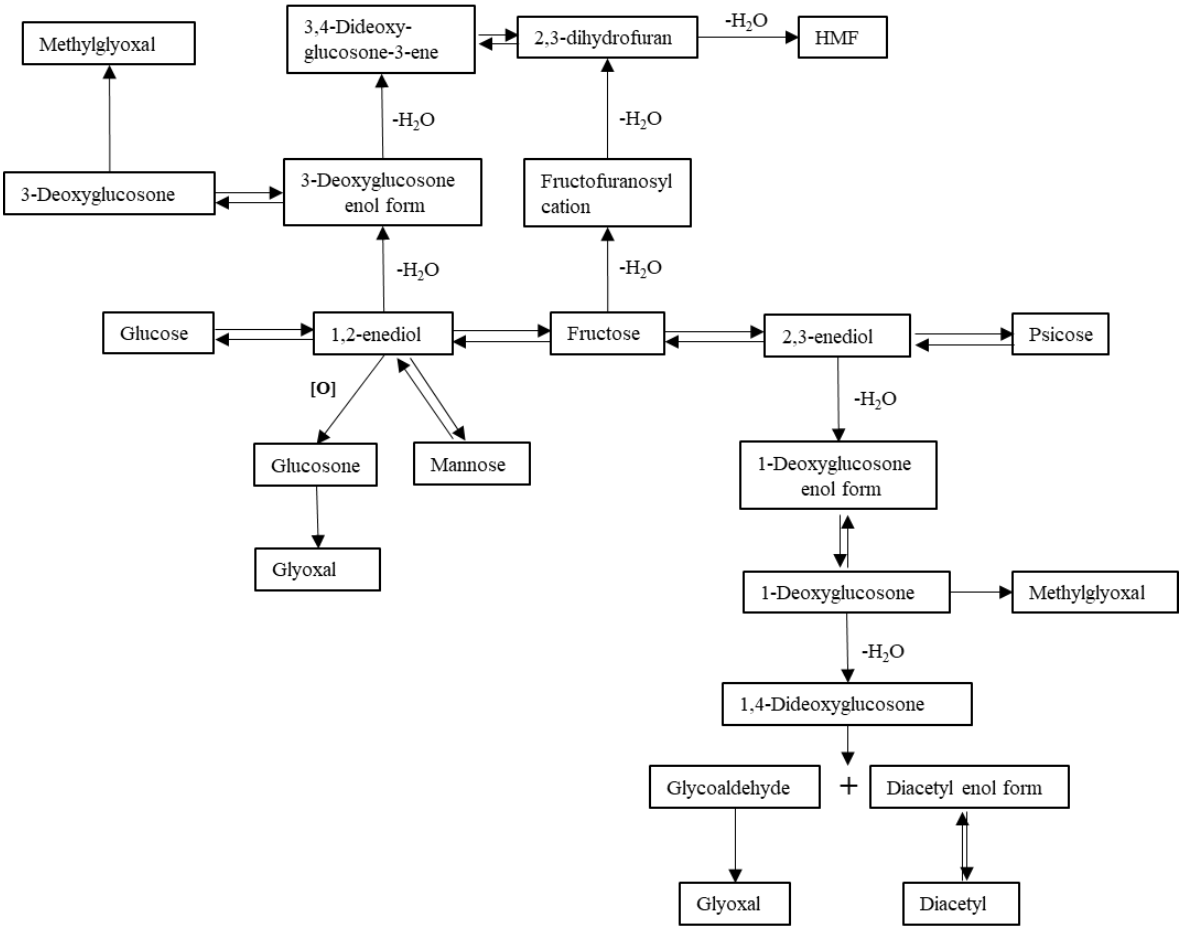


Figure 1.3. Simplified outline of the sugar degradation reactions in relation to α-dicarbonyl compounds formation, adapted from [2, 89-92].

It should be noted here that Maillard reaction and caramelization are the reactions which comprise common reactive intermediates in parallel and consecutive reactions. It is well known that Maillard reaction proceeds effectively in the alkaline, low-moisture medium with high temperature conditions and optimum aw of 0.6-0.8 [2] whereas caramelization prefers temperatures above 120 and/or 9<pH<3 [4].

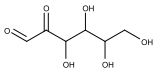
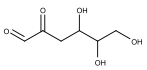
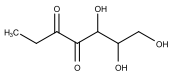
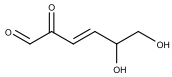
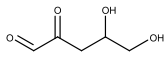
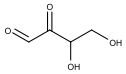
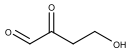
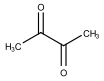
1.2.2. α-Dicarbonyl Compounds

1.2.2.1.Physical and Chemical Properties

There are about 22 kinds of α-dicarbonyl compounds which have been qualitatively and quantitatively detected in a wide range of foods until now [60]. Among them, glucosone, 3-DG, 1-DG, 4-DG, 2-DG, galactosone, 3-deoxygalactosone (3-DGal), 3,4-dideoxyglucosone-3-ene (3,4-DGE), 1,4-deoxyglucosone represents the important intact (C_6-skeletal) α-dicarbonyl compounds formed in foods. Following the formation of intact α-dicarbonyl compounds , *retro-*

aldol reactions, fragmentation, and water elimination reactions lead to the generation of shorter chain α-dicarbonyl compounds [2]. The most common short chain α-dicarbonyl compounds in foods have been stated in the literature as 3-deoxypentosone (3-DP), threosone, 3-deoxythreosone (3-DT), diacetyl (DA), methylglyoxal (MGO) and glyoxal (GO). The physical and chemical properties of these α-dicarbonyl compounds are given in **Table 1.1.**

Table 1.1. The physical and chemical properties of glucosone, 3-DG, 1-DG and 3,4-DGE [2, 60, 93].

Name	Molecular Formula	Structure	Molar mass (g/mol)	Density (g /cm^3)	Boiling point(°C)	Melting point(°C)
Glucosone	$C_6H_{10}O_6$		178.140	1.574	481.0	118-120
3-DG	$C_6H_{10}O_5$		162.141	1.410	400.1	73-75
1-DG	$C_6H_{10}O_5$		162.141			
3,4-DGE	$C_6H_8O_4$		144.130	1.401	381.5	
3-DP	$C_5H_8O_4$		132.14	1.3	316.5	
Threosone	$C_4H_6O_4$		118.09	1.4	305.3	
3-DT	$C_4H_6O_3$		102.09	1.2	200.2	
DA (2,3-butanedione)	$C_4H_6O_2$		86.09	0.981	88	-2 and -4
MGO	$C_3H_4O_2$		72.06	1.046	72	25
GO	$C_2H_2O_2$		58.04	1.27	51	15

1.2.2.2.Formation Mechanisms of Intact α-Dicarbonyl Compounds

Glucosone (D-*arabino*-hexos-2-ulose, 2-keto-D-glucose): During Maillard reaction and/or caramelization, glucosone is generated through the oxidative pathway [3, 94]. The oxidation of glucose and fructose during caramelization and Amadori/Heyns product during Maillard reaction yields glucosone by removal of 2 protons in the presence of oxygen and even small amounts of transition metal ions (**Figure 1.4**) [3, 94]. During caramelization, the LdB-AvE transformation causes the formation of 1,2-enediol intermediate which leads to generation of glucosone. During Maillard reaction, oxidation of Amadori and/or Heyns products follows by the hydrolysis from first carbon of fructosamine and release of amino acid that results in

glucosone formation. In addition to general factors affecting the performance of Maillard reaction and caramelization, the main two factors, presence of molecular oxygen and transition metal ions enhance the formation of glucosone [3]. Besides, glucosone also easily forms in aqueous conditions rather than dry conditions via hydrolysis of Amadori product [94].

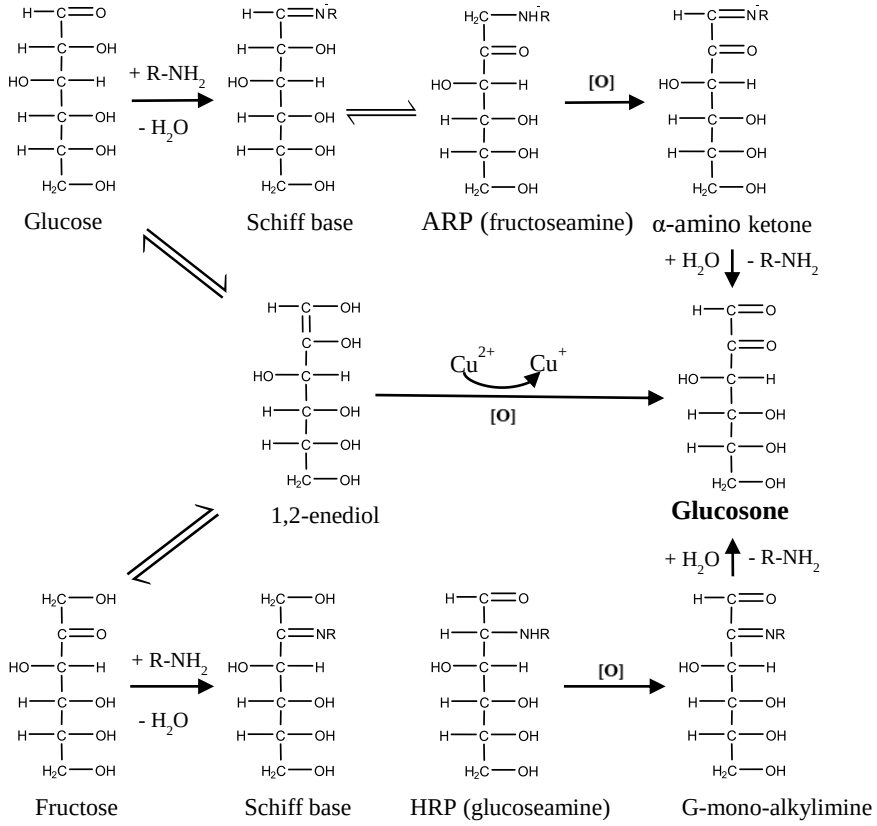


Figure 1.4. Formation of glucosone through oxidation of sugars and Amadori/Heyns products, adapted from [3, 91, 94].

3-Deoxyglucosone (3-DG: 3-deoxy-D-*erythro*-hexos-2-ulose): 3-DG is generated independent from the presence of oxygen during Maillard reaction and caramelization [3]. Dehydration and enolisation reactions yield to the formation of 3-DG during sugar degradation whereas hydrolysis and regeneration of amino acids following enolisation and dehydration reactions give 3-DG during Maillard reaction (**Figure 1.5**). Elimination of a water molecule from the C-3 of 1,2-enediol is analogue to that of 1,2-eneaminol. 3-DG exists in aqueous solutions in many forms, mostly α- / β- pyranose and furanose cyclic structures [95]. Similarly, by elimination of a water molecule from galactosone, 3-deoxygalactosone (3-deoxy-D-*threo*-hexos-2-ulose) is analogously formed [96]. As mentioned before, acidic environment triggers the 1,2-enolization which gives 3-DG and in low moisture conditions, 3-DG level increase since hydrolysis is restricted and dehydration is triggered [2].

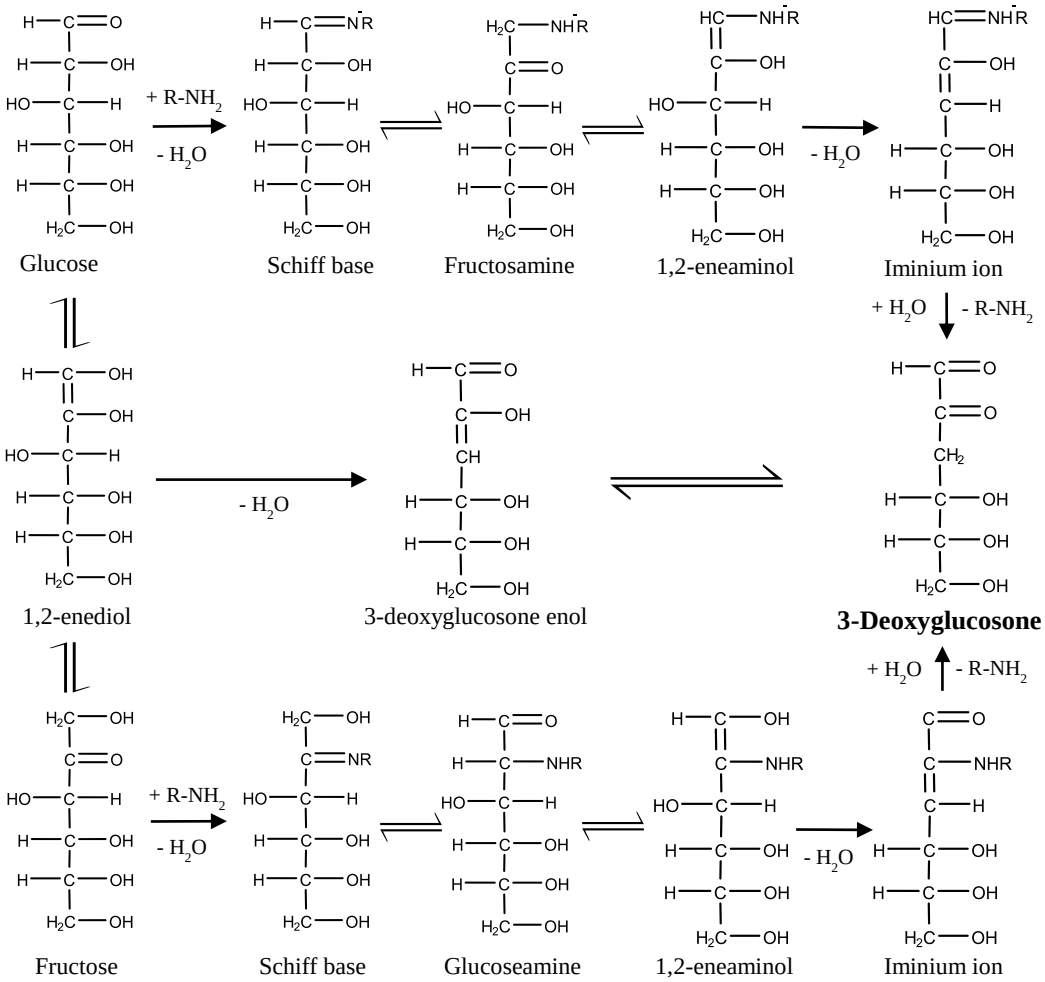


Figure 1.5. Formation of 3-deoxyglucosone through degradation of sugars and Amadori/Heyns products, adapted from [2, 91].

1-Deoxyglucosone (1-DG: 1-Deoxy-D-*erythro*-hexo-2,3-diulose, 1-deoxyhexo-2,3-diulose): 2,3-enolisation reaction of both fructose and Amadori/Heyns products lead to the formation of 1-DG [2, 97]. A water elimination from the first carbon of 2,3-enediol intermediate generates 1-DG while it is formed via regeneration of amino compound from 2,3- eneaminol intermediate (**Figure 1.6**). Similar to 3-DG, 1-DG is found in various cyclic hemiacetal structures in aqueous solutions. Although both 3-DG and 1-DG is formed via non-oxidative pathway, 1-DG decreases under aeration since its redox reactivity is much higher due to its reductone structure [3]. In addition, 1-DG is also a key reactive intermediate in the formation of various important aroma compounds such as maltol and isomaltol which have an intensive odor and caramel taste [98].In alkaline conditions and dry conditions, 1-DG formation is favored since 2,3-enolization requires partial deprotonation of the nitrogen in amino groups [97].

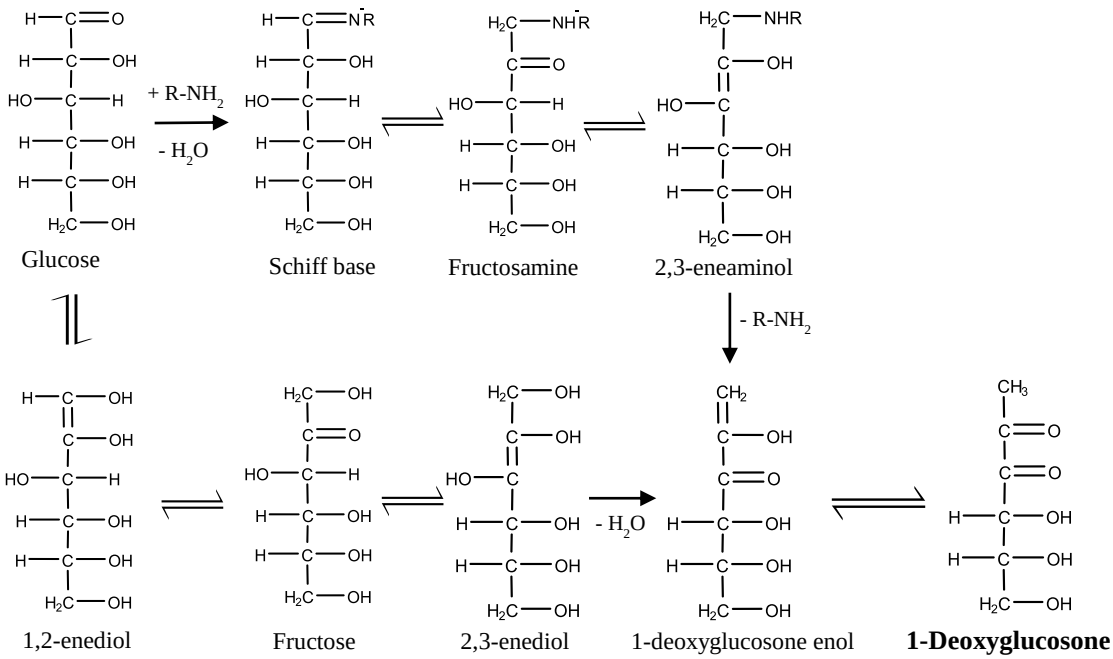


Figure 1.6. Formation of 1-deoxyglucosone through degradation of sugars and Amadori product, adapted from [2, 97].

3,4-Dideoxyglucosone-3-ene (3,4-DGE)*:* Since strong evidences about the cytotoxic effect of 3,4-DGE and its being main precursor of 5-hydroxymethylfurfural, understanding the formation mechanism is getting attention [99]. 3-DG was found to convert to *cis* (Z) and *trans* (E) forms of 3,4-DGE under mild acid conditions by removal a water molecule (**Figure 1.7**) [100]. The unsaturated 3,4-DGE in Z form will subsequently rearrange to the structural favorably cyclic form such as HMF [101]. Unlike the *cis* isomer, (E)-3,4-DGE will not dehydrate to form of HMF due to stereo hindrance [101, 102]. Therefore, the free aldehyde in the (E)-3,4-DGE can later lead to the formation of Aldol condensation products [101]. As expected, low moisture contents and acidic environment increase the formation of 3,4-DGE [101].

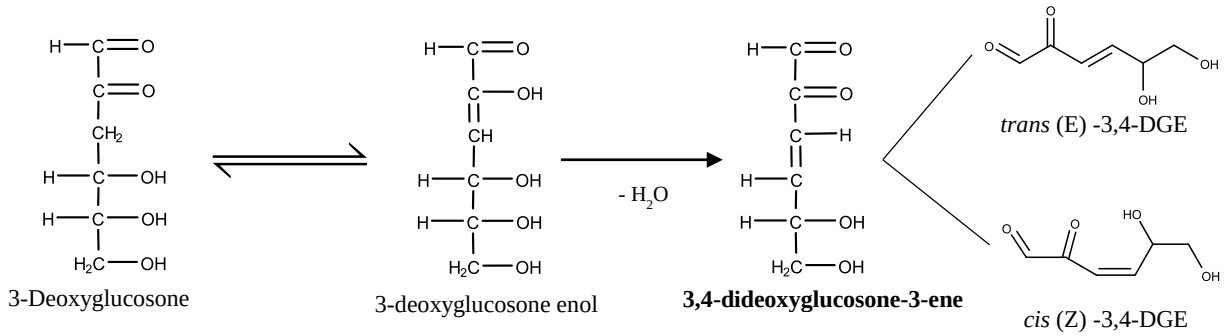


Figure 1.7. Formation of 1-deoxyglucosone through degradation of sugars and Amadori product, adapted from [2, 97].

1.2.2.3.Formation Mechanisms of Short Chain α-Dicarbonyl Compounds

3-Deoxypentosone (3-DP: 4,5-dihydroxy-2-oxopentenal): 3-DP is formed mainly from the degradation of 1,4-glycosidically linked di- and oligosaccharides such as maltose, maltotriose and lactose [3, 103, 104]. During Maillard reaction and/or caramelization, the formation of 1-amino-1,4-dideoxyhexosulose and/or 4-deoxyhexosulose by vinylogous β-elimination from the 2,3-enediol compound is the key reaction for the formation of 3-DP (**Figure 1.8a**) [103, 104]. In addition, it was indicated that no 3-DP was detected whereas 1,4-dideoxyhexosulose was predominant in a dry reaction model [105]. Indeed, Hollnagel and Kroh [103] indicated that hydroxyl ion or carboxylate ion have a critical role in *retro*-aldolization cleavage which causes 3-DP majority in aqueous systems. The *retro*-aldolization is known the requirement of water participation, thus the formation of 3-DP would be higher at high relative humidity conditions [106]. In support, glucosone which is another predominant dicarbonyl in aqueous systems lead to the generation of 3-DP as given in **Figure 1.8b** [3]. Gobert and Glomb [3] suggested that the split of 1,3-tautomer into formic acid and an enediol via β-dicarbonyl cleavage results in the formation of 1,2-enediol which later dehydrates to give 3-DP. Besides, it was stated that small amount of pentosone was formed in aerated glucosone incubations through direct oxidation of enediol intermediate [3]. The type of carbohydrate such as di- and oligosaccharides, the presence of oxygen and aqueous conditions enhance the formation of 3-DP [60]. In addition, Degen, et al. [9] reported that 3-DP was only found in alkali-treated pretzels among various food types. The possible explanation was attributed as the higher fragmentation of carbon chains in alkaline conditions [9].

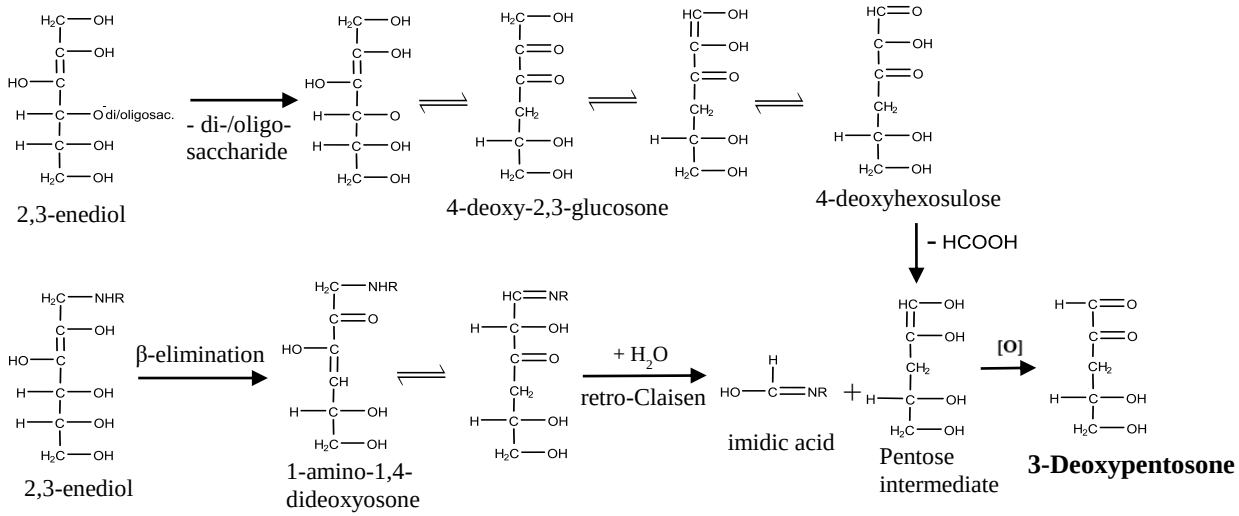


Figure 1.8a. Formation of 3-deoxypentosone through degradation of 2,3-enediol of di-/oligosaccharides and Amadori product, adapted from [103, 104].

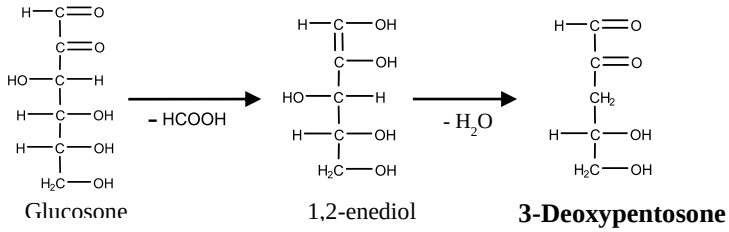


Figure 1.8b. Formation of 3-deoxypentosone through degradation of glucosone, adapted from [3].

Threosone (3,4-dihydroxy-2-oxo-butanal, tetrosone): Although the formation mechanism of threosone is explained in detail during ascorbic acid degradation, much less has been published on the chemistry of threosone formation during Maillard reaction and caramelization [107]. From the limited studies, Usui, et al. [108] reported the formation of threosone from glucosone during degradation of glucose (**Figure 1.9a**). Accordingly, formation of arabinose from glucosone by α-dicarbonyl cleavage lead to the generation of 1,2-enediol intermediate which later gives erythrose [108]. Finally, threosone is formed from erythrose by oxidation. Following that, Voigt and Glomb [98] proposed a formation mechanism of threosone from 1-deoxyglucosone (**Figure 1.9b**). Thus, a hydrolytic β-dicarbonyl cleavage of the 2,4-tautomer of 1-deoxyglucosone gives acetic acid and the C_4-enediol intermediate which leads to the formation of threosone by oxidation [98]. As expected from the suggested mechanisms, aqueous and aerated conditions trigger the formation of threosone [98].

3-Deoxythreosone (3-DT: 4-hydroxy-2-ketobutyraldehyde, 4-hydroxy-2-oxobutanal): The formation mechanism of 3-DT is similar to that of threosone with one difference at the last step. This is the water elimination from erythrose during glucosone degradation [108] and from C_4-enediol intermediate during 1-DG degradation (**Figure 1.9a, b**) [98]. Alternatively, Usui, et al. [108] proposed other formation pathway for 3-DT from 3-deoxyglucosone and 3-deoxypentosone as shown in **Figure 1.9a**. Since the water elimination causes the 3-DT generation, the formation of 3-DT is affected by low-moisture content independently from the presence of oxygen [109]. Additionally, it has been reported that C_4-enediol intermediate may also isomerize to give 1-deoxythreosone (1-DT) with water elimination (**Figure 1.9b**) [98]. However, 1-DT decreases under deaeration and 3-DT becomes prominent since the reductone structure of 1-DT gives high reactivity and short life-time to it [109].

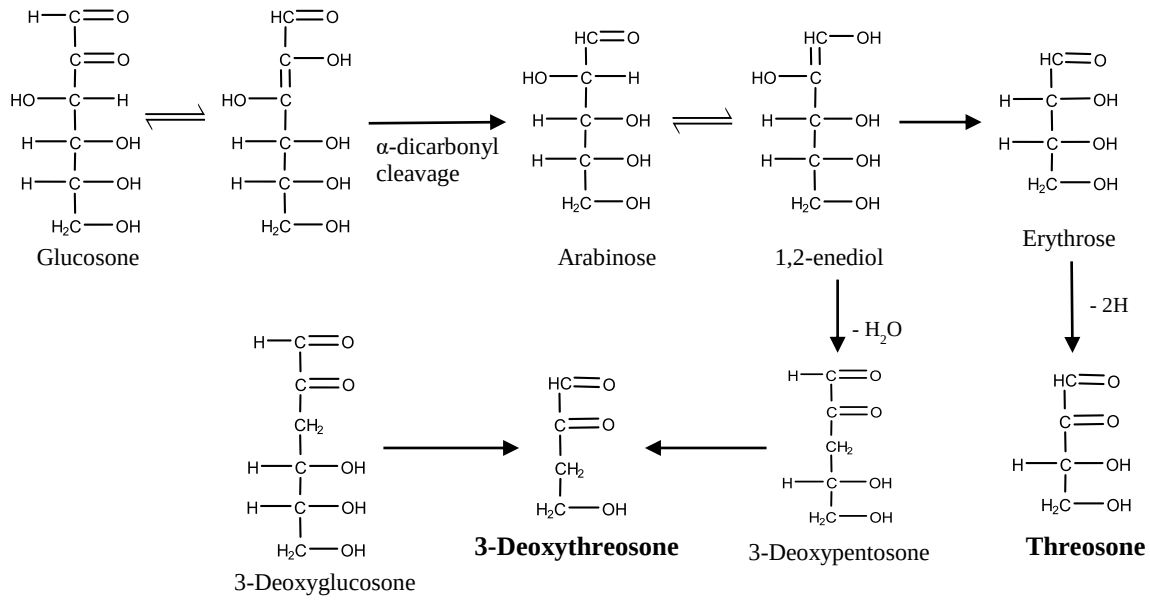


Figure 1.9a. Formation of threosone and 3-deoxythreosone through degradation of glucosone and 3-deoxyglucosone, adapted from [108].

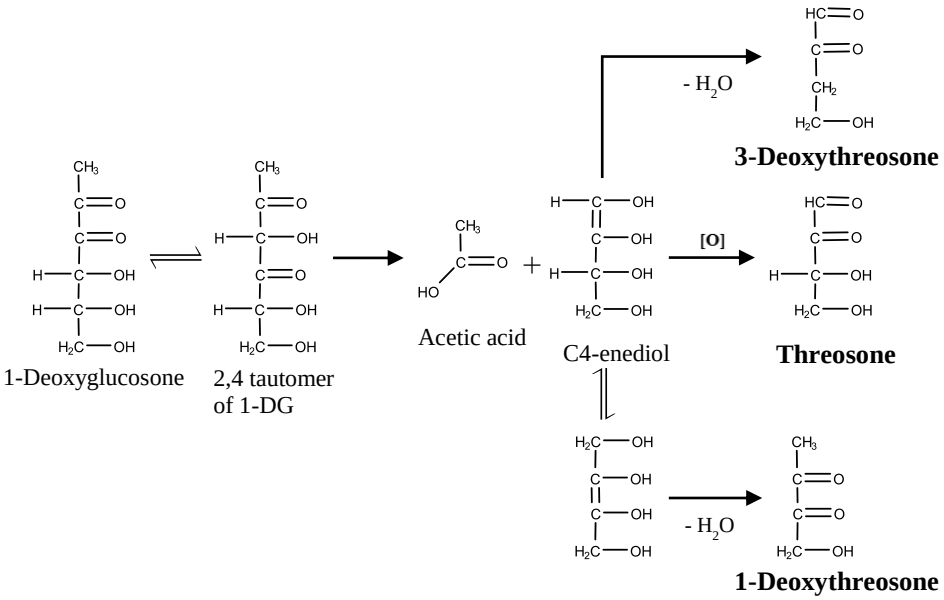


Figure 1.9b. Formation of threosone, 3-deoxythreosone and 1-deoxythreosone through degradation of 1-deoxyglucosone, adapted from [98].

Diacetyl (DA: 2,3-butanedione): Following the formation of 1-deoxyglucosone through 2,3-enolization and water elimination reactions especially in alkaline media during caramelization or Maillard reaction, 1-deoxyglucosone act as a main precursor of diacetyl [92]. In this pathway, 1-deoxyglucosone undergoes a rearrangement to form diacetylformoin which is later reduced (**Figure 1.10**) [91]. Then, water elimination gives 1,4-dideoxyglucosone which generates diacetyl by *retro*-aldol scission [92]. It has been reported that diacetyl mainly formed from sugars in acidic or basic media rather than in neutral conditions [110]. In addition, considering

the formation of 1-deoxyglucosone above pH 7.0, it can be said that diacetyl formation strongly depends on pH [90].

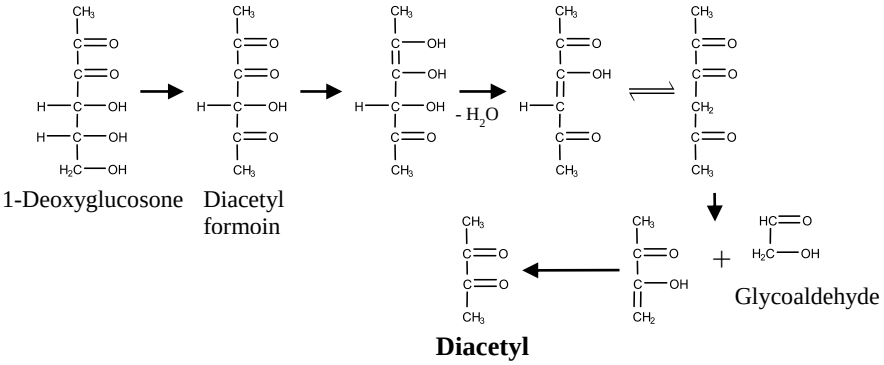


Figure 1.10. Formation of diacetyl via degradation of 1-deoxyglucosone, adapted from [90, 111].

Methylglyoxal (MGO: 2-oxopropanal): Both 1-DG and 3-DG formed during caramelization and/or Maillard reaction are the precursors of MGO (**Figure 1.11**) [90, 112, 113]. Thus, Hollnagel and Kroh [90] proposed that MGO is formed from 1-DG by the cleavage of C3-C4 bond. Weenen [112] suggested and Yaylayan and Keyhani [113] confirmed that MGO is generated also from 3-DG via the same C3-C4 bond cleavage pathway. Additionally, Thornalley, et al. [114] indicated that MGO can also be formed from glyceraldehyde via 2-ene-2,3-diols scission. Besides, it has been stated that there are various pathways of MGO formation from intact dicarbonyl compounds by the cleavage of C1-C3 (32%), C4-C6 (47%), C2-C5 (21%) proved by ^{13}C-labeled glucose incubation experiment [3]. Therefore, MGO formation strongly depends on the factors such as temperature and presence of amino compounds [3]. Temperature has quite significant effect on the formation of MGO that the temperature increase from 100°C to 120°C more than doubled the MGO level [115]. On the other hand, pH and presence of oxygen have no influence on MGO formation since MGO was formed in equal yields under both aerated and deareated conditions [3, 116].

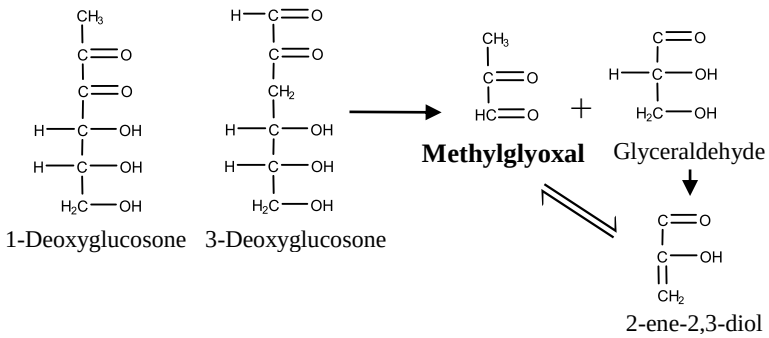


Figure 1.11. Formation of methylglyoxal through degradation of 1-/3-deoxyglucosone, adapted from [90, 111, 112].

Glyoxal (GO: oxaldehyde): As the simplest α-dicarbonyl compound, GO is formed via both oxidative pathway and carbon skeleton cleavage pathway [3]. Therefore, glucosone, 1-deoxyglucosone and 3-deoxyglucosone have been suggested as the precursors of GO [90, 113, 117]. In addition, it has been indicated that GO is also formed from Schiff bases during Maillard reaction through Namiki pathway [83, 118]. Hofmann, et al. [117] proposed a mechanism based on the cleavage of C2-C3 bond of glucosone resulting in the formation of GO as given in **Figure 1.12a**. Besides, a removal of two water molecules from C3-C4 and C5-C6 of aldohexose and retro-aldol cleavage between C2-C3 has been suggested as GO yield [111]. Similarly, 1-deoxyglucosone (**Figure 1.12b**) and 3-deoxyglucosone (**Figure 1.12c**) undergoes C4-C5 retro-aldolization that results in the formation of GO and also diacetyl [91]. As mentioned, Hayashi and Namki [83] proposed that formation of glycoaldehyde *N*-alkylimine from Schiff bases in the early stage of Maillard reaction plays an important role on the formation of GO (**Figure 1.12d**). In this pathway, *N*-glycosylamines (Schiff bases) undergoes a retro-aldol fragmentation that lead to the generation of glycoaldehyde *N*-alkylimine and erythrose [83]. This highly reactive intermediate oxidizes to form glyoxal alkylimine which hydrolyzes with the elimination of amino compound to form GO whereas the hydrolysis of glycoaldehyde *N*-alkylimine yields glycolaldehyde [83, 118]. In addition to that, an isotope labelled study showed that glyoxal was generated from C1-C2 (49%), C5-C6 (31%), and the 20% was attributed to the C2-C5 region which is likely to the fragmentation of C4 and C5 pieces of glucose [3].

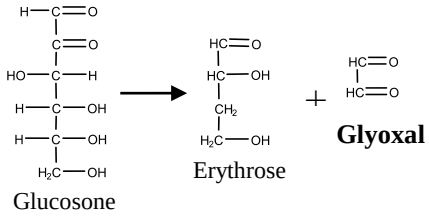


Figure 1.12a. Formation of glyoxal through degradation of glucosone, adapted from [91, 117].

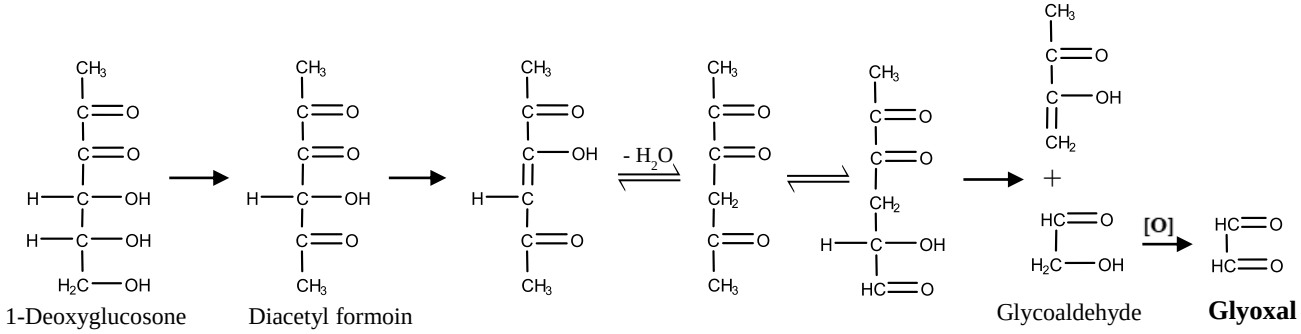


Figure 1.12b. Formation of glyoxal through degradation of 1-deoxyglucosone, adapted from [90, 91, 111, 119].

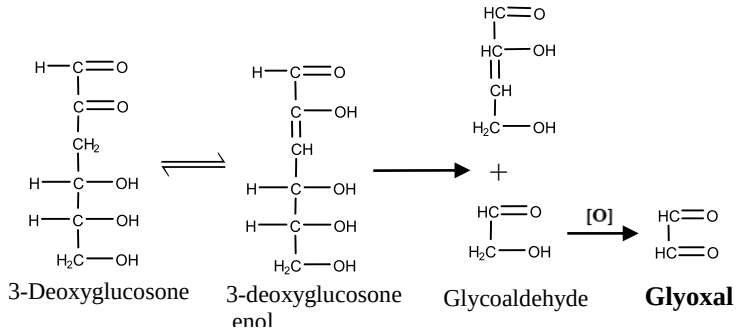


Figure 1.12c. Formation of glyoxal through degradation of 3-deoxyglucosone, adapted from [91, 111].

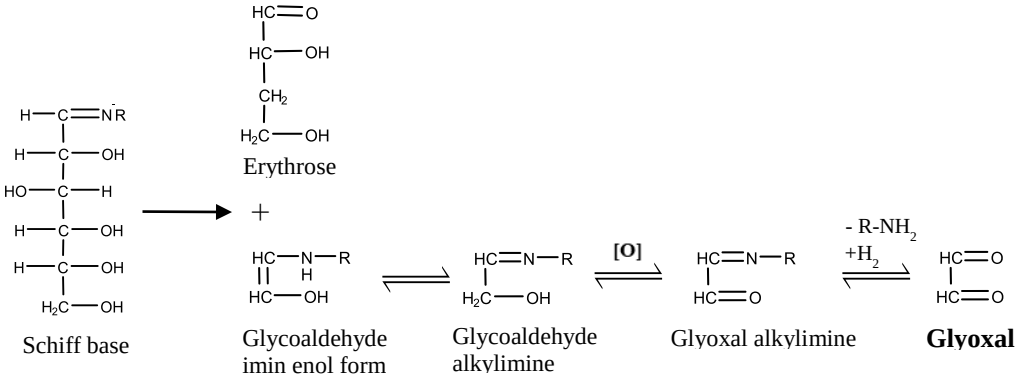


Figure 1.12d. Formation of glyoxal through Namiki pathway, adapted from [83, 118, 120].

1.2.2.4.Toxicity and Exposure of α-Dicarbonyl Compounds

α-Dicarbonyl compounds can be formed *in vivo* by mainly glucose degradation and minor ketone metabolism, threonine catabolism, degradation of glycated proteins or monosaccharides in human body [95]. Although detoxifying systems such as glyoxalase system in the human body can metabolize α-dicarbonyl compounds , the imbalance between the formation and elimination of α-dicarbonyl compounds and also the exposure to exogenous α-dicarbonyl compounds results in the accumulation of those which causes dicarbonyl stress in the body. Dicarbonyl stress is defined as the abnormal accumulation of α-dicarbonyl compounds leading to cell and tissue dysfunction in ageing and disease by protein and DNA modifications [121]. The mechanisms of dicarbonyl toxicity have been suggested mainly by three primary ways: (i) a direct inhibitory effect of α-dicarbonyl compounds on enzymes through the formation of advanced glycation endproducts (AGEs) (ii) the indirect depletion of glutathione and increase in reactive oxygen species (ROS) (iii) the formation of DNA adducts which are genotoxic [122]. Indeed, the formation of AGEs which is involved in various diseases such as the diabetic complications [123], nondiabetic nephropathy [124], cardiovascular diseases [125], Alzheimer's disease [126], cataract [127], the progression of aging and tumor-promoting process [128]. The mechanisms behind the AGEs damage tissues and trigger inflammation can

be explained by irreversible linking to proteins, in particular long-lived extracellular matrix proteins [60, 129], playing a role in signal transduction cascades in the cells as a component [130], activating the receptor for AGEs (RAGE) in the body since AGEs and also α-dicarbonyl compounds are characterized as pro-inflammatory and prooxidant mediators [128]. In addition, formation of AGEs can also cause the loss of nutritional value of proteins. The formation of AGEs occurs through the electrophilic attack of α-dicarbonyl compounds to the nucleophilic sites (thiol, guanidinium, and amino groups) of protein, peptides or amino acids [129]. The most quantitatively and functionally important AGEs in physiological systems has been reported as hydroimidazolone adducts of 3-deoxyglucosone (3DG-H), methylglyoxal (MG-H1) and glyoxal (G-H1) as a result of the reaction with arginine residues [131]. Besides, N_{ε}-carboxymethyl-lysine (CML), N_{ε}-carboxyethyl-lysine (CEL), and pyrraline formed from glyoxal, methylglyoxal and 3-deoxyglucosone with the reaction of lysine residue, respectively, also contribute to the protein modification in the body [121].

In the terms of potential toxicities and prevalence in foods of α-dicarbonyl compounds , the most significant ones have been reported as 3-deoxyglucosone (3-DG), glucosone, 3,4-dideoxyglucosone-3-ene (3,4-DGE) and 1-deoxyglucosone (1-DG), MGO, GO and DA [99]. But in particular, MGO has been stated as a dominant mediator of dicarbonyl stress in vivo due to its higher reactivity [121]. The concentrations of 3-DG, MGO and GO have been found in the range between 1-4 μM in mammalian cells and 50 -150 nM in human plasma under normal conditions [131]. Degen, et al. [132] indicated that only 10-15% of the dietary 3-DG excreted in urine as its metabolite 3-deoxyfructose while the fate of remaining (85-90%) is unknown. It has been reported that approximately 90% of 3-DG is metabolized enzymatically [133]. Although the plasma levels of 3-DG has been controversial, it was clearly demonstrated that 3-DG at 100 μM induces oxidative stress and apoptosis in leukemia cells [134]. Glucosone has been reported as toxic for mice, rats, rabbits, guinea-pigs and cats at toxic doses ranging in 1 to 2 mg per gram body weight [135] In another study, glucosone was found to be cytotoxic on hamster lung cells in the presence of cupric ion (Cu^{2+}) [136] in addition to its the mutagenic effect [137]. On the other hand, 3,4-DGE has shown the strongest cytotoxic effects since only 11 μM of 3,4-DGE caused an almost complete loss of cell viability [138]. 1-DG has been found to easily generate 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-Pyran-4-One (DDMP) which generated active oxygen species to cause DNA strand breaking and mutagenesis in a dose- and time-dependent manner [139, 140]. As the most reactive dicarbonyl *in vivo*, MGO, is formed about 3 mmol per day in normal physiological conditions and 99.7% of it is metabolized by

glyoxalase system [133]. However, plasma concentration of MGO may increase up to 6 fold in diabetic patients [141]. It is reported that MGO has been found to be toxic to human neuroblastoma cells above the concentration of 0.15 mM with a LD50 of approximately 1.25 mM [142]. The accumulation of MGO and GO in cells can lead to the formation of hydrogen peroxide which lead to oxidative stress and tissue damage [143]. Glyoxal has directly genotoxic activity both *in vitro* in bacterial and mammalian cells and *in vivo* in rats [144]. Following the first report about the mutagenicity of DA in 1979 [145], several reports have been revealed that DA causes the mutation in cell gene and loss of chromosome [92]. In addition, the respiratory toxicity of DA has been found in mice [146]. Furthermore, bronchiolitis obliterans occurring among workers exposed to DA vapor has been linked to the respiratory toxicity of DA [92]. For example, in popcorn subclinical changes in lung function, airway obstruction and bronchiolitis obliterans have been found in workers exposed to DA [147]. It should be interestingly noted here that GO, MGO and DA has not been expected to show local cytotoxic effects since they have transient effects on cell viability and they can be measurable at very high doses such as above 200 μM in different intestinal cell lines [148, 149]. Such concentrations may not be reachable with diet.

As mentioned, the exposure to the exogenous α-dicarbonyl compounds is also considered as risk factors for healthy subjects since the increase in the concentration of α-dicarbonyl compounds correlates to the amount of AGEs accumulated in the body. The main source of exogenous α-dicarbonyl compounds has been reported as food and beverages [99]. There are also other exogenous sources for the total exposure of α-dicarbonyl compounds such as peritoneal dialysis fluids [150], cigarette smoke [151], and drinking water [152] but in minor contribution. In general, dietary intake of α-dicarbonyl compounds is calculated by multiplying the mean daily consumption for each food with the corresponding mean or median occurrence level. Thus, it is clearly seen that the estimation of average intake of α-dicarbonyl compounds differs depending the dietary habits of consumers in different countries. However, it can be possible to make a rough estimate of the daily intake from common diets and the recommendations by the authorities such as WHO, USDA, AAP. Therefore, the calculated daily intake of most reported α-dicarbonyl compounds in various food from the literature according to the different dietary habits or healthy diet recommendations is given in **Table 1.2.** 3-DG contributes to the highest daily exposure to α-dicarbonyl compounds among others and dried fruits, honey, balsamic vinegar and infant UHT milk cause a daily intake of more than 10 mg of 3-DG. Despite the low level of daily intake of MGO when compare to 3-DG, the ingestion

of MGO can pose a risk due to the almost 200-fold higher reactivity than 3-DG [153]. Similarly, 3,4-DGE cause an almost complete loss of cell viability in only amount of 11 μM as mentioned [138] while 3-DG needs to be 100 μM to induce apoptosis [134]. The daily consumption of beer can lead to expose to 0.7 mg of 3,4-DGE and this can cause the reach to the mentioned toxic dose of 3,4-DGE [102]. According to hypothetical diets calculated by Degen, et al. [9], the intake of 3-DG and MGO was ranging in 20 - 160 mg/day and 5 – 20 mg/day, respectively. The authors suggested that a diet based on mainly fresh fruits, vegetables, and milk products provides a minimum intake of these α-dicarbonyl compounds , whereas a diet rich in high sugar content foods such as fruit juices, sugar beet syrup, etc can cause the maximum intake [9]. Similarly, Hellwig, et al. [99] estimated daily exposure to dominant α-dicarbonyl compounds , based on a model diet including bread, honey, jam, cheese, coffee, beer, fruit juice, cookies, cooked pasta. Accordingly, the exposure to 3-DG, 3-DGal and MGO were calculated as 61.2, 8.9 and 1.9 mg/day, respectively for a 70 kg body weight adult [99]. For DA exposure, Clark and Winter [154] indicated that a 70-kg consumer of all the foods containing DA at the maximum reported levels would be exposed to about 1.1 mg DA/day. On the other hand, no-observed-adverse-effect-level (NOAEL) of 127 mg GO/kg bw/day is stated for rats [144] and 90 mg DA/kg bw/day for rats [155] due to 90-days oral study.

It is still under debate whether the exposure to exogenous dietary α-dicarbonyl compounds contribute to the endogenous α-dicarbonyl compounds pool which are related to the mentioned diseases. Recent studies indicate that short-chain α-dicarbonyl compounds may not be absorbed in the gastrointestinal tract since they are scavenged during digestion [99]. It has been reported that pancreatic digestive enzymes cause the decrease in the concentrations of GO, MGO and DA without the explanation of the fate of the reaction products [99]. A 3 day manuka honey diet (containing 500 μmol MGO) with four healthy volunteers study by Degen, et al. [156] revealed that no effect of dietary MGO on the level of MGO *in vivo* has been found in 24 h urine. On the contrary, an increase in the plasma concentrations of GO, MGO and d-lactate has been reported after a glucose load in individuals, however, it is not clear whether the postprandial degradation reactions of glucose contribute *in vivo* [157]. A study with mice ingesting high concentrations of either glucose or fructose via the drinking water indicated that an increase in MGO-derived AGEs in the liver tissue was observed in glucose-based diet as well as increase in GO-derived AGEs from fructose-based diet [158]. It should be here bear in mind that a fast conversion of potentially absorbed α-dicarbonyl compounds to AGEs or other compounds make difficult to detect them in urine or plasma. Concerning the intact α-dicarbonyl

compound, 3-DG, about 17-fold and 20-fold increase in urinary excretion of 3-DG and its metabolite 3-deoxyfructose (3-DF), respectively, has been observed when subjects received a diet containing 505 μmol 3-DG [132]. In support, the urinary 3-DG and 3-DF excretion decreased by 60% and 57%, respectively, during the raw food diet avoiding the ingestion of 3-DG and other Maillard reaction products [132]. Although it is still unclear for the fate of exogenous α-dicarbonyl compounds during digestion, high exposure to the dietary α-dicarbonyl compounds can pose a risk for the accumulation of α-dicarbonyl compounds which may be absorbed into systemic circulation and led to the formation of AGEs.

Table 1.2. Calculated daily intake levels for selected α-dicarbonyl compounds in foods, data collected from references [9, 10, 159, 160].

Food Groups	Daily food intake	3-DG (mg)	3-Dgal (mg)	G* (mg)	1-DG (mg)	DA (mg)	MGO (mg)	GO (mg)	HMF (mg)	Ref.
Bakery, Pasta, Potato Products										
Bread	120 g	5.4	0.6				0.4		0.7	[9]
Bread and breakfast cereals	147 g	2					0.8	0.9		[10]
Cookie	50 g	6.5	0.7				0.4		0.1	[9]
Rice	119 g	0.13					0.11	0.07		[10]
Pasta (cooked)	250 g	0.3	nd				nd		nd	[9]
Potatoes (cooked)	250 g	1.7	nd				nd		nd	[9]
Dairy Products										
UHT milk	250 ml	tr		tr	tr	tr	tr	tr	tr	[160]
LH* UHT milk	250 ml	2.1	2.9	0.4	0.2	tr	tr	0.2	tr	[160]
LH Protein fortified milk	250 ml	4.3	4.4	0.7	0.1	tr	0.1	0.6	0.4	[160]
Infant UHT milk	750 ml	11.2	0.8	3.6	0.1	0.1	0.5	1.1	0.1	[160]
Dairy products	381 g	0.24					0.09	0.05		[10]
Meat & Fish Products										
Meat and fish	114 g	0.12					0.26	0.12		[10]
Fat & Oil Products										
Fats and oils	41 g	0.46					0.07	0.16		[10]
Fruit and Nut Products										
Dried fruits	55 g	27.5		14.3	0.3	0.1	1	0.3	2.6	[159]
Fruit puree	150 ml	0.8		1.3			0.1	0.2	0.5	[159]
Snacks and nuts	27 g	0.14					0.07	0.08		[10]
Sweets, Sauces and Others										
Sweets and chocolate	75 g	2.2					0.56	0.52		[10]
Candies	40 g	9.7	0.3				nd		0.3	[9]
Balsamic vinegar	30 ml	10.2	0.4				0.3		3.7	[9]
Soy sauce	30 ml	2.5	0.5				0.2		nd	[9]
Honey	20 g	13	0.7				nd		0.1	[9]
Jam	20 g	3.3	0.2				0.1		0.3	[9]

Table 1.2 continue.

Drinks							
Fruit juices	300 ml	8.1	0.4	nd		0.3	[9]
Soft drinks	300 ml	0.5	nd	nd		0.1	[9]
Coffee	393 g	0.14		0.87	0.11		[10]
Tea	314 g	0.03		0.01	0.01		[10]
Malt beer	500 ml	15	5.7	0.3		2.8	[9]
Wine	200 ml	1.4	nd	nd		nd	[9]
Alcoholic drinks	137 g	1.7		0.8	0.9		[10]

*G, glucosone; LH, lactose hydrolyzed; nd, not detectable; tr, trace
Data are based on the median level of the values.

1.2.2.5.Occurrence of α-Dicarbonyl Compounds in Foods

The concentrations of mostly reported α-dicarbonyl compounds in categorized food groups are summarized in **Table 1.3.** It is clearly seen that 3-deoxyglucosone is generally the dominant α-dicarbonyl compound among others. It is possible to explain that 3-DG is kinetically stable that can accumulate in foods during heating or storage when compare to others [60]. The highest level of 3-DG is given as 2990 mg/kg in dried raisins [10], followed by 2622 mg/L in balsamic vinegar and 1641 mg/kg in honey [9]. On the other hand, MGO as the most reactive dicarbonyl *in vivo*, is only of minor quantitative importance in foods reported, except for Manuka honey (736 mg/kg) [10] and coffee beans (215 mg/kg) [13]. The concentrations of α-dicarbonyl compounds vary a lot depend upon the food types. Foods including high sugar content such as dried fruits, honey, sugar syrups, fruit juices, candies, sweet bakery products contain high amounts of α-dicarbonyl compounds depending the process and storage conditions (**Table 1.3**). For example, sweet wines were found to contain higher levels of 3-DG than dry white wines [161] as well as the higher concentrations of α-dicarbonyl compounds in malt beer than the other types of beer [102]. In support, Lo, et al. [162] stated that the replacement of sugar by sweeteners resulted in a significant decrease in the concentrations of α-dicarbonyl compounds in carbonated soft drinks. On the other hand, Hellwig, et al. [99] indicated that the content of α-dicarbonyl compounds decrease or increase depends on whether soft drinks are sweetened with sucrose or high fructose corn syrup. In addition to sugar content of foods, the type of sugars exerts a major impact on the occurrence of α-dicarbonyl compounds in foods. Monosaccharides such as glucose, fructose are more susceptible to degradation due to their hemi-acetal structure when compare to sucrose which has a full acetal structure [60]. Indeed, this hypothesis was proved by several studies. For example, one of them found that 3-DG content was significantly higher in fruit juices which have glucose and fructose as predominant sugars than soft drinks in which sucrose premodinates as an exogenous sweetener [9]. Another proved that the amounts of 3-DG significantly increased when glucose and fructose syrups were used as additional

sweeteners in soft drinks [9, 162]. However, it was also found that 3-DG content in cookies was smaller amounts than in candies and jams although high amounts of sugar are present in cookies and intensive heat treatment is performed during baking of cookies [9]. The possible explanation is that Maillard reaction plays a role in the reactions of α-dicarbonyl compounds with amine groups to form advanced glycation products. Indeed, the concentrations of α-dicarbonyl compounds in protein-rich foods such as meat, dairy products were comparably lower than others as seen in **Table 1.3**, since α-dicarbonyl compounds can easily react with the side-chains of protein-bound lysine or arginine. Besides the role of sugar and amino groups, intensive heat treatment lead to the formation of high amounts of α-dicarbonyl compounds by reduction of water content. For example, significantly higher amounts of 3-DG were found in the crust where low moisture conditions occur than in the crumb during baking [9]. Other types of processing such as fermentation, ripening or long heat treatment cause the high accumulation of α-dicarbonyl compounds . The amount of 3-DG was significantly higher in vinegar and soy sauce than other type of sauces like pepper sauce, ketchup, or oyster sauce, probably because of the processes like fermentation, ripening during the production of vinegar and soy sauce [9]. In addition, the existence of the highest concentration of 3-DG in dried fruit and balsamic vinegar revealed that acidic and low moisture conditions have important effect on the formation of α-dicarbonyl compounds in foods. In conclusion, the main factors affecting the occurrence of α-dicarbonyl compounds in foods can be summarized as (i) the content and type of sugars, (ii) the presence of amino groups such as amino acids, peptides, (iii) the extent of heat treatment, (iv) pH and aw conditions, (v) the process type like fermentation, ripening and (vi) storage conditions.

Investigations on the occurrence of α-dicarbonyl compounds have been mainly focused on analyzing the commercially available foods. On the other hand, the effects of processing and storage conditions on the formation of α-dicarbonyl compounds are scarce especially in real food systems. Recently, it has been indicated that 3-DG was major dicarbonyl found in apple juice [29] and orange juice [30, 65] with an increasing trend during the storage of them. However, in this study, there has been no information about glucosone concentration which has been found dominant in model sugar solutions [163]. Similarly, Liu and Li [21] stated that during frying and prolonged storage such as 60 days, the increase was observed in the contents of GO and MGO in fried dough twist as well as in Tai fish sauce [164]. Additionally, Zhang, et al. [33] indicated that high temperature storage (40 °C) lead to the increase in the concentrations of 3-DG and 3-deoxygalactosone in UHT milk during 1 year of storage. On the other hand, Tas

and Gokmen [16] reported that the individual contents of α-dicarbonyl compounds either did not change or decreased during the 1 year storage of roasted hazelnuts. This controversy might strongly be related to the content of food such as the presence of sugar, the convenient moisture content and pH conditions. In the case of process effect, it has been generally reported that the increase in process temperature and time triggers the formation of α-dicarbonyl compounds in most foods such as cookies during baking [17, 19, 20], sesame seeds [165], hazelnuts [16] and coffee [13] during roasting. On the contrary, Taş and Gökmen [166] indicated that the concentrations of α-dicarbonyl compounds (3-DG, glucosone, GO and DA) substantially decreased during roasting of alkaline treated cocoa. This finding supports the hypothesis of alkaline conditions stimulate the formation of AGEs from α-dicarbonyl compounds. The leavening agents such as ammonium bicarbonate participates in degradation of sugars during the production of cookies results in the increase in GO, MGO and DA content [7]. Several chemical inhibitors such as sulfur dioxide, sulfites and thiol compounds have been used for the preservation of foods during processing or storage. The use of sulfur compounds lead to the decrease in pH through the formation of hydrogen ion that cause the increase in the sugar degradation [167] and also indirectly the accumulation of α-dicarbonyl compounds. On the other hand, sulfites also block the carbonyl group of the reducing sugar and interact with α-dicarbonyl compounds that results in the decrease in α-dicarbonyl compounds [168]. In support, Wedzicha and Garner [168] reported that 3,4-dideoxyhexosulose converted to form 3,4-dideoxy-4-sulphohexosulose in the presence of sulfites in model glucose-glycine system. Further studies must focus on the effect of processing agents and storage on the formation of α-dicarbonyl compounds in both real and model food systems to clarify this complexity in the literature.

Table 1.3. Occurence of α-dicarbonyl compounds in foods, mg/kg or mg/L, data collected from references [9, 10, 16, 17, 32, 92, 96, 102, 154, 160, 169-171].

Food Groups	3-DG	3-Dgal	Glucosone	1-DG	3,4-DGE	3-DP	DA	MGO	GO	Ref.
Cereal, Bakery, Pasta, Potato Products										
Baby foods (cereal-based)	3.9-827.1		nd-4.8	nd-50.6				0.4-17		[8]
Bread	5.1-619	nd-47						nd-28	1.5-11	[9, 10]
Breakfast cereals	0.3-71							0.7-9.9	0.7-9.7	[10]
Cookie	6-482	tr-88						1.8-81	2.1-31	[9, 10]
Pasta (cooked)	nd-8.8	nd						nd-0.92	nd-0.45	[9, 10]
Potatoes (french fried,cooked,fried)	nd-18	nd						nd-1.4	2.1-3.3	[9, 10]
Rice (boiled, white-brown)	0.2							0.2-0.4	0.1-0.3	[10]
Dairy Products										
Butter	0.10-1.6						0.5-4.0	nd-0.1	nd-0.1	[10, 154]
Cheese	nd-2.1						0.02-4.5	nd-0.96	nd-0.4	[10, 92, 96, 154]
Cream	4.6-16	5.3-19				nd	nd-0.08	nd	nd-1.0	[10, 92, 96]
Egg (boiled, fried)	0.4-0.7							0.1-0.6	0.2-0.3	[10]
Evaporated milk	2.0-2.2	0.9-1.0				1.2-1.5		nd	0.6-0.8	[96]
Milk, whole UHT	0.2-0.4	0.2-0.4	nd-0.03	0.1-0.8			0.01-0.1	nd-0.07	0.3-0.9	[10, 160]
Milk, semi-skimmed UHT	0.1-1.4					nd		nd-0.11	nd-3.2	[10, 172]
Milk, LH UHT	3.1-12.7	4-18	0.8-2.0	0.1-1.8			0.03-0.1	0.1-0.2	0.6-0.9	[160]
Milk, LH Protein fortified UHT	14-22	12-23	0.9-4.1	0.01-0.9			0.03-0.1	0.1-1.0	0.7-5.4	[160]
Milk, Protein fortified UHT	0.5-1.6	0.3-1.8	0.1-0.1	0.3-0.5			0.07-0.2	nd	0.6-0.8	[160]
Milk, Chocolate	3.6							1.2	2.4	[10]
Milk, Infant UHT	5-40	0.5-3	nd-6.7	nd-0.3			nd-0.3	nd-1.2	0.9-1.9	[160]
Whey drink	13	nd				nd		1.1	nd	[96]
Yogurt	0.3-11	nd-0.7				nd	200-3000	0.5-2.3	0.2-0.5	[9, 10, 92, 96]
Fat & Oil Products										
Margarine							0.3-2.3	1.79		[110]
Olive oil	0.05							0.14	0.03	[10]
Safflower oil							0.11	0.034	0.16	[110]

Table 1.3 continue.

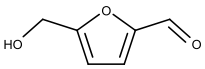
Food Groups	3-DG	3-Dgal	Glucosone	1-DG	3,4-DGE	3-DP	DA	MGO	GO	Ref.
Fruit, Nut and Vegetable Products										
Cashew nuts	2.8-4.4							1.7-2.6	2.6-5.6	[10]
Deep fried snacks	2.6-13							1.8-4.2	1.6-4.2	[10]
Dried fruits	158-2990		nd-15.6					2.5-6.6		[10, 172]
Fresh fruits	0.99-16							0.5-1.2	1.5-4.4	[10]
Fruit puree (apple,infant puree)	26.7-369		0.8-7.6	nd-0.7				0.4-21	37	[8, 10]
Hazelnuts	2.8-3.8		nd	nd-0.3		nd-1.44	nd-1.4	nd-1.9	nd-1.1	[16, 172]
Peanuts	1.5-2.9		nd-0.6					nd-3.5	nd-5.3	[10, 172]
Sesame seed	0.9-3.9			0.5-0.8				1.3-5.7	0.2-1.2	[165]
Vegetable-legumes	0.12-33							0.4-5.1	0.6-7.6	[10]
Meat & Fish Products										
Chicken products (pan-fried,roasted)	0.1-0.9							1.6-2.3	0.3-0.5	[10]
Fish products (pan-fried)	0.04-3.7							0.9-2.1	0.2-1.4	[10]
Meat products (beef, pork, hamburger,salami)	0.62-61							1.7-3.9	1.4-2.6	[10]
Sweets, Sauces and Others										
Candies	141-1011	nd-36						nd-5.2	nd-10	[9, 10]
Chocolate (dark, milk)	13-17							3.3-5.3	5.9-15	[10]
High fructose corn syrup	194-730	10-60	31-401	nd-26	3.5-14			1.4-11		[10, 17]
Honey	271-1641	14-46		2.5-5.3			0.7-2.4	nd-736	9.2-17	[9, 10, 32]
Jam, jellies, sweeteners	1.7-1061	nd-124						nd-13	nd-6.3	[9]
Popcorn							2-24			[154]
Soy sauce	32-832	12-71				37-1054		nd-12	nd-11	[9, 171]
Vinegar	0.1-2622	1.1-162					0.3-14	nd-53	nd-5.7	[9, 169]
Drinks										
Beer	9.0-136	nd-33			13-123		0.03-0.1	nd-1.4	0.2-0.3	[9, 10, 92, 102]
Coffee (drinks or bean)	nd-1419	nd					2.7-2.8	nd-215	nd-47	[9, 10, 13, 154]
Fruit juices	nd-410	nd-60						nd-2.2	1.7-3.2	[9, 10]
Soft drinks	nd-87	nd-7.7	nq-21	nd-2.8	nd-0.9			nd-0.98	nd-1.3	[9, 10, 170]
Tea (black, green)	0.1							0.02	0.03	[10]
Wine	2.2-95	nd-49					0.5-10	nd-4.5	0.1-0.3	[9, 10, 92]

1.2.3. 5-Hydroxymethylfurfural

1.2.3.1.Physical and Chemical Properties

5-Hydroxymethylfurfural (5-hydroxymethyl-2-furaldehyde, HMF) is an intermediate formed during Maillard reaction and also by dehydration of sugars under mild acidic conditions [4, 173]. HMF is widely used as an important quality deterioration marker as a result of heating and/or inadequate storage conditions in foods [173]. Selected physical and chemical properties are given in **Table 1.4**.

Table 1.4. The physical and chemical properties of 5-hydroxymethylfurfural (HMF) [173].

Name	Molecular Formula	Structure	Molar mass (g/mol)	Density (g /cm^3)	Boiling point(°C)	Melting point(°C)
HMF	$C_6H_6O_3$		126.11	1.206	350.97-354.09	32-34

1.2.3.2.Formation Mechanisms

It has been first reported in 1875 that HMF was formed as an intermediate during the reaction between levulinic acid from sugar and sulfuric acid [174]. In 1895, Düll [175] and Kiermayer [176] had been described the conversion of sucrose into HMF. Later on, Middendorp [177] declared the detailed synthesis, physical and chemical characteristics of HMF in 1919. Several years later, a great number of papers concerning the chemistry of HMF have been published. Among them, Haworth and Jones [178] obtained HMF from sucrose treated with oxalic acid in aqueous solution under various conditions. In conclusion, it has been indicated that decomposition of sugars result in the formation of HMF during caramelization, Maillard reaction and/or pyrolysis of hexoses or disaccharides [5, 179, 180]. The main reactions lead to the formation of HMF in foods are summarized in **Figure 1.13**. Thus, two main routes have been attributed to the generation of HMF that involves fructofuranosyl cation (FFC) from sucrose or fructose, and 3-deoxyglucosone from caramelization or Maillard reaction [5, 180]. In the first pathway, glycosidic bond of sucrose could easily cleave to produce glucose and FFC under dry heating conditions at elevated temperatures [5]. Formation of FFC has been lead to the quick conversion of FFC to HMF [5]. Similarly, Antal, et al. [180] has been published that FFC intermediate from hydrolysis of sucrose also produce HMF in high yields in aqueous medium at high temperatures. In the second pathway, formation of 3-deoxyglucosone either from 1,2-enediol during caramelization or from Amadori/Heyns product during Maillard reaction plays a key role on the formation of HMF [5, 179]. A removal of one molecule of water produce 3,4-dideoxyglucosone-3-ene (3,4-DGE) which presents both in *cis* (Z) or *trans* (E)

forms [2, 101]. The unsaturated *cis* form of 3,4-DGE subsequently rearrange to the structural favorably HMF [101, 181]. However, it has been shown that 3-DG route is not the favorite one in the formation of HMF, since HMF formation from sucrose and fructose has been found 4.5 and 2.5 fold more than from 3-DG [5]. In addition, fructose was found to be 31.2 times faster than glucose in the formation of HMF in sugar-catalyst model systems [182]. There are several factors affecting HMF formation such as temperature, type of sugar, pH, water activity, presence of catalysts [173]. Considering the water elimination, acid-catalysis and high temperature causing HMF formation, low-moisture medium, acidic conditions and high temperatures can be accepted as the main factors [173].

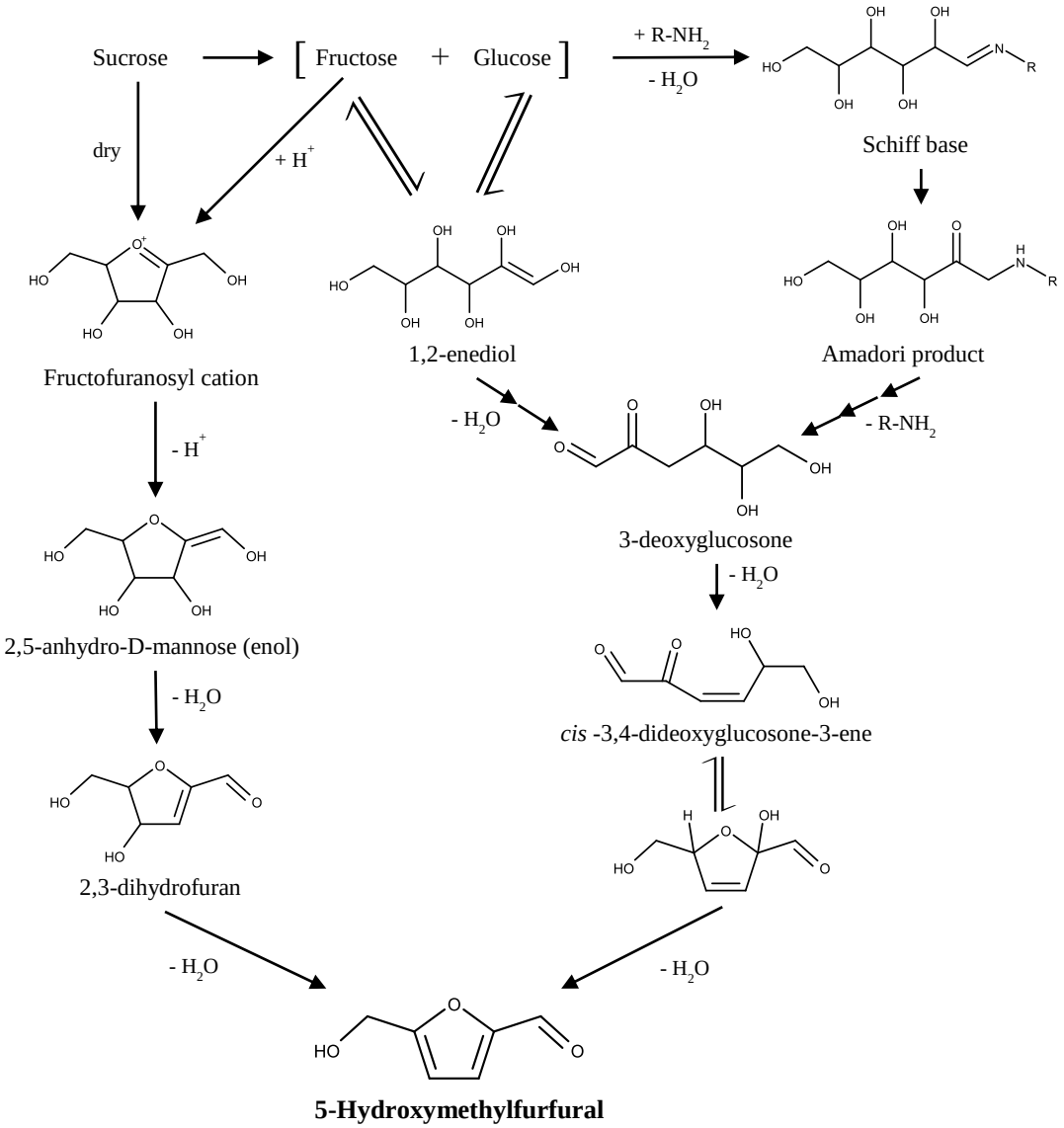


Figure 1.13. Formation of 5-hydroxymethylglyoxal through sucrose and 3-deoxyglucosone degradation during caramelization and Maillard reaction, adapted from [5, 173, 179, 180].

1.2.3.3.Toxicity and Exposure

HMF at high concentrations is known to be cytotoxic, causing irritation to eyes, upper respiratory tract, skin, and mucous membranes. However, it is not confirmed whether HMF exposure has genotoxic effect in humans *in vivo* and also in standard *in vitro* assays [183-185]. The main concern about toxicity of HMF is the metabolite of HMF, 5-sulphoxymethylfurfural (SMF) which was confirmed with genotoxicity and mutagenicity by *in vivo* and *in vitro* studies [186, 187]. The rapid conversion of HMF to SMF has gain concern with respect to genotoxicity despite the genotoxic and tumorigenic effects of SMF on human health remain unclear [173]. On the other hand, Delgado-Andrade, et al. [188] reported that absorption and transport of HMF in Caco-2 cell line becomes higher in the presence of higher HMF concentration in cells. HMF was also attributed to the generation of 5-hydroxymethyl-2-furoic acid (HMFA) which is the main metabolite of HMF in the body and eliminated renally [189]. In addition, HMF can also convert to 5-chloromethylfurfural (CMF) which is much more mutagenic than SMF and a strong hepatocarcinogen in infant male B6C3F1 mice [190]. In recent years, HMF was stated as a critical precursor of acrylamide which has been classified as a "probable human carcinogen" in Group 2A [191, 192]. Nevertheless, HMF has not been yet classified as human carcinogen by the International Agency for Research on Cancer due to the lack of enough animal studies and controversial reports on the mutagenic and genotoxic effects of it [193].

The daily exposure to HMF for humans has been reported in several studies. The estimates for daily HMF intake have ranged between 2.1 mg and 30 mg per person while it can reach to 350 mg per person with consuming the beverages e.g. dried plum juice [185, 194, 195]. Abraham, et al. [196] indicated that no adverse effect levels (NOAEL) are in the range of 80 – 100 mg/kg body weight and day based on acute and subacute toxicity in various animal experiments. Besides the potential genotoxicity of HMF, the daily intake of HMF is of concern since its dietary intake is several orders of magnitude higher than that calculated for other heat-induced food toxicants such as acrylamide and furan [197].

1.2.3.4.Occurrence in foods

Thermal processing (roasting, baking, frying, sterilization, etc) and acidic conditions with low moisture media lead to the excessive accumulation of HMF especially in sugar-rich foodstuffs. As given in **Table 1.5**, balsamic vinegar contains the highest HMF content as 35251 mg/L, followed by chicory coffee, biscuits and dried fruits [197]. In addition to process and food conditions, the contribution of pro-longed storage to the accumulation of HMF has been reported in several studies. For example, Selen Burdurlu and Karadeniz [57] indicated that HMF

concentration in apple juice concentrations increased from 0.52 mg/kg to 963 mg/kg during the storage depending the temperature. Similar to the formation of α-dicarbonyl compounds, mainly the type and concentration of sugars, pH, moisture content, processing and storage conditions have a huge impact on the formation of HMF.

HMF level is used as an indicator of thermal damage during thermal process or unsuitable storage conditions in various foods such as processed fruits, coffee, honey, and milk [197]. For instance, 40 mg/kg, 10 mg/L and 25 mg/kg for HMF in honey, fruit juices and fruit concentrates, respectively has been declared as the upper limits for heat damage [198, 199]. In addition, HMF is also used for monitoring the thermal treatment of cereal products such as pasta drying, bread baking, extrusion of baby cereals and breakfast cereals [197].

Table 1.5. Occurence of HMF in foods, mg/kg or mg/L, data collected from references [16, 197, 200-203].

Food Product	HMF content (mg/kg or mg/L)	Ref.
Cereal, Bakery, Pasta, Potato Products		
Baby food (cereal-based)	0-57.2	[197]
Bread	3.4-68.8	[197]
Breakfast cereals	6.9-240.5	[197]
Biscuits	3.9-3783.3	[201, 203]
Cookies	0.5-74.5	[197]
Potato chips	35.0-75.0	[202]
Dairy Products		
Baby food (milk-based)	0.18-0.25	[197]
Powdered infant milk	1.89-4.38	[200]
Fruit, Nut and Vegetable Products		
Dried fruits	25-2900	[197]
Roasted almond	9	[197]
Roasted hazelnut	0.9-8.5	[16]
Sweets, Sauces and Others		
Honey	10.4-58.8	[197]
Jam	5.5-37.7	[197]
Vinegar (balsamic)	316.4-35251.3	[197]
Drinks		
Beer	3.0-9.2	[197]
Coffee (roasted,instant,decaffeinated)	100-4100	[197]
Chicory coffee	200-22500	[197]
Fruit juices	2.0-22.0	[197]
Wine	1.0-1.3	[197]

1.3.MULTIRESPONSE KINETIC MODELING OF CHEMICAL REACTIONS IN FOODS

During processing or storage, monitoring the chemical, physical and microbiological changes provides controlling the quality of foods. For this, kinetic modelling gives an opportunity for the investigation of these changes on a time dependent manner. Kinetic modelling has been applied to food microbiology, thus predict how microorganisms behave in foods by using mathematical models [204]. For chemical changes in foods, for example color is used as a function of time and temperature. If the rate and temperature is known for a reaction, its formation can be predicted and also controlled via kinetic modelling.

To the general rate law, chemical reaction kinetics is described with the reaction rate depending the reactant concentrations and constant parameters. In a closed system, the rate of the decrease in the concentration of a compound is given as

$$\frac{\mathrm{d[A]}}{\mathrm{d}t} = -k[\mathrm{A}]^n$$

in which the concentration of component A decreases over time (*t)*, where *k* is the reaction rate constant and *n* is the reaction rate order which is usually ranging in 0 and 2 in foods. The order of a reaction is used for mathematical description of time- or concentration-dependence whereas it is not useful for understanding chemical reaction mechanism.

If a kinetic model describes the changes in the concentration of only one product with time, it is called a uniresponse kinetic model and it cannot give information about the whole mechanism. In the case of complex reactions such as Maillard reaction and caramelization, applying uniresponse kinetic model to such reactions is just a fitting procedure. There have been several studies in chemical reaction kinetics in which the rate of browning, the degradation rates of sugars and amino acids, or the vitamin C degradation rate is investigated with fixed ordered kinetics [55, 205, 206]. To understand the limitations of uniresponse kinetic modelling, van Boekel [207] gives an example in which a compound A degrades to compound B following a first-order reaction as given below:

$$\mathrm{A} \xrightarrow{k} \mathrm{B}$$

Since only the concentration of A can be measured in a uniresponse model, it cannot be ever possible to know whether A completely convert to B or not. However, in the multiresponse kinetic modelling of the same reaction, the conversion percentage can be calculated since the

concentration of B also measured. If the conversion is not 100%, other routes for further reactions of B or decomposition of A can be investigated to clarify the mechanism of reaction. In the case of complex reaction kinetics, changes in only one compound does not give insight in the reaction mechanism.

Multiresponse kinetic modelling approach includes analyzing and modeling more than one component simultaneously [208]. Martins, et al. [209]stated the essential steps to be followed for multiresponse kinetic modelling. Thus, the first step should be identification of the most important reactants and products, and then calculation the mass balance. The mass balance of reactants and products is calculated as the relative ratio of each compound and it gives the information about the percentage of conversion of reactants to products that will be helpful in model discrimination to reach the best model fit. Following steps will be defining the co-products of the same reaction pathway, differentiating between primary and secondary routes, identifying the effect of critical process parameters such as pH, temperatures etc, and determining the influence of reactant concentrations [209]. The last steps include the proposition a model mechanism for the reaction network and testing the hypothesized mechanism. Following the proposition of model mechanism, every reaction step is translated to ordinary differential equations to determine the reaction rate constants and statistical parameters [208]. Hydrolysis of sucrose and sugar degradation can be used for illustration to understand the mathematical steps.

$$\text{Suc} \xrightarrow{k1} \text{Glu} + \text{Fru} \xrightarrow{k2} \text{P}$$

Thus, sucrose (Suc) is hydrolyzed to form glucose (Glu) and fructose (Fru) while glucose and fructose also degrades to products (P). In a closed system, the differential equations for sucrose, glucose, fructose and products are written as

$$\frac{\mathrm{d[Suc]}}{\mathrm{d}t} = -k1[\mathrm{Suc}]$$

$$\frac{\mathrm{d[Glu]}}{\mathrm{d}t} = k1[\mathrm{Suc}] - k2[\mathrm{Glu}]$$

$$\frac{\mathrm{d[Fru]}}{\mathrm{d}t} = k1[\mathrm{Suc}] - k2[\mathrm{Fru}]$$

$$\frac{\mathrm{d[P]}}{\mathrm{d}t} = k1([\mathrm{Glu}] + [\mathrm{Fru}])$$

Following the equations, the mathematical model is tested by fitting to experimental data and by checking the model parameters obtained. To criticize the kinetic models, the goodness of fits of the models to experimental data and the highest posterior density (HPD) intervals of the estimated parameters are used. If the model is not acceptable, the proposed model needs to be revised by including other possible reaction routes or excluding insignificant steps.

Although a few studies have been used multiresponse kinetic modelling approach in complex chemical reactions in real food systems, there have been several studies in many different model systems. For the Maillard reaction chemistry, it has been possible to develop the reaction mechanism models in heated monosaccharide-casein model systems [210], as well as in heated disaccharide-casein model systems [211]. Martins, et al. [115] and Martins and Van Boekel [212] have been proposed a kinetic model for the fate of the Amadori compound *N*-(1-deoxy-d-fructos-1-yl)glycine in aqueous model systems. As a result of these studies, the decrease in pH by 1.3 unit have the same effect with the increase in temperature of 20 °C on the degradation of the Amadori product. Another striking result in these studies has been reported that lower pH triggers the 1,2-enolization of Amadori compound while higher pH values encourage the 2,3-enolization [212]. Moreover, the reason of the lower amounts of 1-DG determined in food samples have been explained in the same study as the higher reactivity of 1-DG [212]. Another multiresponse kinetic modeling is studied by Martins and Van Boekel [213] in aqueous glucose/glycine model systems. Following this, the authors investigated the effect of pH and reactant initial concentrations on the proposed Maillard reaction mechanism model in the same aqueous glucose/glycine model systems [214]. The results from these studies suggested that the reversible reaction of Amadori product to its precursors, glucose and glycine, is not quantitatively important. In addition, 3-deoxyglucosone was found to be the important precursor of color formation and carbohydrate fragmentation and acetic acid as a stable end product was an important indicator of Maillard reaction at pH 6.8 [213]. Furthermore, the proposed model for the Maillard reaction in glucose/glycine systems was found to be robust for changes in initial reactant concentrations and pH [214]. Another kinetic model of formation of N^{ε}-carboxymethyl-lysine (CML) was proposed by Nguyen, et al. [215] in aqueous sugar-casein model systems. One of the interesting result from this study is the formation of CML from Amadori product rather than from directly reducing sugar. As mentioned, there have been numerous study on complex reactions in model systems, e.g. formation of pyrraline in lysine-glycine/glucose model systems [216], formation of acrylamide and HMF in different model systems [217-220], generation of acrylamide, beta-carboline heterocyclic amines and advanced

glycation end-products in aqueous Maillard reaction model system [221], furan and furfural generation in cake model systems [222]. In the case of α-dicarbonyl compounds formation and elimination, Kocadagli and Gokmen [6] investigated the formation of α-dicarbonyl compounds during Maillard reaction and caramelization in heated glucose/wheat flour system. The results from this study suggested that 1-deoxyglucosone was formed mainly from Amadori product while 3-deoxyglucosone was generated from both glucoses itself and Amadori product also. In addition, short-chain products, methylglyoxal and diacetyl was generated via 1-deoxyglucosone whereas glyoxal was formed from glucosone [6]. Besides, fructose was found to be the main precursor of HMF formation in this study [6]. Moreover, Kocadağlı and Gökmen [223] investigated the effect of sodium chloride on the formation of α-dicarbonyl compounds and HMF in glucose and glucose-sodium chloride mixture during heating under caramelization conditions by using multiresponse kinetic modelling approach. The authors indicated that the presence of NaCl led to the decrease in rate constants of 3-deoxyglucosone and 1-deoxyglucosone formations whereas the rate constants of the formation of HMF increased 4-fold in the presence of NaCl [223].

Although studying in model systems gives insight into the reactions, real food systems should be used for a deep understanding of the reactions under the effect of food matrices. In recent years, using multiresponse kinetic modeling approach in real foods has increasingly get attention, but still with few studies as mentioned before [11, 13, 203, 224-226]. Among these studies, mechanistic models have been proposed for the formation of acrylamide and HMF in sesame [224], in coffee [13], in biscuits [203, 227], and in french fries [226] during heat treatments. For the Maillard reaction and caramelization chemistry, multiresponse kinetic modeling approach is used for the formation of α-dicarbonyl compounds and glycation products in sesame seeds [11] and hazelnuts [225] during roasting. For the formation of α-dicarbonyl compounds in sesame seed, a low moisture and sucrose poor system, the most kinetically important steps were stated as formation of 3-deoxyglucosone from glucose itself, 1-deoxyglucosone generation from Amadori product rather than Heyns product and methylglyoxal and diacetyl formation from 1-deoxyglucosone [11]. The authors also indicated that dicarbonyl compounds could be formed in a water-limited medium under dry heating process [11]. In addition to these findings, Tas and Gokmen [225] indicated that HMF formation from fructofuranosyl cation rather than 3-deoxyglucosone pathway, and glyoxal formation through glucose degradation were also important reaction steps while 3,4-dideoxyglucosone formation from 3-deoxyglucosone was a rate-determining step in the formation of HMF during

roasting of hazelnut. The temperature dependence of the reactions was also stated by the authors as more complicated than defined by the Arrhenius equation in a real food system [225]. Multiresponse kinetic modeling approach has been also used for vitamin C loss in mango [228], formation of aroma compounds in heated beef liver extract [229] and in milk [230], enzyme activity in hydrolysis of whey proteins [231], degradation of color compound (chlorophyll) in olives [232], and optimization of thermal process conditions in milk [233]. On the other hand, there has been no study on the multiresponse kinetic modeling of the Maillard reaction and/or caramelization during storage of a real food. Since these reactions occur simulteanously in foods during processing or storage, there is still a great need to investigate such complicated reactions in complex real foods in terms of quality and safety issues. Thus, multiresponse kinetic modeling can be powerful approach to unravel the sophisticated reaction mechanisms in both model systems and real foods.

CHAPTER 2

A SURVEY OF THE OCCURRENCE OF α-DICARBONYL COMPOUNDS AND 5-HYDROXYMETHYLFURFURAL IN FRUIT PRODUCTS

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2.1. INTRODUCTION

α-Dicarbonyl compounds and 5-hydroxymethylfurfural (HMF) are easily generated from sugars under acidic and low moisture conditions during processing or storage of foods as mentioned in detail in Chapter 1 [4, 50]. Since these compounds are the precursors of toxic compounds such as acrylamide and advanced glycation end-products (AGEs), their occurrence in foods is of importance in terms of quality deterioration and safety evaluation of foods. Thus, several studies reported the level of most abundant α-dicarbonyl compounds and HMF found in various foods have been published in the literature as given in **Table 1.4** and **1.5**. According to these studies, foods including high sugar content and acidic pH such as dried fruits, vinegar, honey, sugar syrups contain high amounts of α-dicarbonyl compounds and/or HMF depending the process and storage conditions. In the case of α-dicarbonyl compounds in foods, the studies have been mostly focused on the occurrence of certain α-dicarbonyl compounds such as 3-deoxyglucosone, methylglyoxal and glyoxal. However, other α-dicarbonyl compounds such as glucosone, 1-deoxyglucosone, 3-deoxypentosone, threosone, 3-deoxythreosone, 3,4-dideoxyglucosone-3-ene might be the major α-dicarbonyl compound depending the food type. For example, it has been reported that glucosone was the dominant one in model sucrose solutions heated at below 100 °C [163]. Only a little information about the level of α-dicarbonyl compounds in fruit products is available in the literature, although fruit products are highly suitable for the formation of α-dicarbonyl compounds due to their acidic and sugary nature. In addition, there has been no study in the literature on the calculation of the daily intake level of α-dicarbonyl compounds and HMF from fruit products despite their high potential adverse effects on human health.

At the beginning of this thesis study, there was a need to create a comprehensive database reporting the α-dicarbonyl compounds and HMF concentrations in the full scale of fruit products. So that, it was possible to make a reliable estimation of the dietary exposure to α-dicarbonyl compounds and determine the major α-dicarbonyl compounds in fruit products. For this purpose, a number of dried fruits, fruit juices, purees, and concentrates were analyzed in order to assess variations of the levels of α-dicarbonyl compounds and HMF in different categories of fruit products.

2.2. EXPERIMENTAL

2.2.1. Chemicals and consumables

Formic acid (98%) was purchased from JT Baker (Deventer, The Netherlands). HMF (98%)

was purchased from Acros (Geel, Belgium). 3-DG (75%), glucosone (≥98%), quinoxaline (99%), 2-methylquinoxaline (97%), 2,3-dimethylquinoxaline (97%), o-phenylenediamine (98%), diethylenetriaminepentaacetic acid (DETAPAC) (98%), methanol, and acetonitrile were purchased from Sigma-Aldrich (Steinheim, Germany). Disodium hydrogen phosphate anhydrous and sodium dihydrogen phosphate dihydrate were purchased from Merck (Darmstadt, Germany). The Carrez I and Carrez II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 ml of water, respectively. Ultra-pure water was used throughout the experiments (Milli Q-System, Millipore, Millford, MA). Syringe filters (nylon, 0.45 lm) and Oasis HLB cartridges (30 mg, 1mL) were supplied by Waters (Milford, MA). Atlantis dC18 column (250 × 4.6 mm, 5 μm), Acquity UPLC BEH C18 (100 × 2.1 mm, 1.7 μm) were supplied by Waters (Millford, MA).

2.2.2. Sample preparation

Dried fruits were obtained from different local markets in Turkey. Fruit juices, juice concentrates, purees, and puree concentrates were obtained as soon as they were produced from a local fruit processing company in Turkey. All samples were kept frozen at -18^{o}C prior to analysis.

Dried fruits, purees, and puree concentrates (2 g) were triple extracted with water (20-10-10 mL) by using firstly ultra-turrax homogenizing and then vortexing for 3 min. After centrifugation at 10,000 x g for 5 min, combined supernatants were used as aqueous extract for analysis. Fruit juice concentrates were only diluted with water prior to analysis. Aqueous extracts or dilutes of the samples were used for the determination of α-dicarbonyl compounds in the samples.

However, aqueous extracts and dilutes were cleaned up for HMF analysis. For Carrez clarification, 1 mL of extract was mixed with 50 μL of Carrez I and 50 μL of Carrez II solutions. The mixture was centrifuged at 10,000 x g for 5 min. The clear supernatant was used for the determination of HMF in the samples.

2.2.3. Analysis of α-dicarbonyl compounds

Derivatization. Derivatization of α-dicarbonyl compounds was carried out with o-phenylenediamine according to the published procedure [8]. Five hundred μL of supernatant was mixed with 150 μL of 0.2% o-phenylenediamine solution containing 11 mM diethylenetriaminepenta acetic acid and 150 μL of 0.5 M sodium phosphate buffer (pH 7). The

mixture was immediately filtered through a 0.45 μm syringe filter into an autosampler vial. It was kept at room temperature, at dark for 2 h prior to measurement.

UPLC-ESI-MS Measurement. α-Dicarbonyl compounds were determined by using a Waters TQD LC-MS/MS system according to the method described previously with minor modifications [8].The chromatographic separation was performed on an Acquity UPLC BEH C18 column using a gradient mixture of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile as the mobile phase at a flow rate of 0.4 mL/min at 60 °C. The gradient mixture was started from 5% B and increased to 25% B in 8 min, then it was increased to 60% B in 1 min and then it was decreased to 5% B in 1 min, then 5% B remained for 2 min. The chromatographic run was completed in 12 min. The injection volume was 10 μL. Waters TQD LC-MS/MS system was operated in positive ionization mode using the following interface parameters: source temperature of 120 °C, desolvation temperature of 370 °C, collision energy 12 V, desolvation gas flow of 900 L/h, capillary voltage of 3.50 kV, cone voltage of 20 V, and extractor voltage of 3 V. The SIM ions of the quinoxaline derivatives of α-dicarbonyl compounds were used for quantitation. Data acquisition was performed by monitoring m/z ratios for quinoxaline derivatives of glucosone: 251; 1- or 3-deoxyglucosone: 235.2; 3-DP: 205; DA: 159.2; threosone: 191; MGO: 145; and GO: 131. Dwell time was set at 97 ms for each. 5-methylquinoxaline was used as an internal standard.

Working solutions of glucosone and 3-DG were derivatized and then the concentrations of glucosone, 3-DG, quinoxaline, and 2-methylquinoxaline were calculated by means of external calibration curves built in the range between 0.1 and 5 mg/L (0.1, 0.5, 1, 2, 5 mg/L). Also, the calibration curve of glucosone was used for semi-quantitation of threosone derivatives and 3-DG calibration curve was used for semi-quantitation of 1-DG and 3-DP, since both have same proton-accepting groups. All working solutions were prepared in water.

2.2.4. Analysis of 5-Hydroxymethylfurfural

One mL of clear supernatant was filtered through a 0.45 μm syringe filter and put into an autosampler vial. The filtered sample was injected onto an Agilent 1200 series HPLC system consisting of a quaternary pump, an autosampler, a diode array detector, and a temperature-controlled column oven. The chromatographic separations were performed on an Atlantis dC18 column using a gradient mixture of (A) 10 mM formic acid in water and (B) acetonitrile as the mobile phase at a flow rate of 1 mL/min at 30 °C. The gradient mixture was started from 10% B and increased to 30% B in 10 min, 30% B remained for 2 min, then it was decreased to 10%

B in 2 min and then 10% B remained for 6 min. The chromatographic run was completed in 20 min. The injection volume was 10 μL. Data acquisition was performed by recording chromatograms at 285 nm. The concentration of HMF was calculated by means of a calibration curve built in the range between 0.1 and 20 mg/L (0.1, 1, 2, 5, 10, 20 mg/L).

2.2.5. Analysis of pH and Brix

The pH of the juice samples was measured using a PHM210 model pH meter (MeterLab, France) and the brix of the juice samples was measured using a Pocket Pal-3 model refractometer (Atago, Japan).

2.2.6. Statistical analysis

All analyses were run in duplicate and the data were subjected to analysis of variance (one-way ANOVA). The Statistical Package for the Social Sciences (SPSS 17.0) was used for the evaluation of statistical significance of the differences between mean values by Duncan test. $P<0.05$ was considered to be statistically significant for the results.

2.3. RESULTS AND DISCUSSION

2.3.1. Levels of α-dicarbonyl compounds and HMF in processed fruits

In this study, a number of fruit products were analyzed for the occurrence of α-dicarbonyl compounds and HMF. Dried fruits, fruits juices and purees, and their concentrates were selected as sugar rich and acidic products, but with low or high moisture contents. Significantly higher levels of α-dicarbonyl compounds were determined in dried fruits than in other fruit products. Statistical variations in the concentrations of α-dicarbonyl compounds and HMF in various dried fruits are given in **Table 2.1**. 3-DG was the dominant α- dicarbonyl compound in all dried fruits. The highest concentration of 3-DG was found as 4117.0 mg/kg in raisin. This level was significantly higher than the maximum concentration reported for 3-DG (2622 mg/L) in balsamic vinegar in the literature [9]. α-Dicarbonyl compounds are highly reactive intermediates which form from sugars during storage or thermal treatment of foods by caramelization or Maillard reaction [4, 234]. It is reported that the formation of α-dicarbonyl compounds is accelerated in acidic and low moisture conditions [235].

Table 2.1. Concentrations of α-dicarbonyl compounds and 5-hydroxymethylfurfural in dried fruit samples (mg/kg).

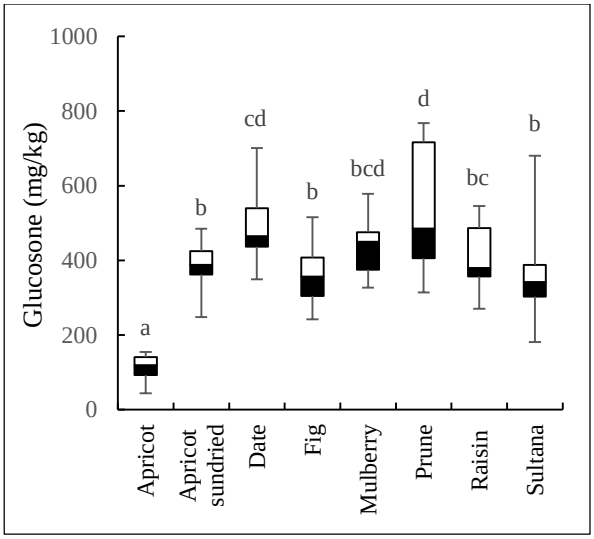
Dried fruits	n	pH		3-DG		Glucosone		1-DG		Threosone	
		Range	Median	Range	Median	Range	Median	Range	Median	Range	Median
Apple	3	4-4.58	**4.4**	89.6-248.6	**181.4**	48.6-134.7	**72.8**	0.8-1.2	**1.1**	8.4-12.7	**10.0**
Apricot	12	3.69-5.24	**4.0**	190.2-1740.3	**649.6**	43.5-154.1	**121.5**	1.5-7.3	**4.8**	4.6-21.7	**13.8**
Apricot-sundried	9	5.2-5.8	**5.6**	807.8-1831.8	**1077.5**	248.3-484.7	**388.2**	3.7-17.7	**7.7**	12.1-80.8	**41.8**
Blueberry	4	3.25-3.51	**3.3**	1476.5-1698.1	**1509.9**	243.9-300.7	**281.8**	4.3-8.6	**5.9**	7.4-36.7	**9.3**
Cape-gooseberry	2	3.89-3.95	**3.9**	1918.4-2033.0	**1975.7**	454.2-563.7	**509.0**	13.0-20.3	**16.6**	12.8-17.9	**15.4**
Cherry	1	3.64-3.73	**3.7**	1647.4-1647.4	**1647.4**	649.5-649.5	**649.5**	35.1-35.1	**35.1**	16.4-16.4	**16.4**
Coconut	2	3.85-4.21	**4.0**	192.5-780.2	**486.4**	18.5-373.4	**195.9**	0.6-5.6	**3.1**	2.8-17.7	**10.3**
Cranberry	5	2.96-3.14	**3.0**	547.3-888.9	**800.6**	333.4-432.4	**397.5**	3.7-5.1	**4.0**	24.4-30.8	**25.8**
Date	11	5.5-6.96	**6.2**	949.3-3185.8	**2286.3**	349.4-700.7	**464.4**	4.7-25.0	**19.1**	30.8-157.7	**40.0**
Fig	10	4.38-5.39	**5.1**	369.1-2053.5	**609.8**	242.0-516.0	**356.2**	2.1-15.2	**3.0**	4.6-120.2	**69.7**
Ginger	2	4.14-4.32	**4.2**	380.8-461.1	**421.0**	128.7-143.2	**136.0**	2.6-2.6	**2.6**	8.4-9.7	**9.0**
Kiwi	3	3.37-3.55	**3.5**	391.8-549.8	**499.4**	204.0-316.6	**222.5**	1.8-2.2	**2.0**	10.4-22.8	**13.5**
Kumquat	2	4.41-4.98	**4.7**	166.9-197.8	**182.3**	65.1-92.3	**78.7**	0.7-0.8	**0.7**	3.9-4.9	**4.4**
Mango	3	3.45-3.75	**3.5**	277.1-540.2	**317.2**	57.4-122.2	**117.6**	1.7-2.6	**2.3**	7.0-9.0	**8.2**
Melon	1	5.71-5.87	**5.8**	80.1-80.1	**80.1**	39.4-39.4	**39.4**	9.0-9.0	**9.0**	11.4-11.4	**11.4**
Mulberry	9	5.54-6.05	**5.9**	267.5-1810.4	**677.3**	326.8-577.9	**458.7**	7.1-31.5	**17.0**	55.1-281.9	**80.5**
Orange	2	4.03-4.2	**4.1**	258.0-364.3	**311.1**	134.4-214.2	**174.3**	7.9-15.9	**11.9**	18.6-20.7	**19.7**
Papaya	3	3.94-4.79	**4.6**	447.4-539.0	**499.7**	164.6-194.3	**170.5**	2.5-3.6	**2.7**	12.7-18.5	**17.0**
Peach	1	4.09-4.12	**4.1**	464.2-464.2	**464.2**	167.1-167.1	**167.1**	13.8-13.8	**13.8**	13.5-13.5	**13.5**
Pear	2	4.77-4.95	**4.8**	224.7-318.7	**271.7**	329.9-360.8	**345.4**	2.7-7.9	**5.3**	46.2-54.6	**50.4**
Persimmon	2	5.52-5.79	**5.7**	21.9-273.3	**147.6**	234.3-246.2	**240.2**	1.7-7.6	**4.6**	33.4-62.9	**48.1**
Pineapple	2	4.22-4.68	**4.5**	356.1-501.6	**428.8**	13.0-18.4	**15.7**	0.6-1.0	**0.8**	1.5-1.8	**1.7**
Prune	8	3.86-4.2	**4.1**	232.5-2541.4	**1866.4**	313.6-768.1	**484.9**	1.8-47.8	**14.0**	14.3-25.0	**19.9**
Plum	3	3.33-3.46	**3.4**	145.3-255.9	**188.3**	319.4-434.0	**321.9**	1.8-2.9	**2.3**	34.4-44.3	**35.9**
Pomelo	2	4.16-4.22	**4.2**	408.1-413.1	**410.6**	168.2-173.3	**170.7**	1.6-1.6	**1.6**	13.4-16.3	**14.8**
Quince	1	4.37-4.39	**4.4**	432.6-432.6	**432.6**	235.2-235.2	**235.2**	5.9-5.9	**5.9**	40.1-40.1	**40.1**
Raisin	15	3.85-4.68	**4.2**	554.2-4117.0	**1311.8**	270.6-545.9	**379.4**	2.6-37.5	**17.8**	4.0-88.5	**34.2**
Silverberry	4	3.61-4.88	**4.7**	515.1-662.4	**576.0**	311.2-481.5	**390.8**	4.4-19.5	**18.0**	18.0-69.8	**20.8**
Strawberry	3	3.61-3.94	**3.8**	351.9-903.4	**577.4**	124.6-315.2	**259.8**	4.0-20.5	**7.7**	24.3-49.0	**40.2**
Sultana	11	3.89-4.85	**4.2**	476.8-1940.9	**985.6**	180.9-680.7	**341.8**	2.6-30.4	**15.9**	6.8-80.2	**45.9**
Tomato	3	3.94-4.85	**4.5**	559.0-960.2	**657.3**	320.8-566.6	**357.6**	4.2-24.7	**7.1**	26.5-39.4	**35.6**

n: number of samples; nd: not detectable.

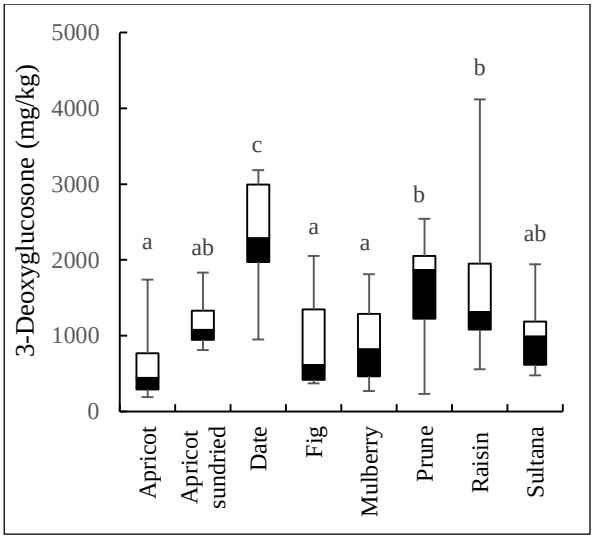
Table 2.1 continue**.**

Dried fruits	n	DA		MGO		GO		HMF	
		Range	Median	Range	Median	Range	Median	Range	Median
Apple	3	1.4-1.7	**1.5**	3.0-6.5	**3.1**	1.0-2.5	**1.8**	5.3-6.9	**6.6**
Apricot	12	2.6-8.8	**3.1**	22.6-254.1	**127.3**	2.4-7.9	**3.9**	4.8-119.0	**6.0**
Apricot-sundried	9	5.7-18.4	**13.8**	26.4-64.3	**40.3**	2.8-11.8	**3.4**	4.5-8.1	**6.0**
Blueberry	4	3.5-15.7	**3.6**	44.4-56.7	**53.5**	8.6-10.4	**9.3**	1820.5-2400.9	**2136.5**
Cape-gooseberry	2	3.4-6.9	**5.1**	46.4-52.0	**49.2**	20.4-31.3	**25.8**	225.7-289.6	**257.6**
Cherry	1	4.7-4.7	**4.7**	48.6-48.6	**48.6**	37.1-37.1	**37.1**	218.0	**218.0**
Coconut	2	1.6-3.0	**2.3**	7.9-28.8	**18.3**	1.3-9.1	**5.2**	21.4-25.6	**23.5**
Cranberry	5	1.7-2.1	**2.1**	8.8-24.5	**18.9**	8.1-11.1	**9.8**	233.5-542.3	**484.4**
Date	11	2.2-5.5	**3.4**	7.729.5	**15.1**	2.1-11.4	**2.8**	6.6-18.0	**9.4**
Fig	10	1.6-4.2	**2.8**	13.0-84.0	**30.1**	2.4-8.6	**6.2**	nd-7.4	**6.6**
Ginger	2	0.8-0.8	**0.8**	24.0-36.9	**30.4**	2.5-3.8	**3.2**	106.5-115.2	**110.9**
Kiwi	3	0.9-1.2	**1.0**	14.3-21.5	**19.8**	9.2-18.2	**9.9**	34.2-126.4	**87.8**
Kumquat	2	0.7-0.8	**0.8**	9.7-12.2	**11.0**	1.4-4.0	**2.7**	nd-26.8	**26.8**
Mango	3	0.9-1.2	**1.1**	19.0-24.3	**21.1**	2.1-6.5	**4.9**	117.4-207.7	**131.2**
Melon	1	1.4-1.4	**1.4**	9.8-9.8	**9.8**	0.2-0.2	**0.2**	nd	**nd**
Mulberry	9	4.6-13.9	**8.7**	12.0-31.7	**22.2**	3.8-5.9	**4.0**	nd-7.4	**7.3**
Orange	2	3.1-3.1	**3.1**	4.3-6.4	**5.3**	5.6-9.2	**7.4**	16.6-18.1	**17.3**
Papaya	3	1.1-1.3	**1.1**	38.6-65.2	**52.9**	3.2-6.3	**3.5**	53.6-112.3	**74.0**
Peach	1	3.2-3.2	**3.2**	6.9-6.9	**6.9**	11.2-11.2	**11.2**	nd	**nd**
Pear	2	1.6-1.7	**1.6**	12.9-23.0	**18.0**	7.4-11.4	**9.4**	nd	**nd**
Persimmon	2	2.3-6.2	**4.2**	1.4-1.8	**1.6**	0.9-2.0	**1.4**	nd	**nd**
Pineapple	2	1.4-1.6	**1.5**	6.1-17.6	**11.9**	0.2-0.9	**0.5**	9.1	**9.1**
Prune	8	0.5-4.8	**2.5**	5.9-46.2	**16.0**	3.6-48.7	**7.7**	10.8-1037.6	**158.6**
Plum	3	2.2-4.5	**2.4**	47.5-96.8	**95.5**	3.9-5.4	**4.8**	nd	**nd**
Pomelo	2	0.9-0.9	**0.9**	25.1-36.1	**30.6**	5.4-10.9	**8.1**	99.4-115.8	**107.6**
Quince	1	2.0-2.0	**2.0**	7.4-7.4	**7.4**	7.4-7.4	**7.4**	nd	**nd**
Raisin	15	2.8-15.3	**6.1**	4.7-19.3	**7.9**	1.6-10.5	**5.0**	9.9-146.3	**28.0**
Silverberry	4	0.9-5.3	**0.9**	2.8-45.6	**6.4**	4.1-23.5	**6.9**	nd	**nd**
Strawberry	3	0.7-4.9	**4.0**	3.9-24.4	**20.4**	4.5-25.2	**11.9**	22.9-73.6	**67.6**
Sultana	11	1.7-4.9	**3.3**	8.0-24.3	**17.7**	2.4-8.8	**4.6**	9.5-37.5	**18.6**
Tomato	3	1.6-8.4	**2.2**	17.1-89.4	**26.2**	4.3-15.8	**11.8**	8.5-103.0	**79.0**

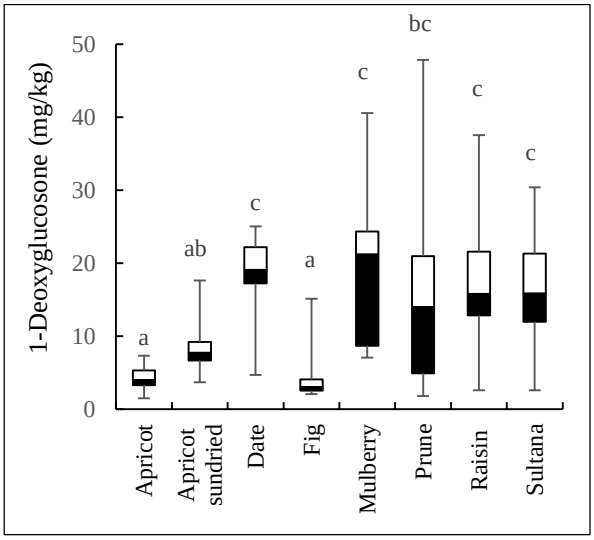
The box-and-whisker plots of dried fruits (n ≥ 8) summarizing the concentrations of α-dicarbonyl compounds (glucosone, 3-DG, 1-DG, DA, threosone, MGO, GO) and HMF are shown in **Figure 2.1**. The concentrations of 3-DG and glucosone were observed in a broad range in dried fruits, especially with the highest median level of 2286.3 mg/kg of 3-DG in dried date fruit and 509.0 mg/kg of glucosone in dried cape-gooseberry. Despite the lower levels compared to 3-DG and glucosone, 1-DG and the breakdown products (threosone, DA, MGO and GO) were observed in dried fruits. Threosone and MGO were the main breakdown products in a range between 1.5 and 281.9 mg/kg, 1.4 and 254.1 mg/kg in dried fruits, respectively.



(a)



(b)



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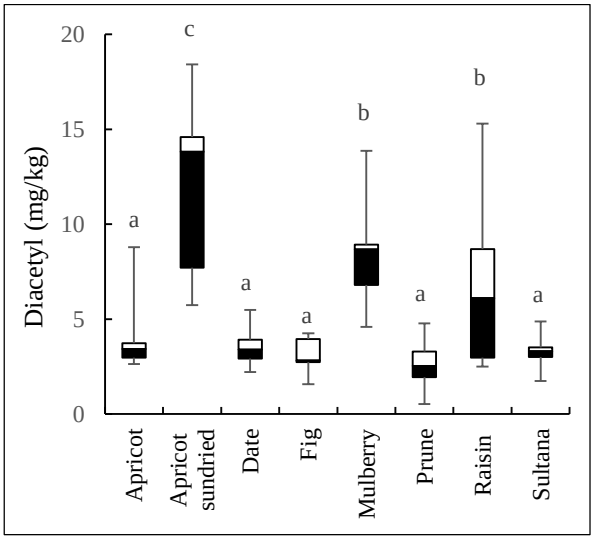

(d)

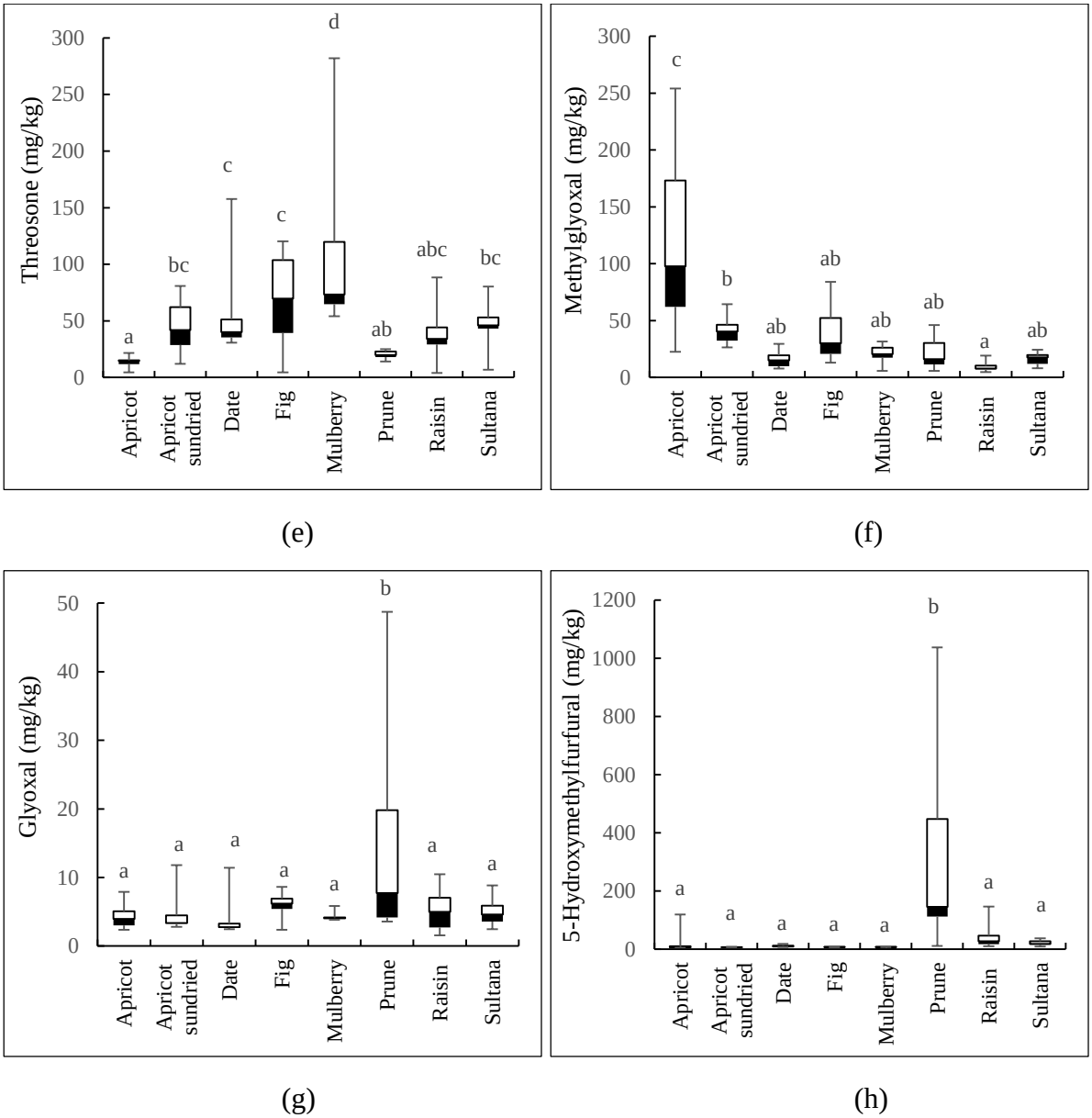

Figure 2.1. Box-and-whisker plots for α-dicarbonyl compounds and 5-hydroxymethylfurfural contents in dried fruit products marketed in Turkey. The center horizontal line of the box is the median of the data. The top and bottom of the box represent the 25th and 75th percentiles (quartiles), while the ends of the whiskers are the 10th and 90th percentiles, respectively. (a) Glucosone (b) 3-Deoxyglucosone (c) 1-Deoxyglucosone (d) Diacetyl (e) Threosone (f) Methylglyoxal (g) Glyoxal (h) 5-Hydroxymethylfurfural.

Fruit juices, as acidic and high-water activity systems, are suitable for the formation of α-dicarbonyl compounds due to sugar degradation and dehydration reactions during processing and/or storage. Different types of fruit juices (clear, cloudy, organic, fresh-squeezed, from concentrate, from puree) were collected from a local juice processing company immediately after processing. Statistical variations in the concentrations of α-dicarbonyl compounds and

HMF in various juices are given in **Table 2.2**. 3-DG and glucosone were the dominant α-dicarbonyl compounds in all types of juices. One unanticipated finding was that the highest level of 3-DG (37.0 mg/L) was determined in tart cherry puree (not from concentrate, organic). The maximum concentration of glucosone (25.7 mg/L) was found in beetroot juice (not from concentrate). The results showed that there was no correlation between the concentrations of α-dicarbonyl compounds and fruit type or content. This finding was in accordance with literature reports in which the concentrations of 3-DG in fruit juices and juices from concentrate showed no significant difference [9]. The levels of threosone, MGO and GO were much lower than those measured for 3-DG and glucosone, concordant with the literature [29, 30, 236, 237]. The concentrations of glucosone and 3-DP in juices were reported for the first time.

Only a little information about α-dicarbonyl compounds is available for fruit juice concentrates, although several studies on α-dicarbonyl compounds in model systems reported in the literature. It was indicated that three major α-dicarbonyl compounds (3-DG, glucosone and MGO) were determined in concentrated sucrose solutions (65%) as a thick juice model system [238]. The authors indicated that 3-DG which was the dominant α-dicarbonyl compound reached a concentration of 640 mg/kg. Concordantly, the main α-dicarbonyl compound found in juice concentrate was 3-DG ranging between 3.4 – 198.4 mg/L. Similar to fruit juices, glucosone was the second dominant α-dicarbonyl compound found in juice concentrates ranging between 0.6 – 69.5 mg/L **(Table 2.2)**.

The concentrations of α-dicarbonyl compounds found in fruit puree were lower than those found in other types of fruit products. A possible explanation for this might be the lower heat treatment during the processing of fruit purees. The dominant α-dicarbonyl compounds were also 3-DG and glucosone in purees and puree concentrates as seen in **Table 2.2**. The levels of 3-DG were ranging 4.7 – 20.8 mg/kg and 54.6 – 157.1 mg/kg in purees and puree concentrates, respectively. The breakdown products of α-dicarbonyl compounds were determined also in fruit puree and puree concentrates with the lower levels compared to the intact α-dicarbonyl compounds. Similarly, Kocadağlı and Gökmen [8] reported that 3-DG was the predominant α-dicarbonyl compound ranging between 26.7 – 92.3 mg/kg in fruit purees. In the meantime, the authors reported that MGO was not detectable in fruit purees contradict to our study. It seems possible that these results are due to the type of fruit purees, and differences in their processing and storage conditions.

Table 2.2. Concentrations of α-dicarbonyl compounds and 5-hydroxymethylfurfural in fruit juices, juice concentrates, purees (mg/L) and puree concentrates (mg/kg).

Juices, Purees and Concentrates	pH	Brix	3-DG	Glucosone	3-DP	Threosone	MGO	GO	HMF
Apple juice clear[a]	3.9	11.9	10.4±0.3	23.5 ± 0.1	0.4 ± 0	0.7 ± 0	1.1 ± 0	2.2±0	1.3±0
Apple juice cloudy	3.7	13.0	7.1±0	14.2 ± 0	0.5 ± 0	0.8 ± 0.1	1.4 ± 0	2.1±0	2.2±1.4
Apple juice cloudy[a]	3.5	15.0	nd	nd	nd	nd	nd	nd	nd
Beetroot juice[a]	4	10.1	13.6±0	25.7 ± 0	0.6 ± 0	0.7 ± 0	1.1 ± 0	5.7±0.2	3.6±0.1
Black grape juice[b]	3.2	15.7	10.5±0	7.2 ± 0	0.1 ± 0	0.4 ± 0	1.1 ± 0	1.9±0	6±0.1
Black mulberry juice[a]	3.8	15.5	nd	nd	nd	nd	nd	nd	15.9±0.5
Highbush cranberry juice cloudy	3.1	10.2	nd	nd	nd	nd	nd	nd	nd
Orange Juice	3.6	15.0	2.1±0	21.5 ± 0.2	0.6 ± 0	0.7 ± 0	2.1 ± 0	4.2±0.1	nd
Pomegranate juice cloudy	3.0	12.8	nd	nd	nd	nd	nd	nd	nd
Pomegranate puree juice[a]	3.1	15.2	14.8±0.2	12 ± 0.2	0.1 ± 0	0.4 ± 0	1.6 ± 0	2±0	7.7±0
Pur juice[b]	3.4	14.7	8.9±0	24.4 ± 0.1	0.4 ± 0	0.7 ± 0	1.4 ± 0	5.4±0.1	0.9±0.1
Purple carrot puree juice[ab]	3.8	7.2	1.5±0	4.5 ± 0	0.2 ± 0	0.6 ± 0	0.8 ± 0	2.4±0	nd
Tart cherry juice[b]	3.5	14.8	12±0	16.6 ± 0.1	0.4 ± 0	0.6 ± 0	2.4 ± 0	3.1±0.1	2.1±0
Tart cherry puree juice[ab]	3.5	15.1	37±0.4	12.8 ± 0	0.3 ± 0	0.3 ± 0	3.3 ± 0	1.4±0	19.9±1.4
Apple juice concentrate	3.4	66.6	58.4±0.3	35 ± 0	2.5 ± 0.1	1 ± 0	9.8 ± 0.1	9.5±0.1	6.9±0.1
Black carrot juice concentrate	3.9	62.8	3.4±0.1	0.6 ± 0	0.1 ± 0	0 ± 0	0.2 ± 0	0.3±0	nd
Black grape juice concentrate	3.8	60.8	102.8±2.7	69.5 ± 2.6	1.4 ± 0.1	1.4 ± 0	4.4 ± 0	5.6±0.3	15.2±0.1
Grapefruit juice concentrate	2.8	59.8	11.4±0	10.6 ± 0	0.7 ± 0	0.3 ± 0	7.9 ± 0.1	10.7±0.1	5.9±0.1
Lemon juice concentrate clear	1.7	51.7	15.7±0.3	19.1 ± 0.1	1 ± 0	0.8 ± 0	22.7 ± 0.3	11.8±0	7.7±0.1
Mandarin juice concentrate	3.1	63.2	15.4±0.1	37.7 ± 0.6	0.9 ± 0	0.7 ± 0	4.3 ± 0	7.7±0	6.3±0
Orange juice concentrate 1	3.2	65.4	13.8±0.2	27.1 ± 0.3	0.6 ± 0	0.4 ± 0	6.1 ± 0.1	9±0	1.2±0
Orange juice concentrate 2	3.1	63.7	15.4±0.1	33.4 ± 0.1	0.7 ± 0	0.6 ± 0	5.4 ± 0	8.1±0.1	2.3±0
Orange juice concentrate 3	3.1	65.1	16.8±0.1	25.1 ± 0.5	0.7 ± 0	0.4 ± 0	6.1 ± 0.1	9.7±0	2.7±0.2
Orange juice concentrate 4	3.2	65.3	14.6±0.8	27.4 ± 1.1	0.6 ± 0	0.3 ± 0	4.4 ± 0.1	5±0.6	3.1±0
Orange juice concentrate clear 1	3.8	62.0	33.3±0.2	15.7 ± 12.9	2.3 ± 0	0.9 ± 0	4.2 ± 0.1	2.9±2.9	3.7±0.1
Orange juice concentrate clear 2	3.8	63.4	34.3±0.6	26.9 ± 0.7	2.5 ± 0	0.9 ± 0	4.3 ± 0	5.7±0.1	4.7±0
Peach juice concentrate	3.4	63.2	51.4±0.8	25.4 ± 1.4	3 ± 0.1	1.8 ± 0	6.2 ± 0	10.7±0.1	1±0.1
Pear juice concentrate	3.9	68.0	26.4±0	51.8 ± 0.3	3.8 ± 0.1	3.6 ± 0.1	12.4 ± 0.3	9.2±0.1	0.7±0
Pineapple juice concentrate	3.3	65.2	176.2±0.3	1.5 ± 0	0.5 ± 0	0 ± 0	1.2 ± 0	1.2±0	512.1±2.7
Pomegranate juice concentrate 1	2.9	62.4	25.4±0.3	18.8 ± 0	2.3 ± 1.9	0.7 ± 0	6.3 ± 0	7.2±0.1	36.1±0
Pomegranate juice concentrate 2	2.9	62.6	46.5±0.3	19.6 ± 0.2	0.7 ± 0	0.6 ± 0	7.6 ± 0	7.8±0.1	43.1±0.6
Strawberry juice concentrate	3.6	62.1	33±0.3	20.5 ± 0.1	3.7 ± 0.1	0.5 ± 0	5.2 ± 0.1	5.1±0	8.3±0.1
White grape juice concentrate	4.2	63.3	198.4±0.6	27.3 ± 0.1	2.2 ± 0	0.7 ± 0	9.1 ± 0.1	3.7±0.1	194.6±3.3
Apple puree	3.9	13.5	7.3±6.3	6.1 ± 0	0.5 ± 0	0.1 ± 0	0.5 ± 0	0.9±0	1.2±0
Apricot puree	4.7	21.2	4.7±0.4	4.4 ± 0.4	0.2 ± 0	2.5 ± 0	0.8 ± 0	1.5±0	nd
Pear puree	3.9	13.3	20.8±0.6	11.7 ± 0.6	0.9 ± 0	0.9 ± 0	0.8 ± 0	2.2±0	4.8±0
Strawberry puree	3.6	8.7	5.1±0.3	8.3 ± 0.5	0.5 ± 0	0.2 ± 0	0.8 ± 0	1.4±0	nd
Tomato puree	4.2	7.4	5.2±0.1	9.5 ± 0.1	0.2 ± 0	0.2 ± 0	0.8 ± 0	1.4±0	nd
Apple puree concentrate	3.8	25.3	157.1±8	52.9 ± 6.7	5.6 ± 0.6	0.3 ± 0.1	1.9 ± 0.1	8.6±2.4	nd
Apricot puree concentrate	3.9	27.2	83.8±1.7	96.5 ± 4	4 ± 0.2	3.4 ± 0.2	9.6 ± 0.9	16.6±0.6	nd
Peach puree concentrate 1	3.9	31.4	54.6±0	23.5 ± 1.4	4.2 ± 0.2	0.2 ± 0.1	2.7 ± 0.5	10±0.3	nd
Peach puree concentrate 2	3.8	27.4	130.2±5.3	38.3 ± 4.4	7.2 ± 0.3	0.3 ± 0.1	3.2 ± 0.1	11.2±1.3	nd
Tomato puree concentrate	4.3	26.6	116.2±0.7	81.8 ± 4.5	4.6 ± 0.2	1.4 ± 0.2	6.2 ± 1	11.6±0.2	nd

[a] indicates the juice not from juice concentrate. [b] indicates the organic juice. nd: not detectable

HMF, which is a key intermediate of Maillard reaction and caramelization, can be formed by enolisation and dehydration of sugars [182]. It is reported that lower pH and lower moisture favors yielding HMF and 3-DG due to sugar dehydration [8]. The highest concentration of HMF was determined in dried fruit samples, reaching up to 2400.9 mg/kg in dried blueberry (**Table 2.1**). These results are consistent with previous studies reporting high amounts of HMF in dried fruits (25 to 2900 mg/kg) [239, 240]. The concentrations of HMF were found to range between not detectable to 512.1 mg/L in fruit juice concentrates (**Table 2.2**). There are several studies in the literature reported that the HMF levels were quite low such as 0.17 to 4.5 ppm in various juice concentrates [55, 57, 241].

The Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union (AIJN) has declared a maximum HMF level of 10 mg/L for fruit juices [198]. To our results, in fruit juices, puree and puree concentrates, HMF was not determined or found very low concentration with the maximum level of 19.9 mg/L in tart cherry puree juice. This finding is in accordance with literature reports, revealing low amounts of HMF in fruit juices [57, 64, 242].

2.3.2. Daily intake levels of α-dicarbonyl compounds and HMF from processed fruits

α-Dicarbonyl compounds might be responsible for lots of diseases such as diabetes, cataract, Alzheimer disease, mortal allergenicity in young children [1, 5, 59, 243]. From this point of view, investigation of the occurrence of α-dicarbonyl compounds in foods and their daily intake levels is great importance. On the other hand, there is still no regulation on their tolerable daily intake levels, although the latest reports about the physiological consequences of α-dicarbonyl compounds cause an increasing concern.

Fruit and fruit products have a significant role in a healthy diet due to their nutritional values like vitamins, minerals, antioxidants, phytochemical compounds. After WHO declared a recommendation for consuming a minimum of 400 g of fruit and vegetables per day [35], there has been a campaign named “5 a day” which encourages the consumption of at least five portions of fruit and vegetables each day in developed countries. The campaign contains not only consume fresh fruits but also fruit juices and dried fruits. As it is well-known, children are the largest group of consumers toward fruit juices, purees, and other fruit products (e.g. yogurts with dried fruits) in the world.

As given in **Table 2.3**, the daily take levels of α-dicarbonyl compounds and HMF for adults and children were calculated by taking into consideration the recommendations of World Health

Organizastion (WHO), United States Department of Agriculture (USDA), American Academy of Pediatrics (AAP) and the studies in the literature [9, 35, 244, 245]. Daily serving sizes of dried fruit, fruit juice, and fruit puree recommended for healthy adults at ages of 20 – 60 years were ½ serving (55g), ½ cup (150 ml) and ½ cup (150 ml), respectively. For children at 6 – 18 years of age, the recommended daily serving sizes of those were 2 servings (110 g), 1 cup (300 ml) and 1 cup (300ml), respectively [9, 35, 244, 245]. The intake levels of α-dicarbonyl compounds and HMF were calculated on the basis of the range (minimum and maximum levels) and the median levels in parenthesis since intake per serving size can vary strongly depending on the brand and the type of fruit products (**Table 2.3**). The broad range of 3-DG levels in dried fruits as 4.8 – 905.7 mg per serving size is a good illustration of the variety of intake levels in this study.

Table 2.3. Calculated daily intake (mg) of α-dicarbonyl compounds and 5-hydroxymethylfurfural for dried fruits, fruit juices and purees.

	Dried Fruits (n=141)		**Fruit Juice (n=14)**		**Fruit Puree (n=5)**	
	Adult	*Children*	*Adult*	*Children*	*Adult*	*Children*
3-DG	1.2-226.4 (27.5)	4.8-905.7 (109.9)	0.2-5.5 (1.6)	0.5-11.1 (3.1)	0.7-3.1 (0.8)	1.4-6.2 (1.6)
Glucosone	0.7-42.2 (14.3)	2.9-169 (57.2)	0.7-3.8 (2.3)	1.3-7.7 (4.6)	0.7-1.8 (1.3)	1.3-3.5 (2.5)
Threosone	0.1-15.5 (1.1)	0.3-62 (4.3)	0-0.1 (0.1)	0.1-0.2 (0.2)	0-0.4 (0)	0-0.7 (0.1)
MGO	0.1-14 (1)	0.3-55.9 (4.2)	0.1-0.5 (0.2)	0.2-1 (0.4)	0.1-0.1 (0.1)	0.1-0.3 (0.2)
GO	0-2.7 (0.3)	0-10.7 (1.4)	0.2-0.8 (0.3)	0.4-1.7 (0.7)	0.1-0.3 (0.2)	0.3-0.7 (0.4)
1-DG	0-2.6 (0.3)	0.1-10.5 (1.3)	-	-	-	-
DA	0-1 (0.1)	0.1-4.1 (0.5)	-	-	-	-
3-DP	-	-	0-0.1 (0.1)	0-0.2 (0.1)	0-0.1 (0.1)	0.1-0.3 (0.2)
HMF	0.2-132.1 (2.6)	1-528.2 (10.5)	0.1-3 (0.4)	0.3-6 (0.9)	0.2-0.7 (0.5)	0.4-1.4 (0.9)

Daily serving sizes of dried fruit, fruit juice, and fruit puree recommended for healthy adults at ages of 20 – 60 years were ½ serving (55g), ½ cup (150 ml) and ½ cup (150 ml), respectively. For children at 6 – 18 years of age, the recommended daily serving sizes of those were 2 servings (110g), 1 cup (300ml) and 1 cup (300ml), respectively [9, 35, 244, 245].

Degen, et al. [9] reported a rough estimation of the daily intake level of α-dicarbonyl compounds in various food. Hereunder, the authors indicated that the median intake level of 3-DG was calculated as 8.1 mg per serving size (300 ml) in fruit juices. Similarly, the intake level of 3-DG in fruit juices changed between 0.5 – 11.1 mg per serving size (300 ml) with a median level of 3.1 mg per serving size in our study. Hellwig, et al. [59] showed the estimated daily exposure to dominant α-dicarbonyl compounds (3-DG, MGO, 3-deoxygalactosone) based on a daily model diet containing bread, honey, jam, cheese, coffee, beer, fruit juice, cookies, cooked pasta, for adults and children in varying levels. The results indicated that the total intake level of these three α-dicarbonyl compounds through a model diet was 72 mg for adults and 51.3 mg for children [59]. According to our results, the median level of daily intake of 3-DG was 27.5 mg

through dried fruits for adults while it was 109.9 mg for children just by consuming dried fruit. As it is clearly seen that the concentration of α-dicarbonyl compounds in fruit products was at a worrying level since the daily intake of only 3-DG from dried fruits was much more than the level of total dominant α-dicarbonyl compounds from a whole diet recommended for children, particularly. In addition to this, another concern is the daily exposure to glucosone which is the other dominant α-dicarbonyl compound following 3-DG in the present study. The daily intake median levels of glucosone through dried fruits, fruit juices, and fruit purees were calculated for children as 57.2 mg, 4.6 mg and 2.5 mg per serving size, respectively (**Table 2.3**). Similarly, Aktağ, et al. [160] reported that the glucosone intake through follow-on infant UHT milk was calculated as 3.6 mg/day following 3-DG (11.2 mg/day). Daily intake levels calculated for other α-dicarbonyl compounds in this study were comparably lower than that of 3-DG and glucosone, nevertheless, their concentrations in fruit products were still an important concern **(Table 2.3)**. Despite the doubt about the possible health consequences of ingested α-dicarbonyl compounds, recent *in vivo* studies present that α-dicarbonyl compounds including MGO, GO have toxic effects for humans [59, 246].

The physiological effects or the possible carcinogenicity of HMF are doubtful. On the other hand, 5-sulfoxymethylfurfural, which is directly formed from HMF *in vivo*, has genotoxic effects [247]. A daily intake of HMF up to 150 mg per day for a 60 kg weight adult (2.5 mg/day/kg body wt) is recommended as safe [195]. Fruit juices and fruit purees had a minor contribution to daily exposure to HMF, however, the HMF levels in dried fruits were quite high as mentioned. To the results, the daily intake level of HMF from dried fruits reached to 132.1 mg for adults and 528.2 mg for children **(Table 2.3).** From these values, it is particularly obvious that the exposed amount of HMF exceeds the recommended level of HMF as 100 mg/day for children about 5-fold. These results make noteworthy contributions to the obligatory to prepare regulations and limitations for the levels of α-dicarbonyl compounds and HMF in foods, contrary to the studies which indicate that the undesired compounds metabolized *via* catabolic systems of the human body [9].

2.4. CONCLUSION

It is a fact that compositional characteristics and ambient conditions may affect the formation of sugar decomposition products in fruit products. There are a lot of studies on the occurrence of HMF in processed foods including fruits and vegetables. However, to the best of our knowledge, this study is the first study reporting comprehensive data on the occurrence of α-dicarbonyl compounds in a large number of processed fruit products. The results revealed the

fact that the occurrence of α-dicarbonyl compounds and HMF was the highest in dried fruits. In addition, the results indicated that the concentrations of α-dicarbonyl compounds may be significantly higher than that of HMF in certain fruit products. 3-DG was found as the main α-dicarbonyl compound in all types of fruit products. It is concluded from the results that α-dicarbonyl compounds should be measured together with HMF in order to better evaluate the quality and safety of processed fruit products. The daily intake levels of α-dicarbonyl compounds and HMF through fruit products were calculated according to the recommended healthy diet for adults and children. Considering their potential adverse effects on human health, it is considered that the exposure levels calculated for α-dicarbonyl compounds through the consumption of processed fruit products cannot be neglected.

CHAPTER 3

CHANGES IN α-DICARBONYL COMPOUNDS AND 5-HYDROXYMETHYLFURFURAL DURING STORAGE OF FRUIT JUICES: A MULTIRESPONSE KINETIC MODELLING APPROACH

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3.1. INTRODUCTION

As mentioned in Chapter 1, fruit products in particular fruit juices have long shelf-lives exceeding 1 year with no requirement of chilling. Thus, unsuitable and/or pro-longed storage conditions have a great impact on the accumulation of α-dicarbonyl compounds and 5-hydroxymethylfurfural (HMF) which can cause the formation of toxic compounds and the nutritional loss in fruit juices [2, 50]. Maillard reaction and caramelization have been mainly responsible for the formation of α-dicarbonyl compounds and HMF through sugar decomposition. However, these simultaneous and complex reactions make difficult to understand the formation mechanisms of α-dicarbonyl compounds and HMF [4, 50]. For example, there is no answer to the question of whether the pathway of 3-deoxyglucosone (3-DG) dehydration or the pathway of fructose dehydration is more favorable for the formation of HMF in fruit juices. Indeed, the detailed mechanim of sugar decomposition reactions during storage of fruit juice, a sugar-rich, acidic and aqueous product, has not been clear yet. Therefore, a kinetic description with estimating the elementary reaction rate constants is required to clarify such complicated reactions in terms of quality and safety issues. Multiresponse kinetic modeling is a powerful approach to unravel such sophisticated reaction mechanisms as it considers all the reactants and products at the same time, as disccused in Chapter 1.

In this section of this thesis study, impact of storage conditions on the formation of α-dicarbonyl compounds and HMF in mostly consumed fruit juices have been investigated with using multiresponse kinetic modelling approach. For this purpose apple juice, orange juice, and peach nectar were selected as typical examples of clear, cloudy and added-sugar products, respectively. Changes in the concentrations of glucosone, 3-DG, threosone, methylglyoxal (MGO), glyoxal (GO), HMF, sugars and free amino acids were determined during storage in order to build a multi-response kinetic model describing the most possible pathway responsible for the formation of α-dicarbonyl compounds and HMF.

3.2. EXPERIMENTAL

3.2.1. Chemicals and consumables

High purity (>99%) sucrose, glucose and fructose were purchased from Sigma-Aldrich (Diesenhofen, Germany). Phosphoric acid (85%) was purchased from Merck (Darmstadt, Germany). HMF (98%) was purchased from Acros (Geel, Belgium). Formic acid (98%) was purchased from JT Baker (Deventer, The Netherlands). 3-DG (75%), glucosone (≥98%), quinoxaline (99%), 2-methylquinoxaline (97%), 2,3-dimethylquinoxaline (97%), o-

phenylenediamine (98%), diethylenetriaminepentaacetic acid (DETAPAC) (98%), methanol, and acetonitrile were purchased from Sigma-Aldrich (Steinheim, Germany). Disodium hydrogen phosphate anhydrous and sodium dihydrogen phosphate dihydrate were purchased from Merck (Darmstadt, Germany). All amino acids (>98%) were purchased from Merck Co. (Darmstadt, Germany). Ultra-pure water was used throughout the experiments (Milli Q-System, Millipore, Millford, MA). Syringe filters (nylon, 0.45 lm) and Oasis HLB cartridges (30 mg, 1mL) were supplied by Waters (Milford, MA).

3.2.2. Preparation of Juice Samples

Golden delicious variety of apples, Washington variety of oranges and Bursa variety of peaches were obtained from local markets. Apple juices, orange juices and peach nectars were produced in the laboratory using the flowchart shown in **Figure 3.1**. The juice samples were stored at 4, 27 and 37°C for 24 weeks. Sub-samples were taken from the stored samples 3 parallel in every 2 weeks, and kept frozen at -18°C prior to analysis. The samples were cleaned up by Carrez clarification for the analysis of HMF and sugars, and by adding acetonitrlile for the analysis of α-dicarbonyl compounds and free amino acids. In Carrez clarification, 1 mL of juice sample was mixed with 50 µl of Carrez I and 50 µl of Carrez II solutions. In acetonitrile clarification, 500 µL of juice sample was mixed with 500 µL of acetonitrile. The mixture was centrifuged at 10,000 x g for 5 min.

3.2.3. Analysis of sugars

One mL of the clear supernatant was passed through a preconditioned (by passing 1 mL methanol and 1 mL water) OASIS HLB cartridge. The first 8 drops of the eluent were discarded and the rest was collected into a vial for analysis. Analysis was performed on an Agilent 1200 HPLC system (Waldbronn, Germany) equipped with quaternary pump, and autosampler coupled with an Agilent 1100 refractive index detector and temperature-controlled column oven. The chromatographic separations were performed on a Shodex Sugar SH-1011 column (300 mm x 8 mm i.d., 6 µm) conditioned at 50 °C. The mobile phase was 5 mM H_2SO_4 in water (v/v) at a flow rate of 1 mL/min. The injection volume was 10 µL. The concentrations of sucrose, glucose, and fructose were calculated from the calibration curves built for each compound in the range between 0.25 and 2.5 g/L (0.25, 0.5, 0.75, 1 and 2.5 g/L). The LOD and LOQ values for sugars were 1.0 mg/L and 3.0 mg/L, respectively.

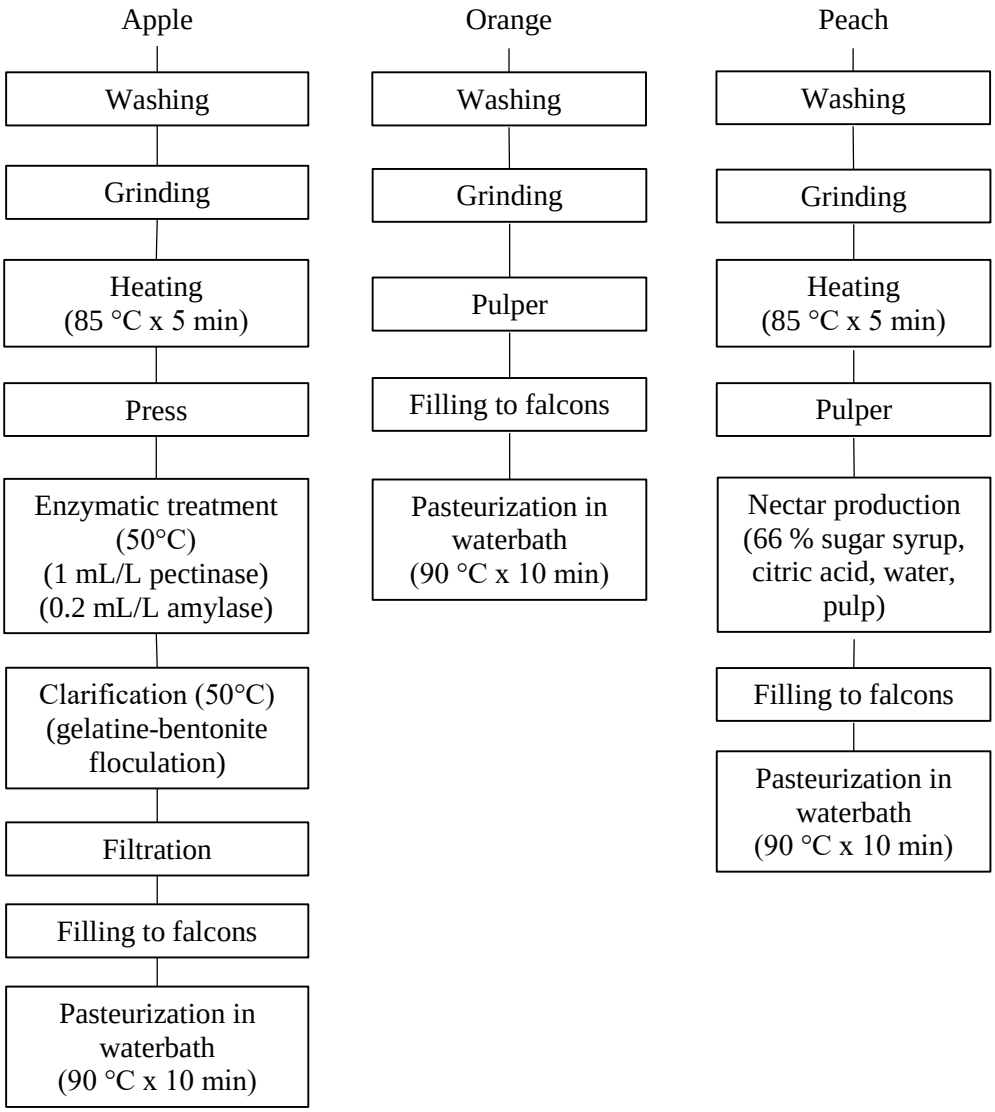

Figure 3.1. Flowcharts of apple juice, orange juice and peach nectar production in the laboratory.

3.2.4. Analysis of HMF

One mL of clear supernatant from juice samples was immediately filtered through 0.45 μm syringe filter and put into an auto sampler vial. The filtered sample was injected onto a Shimadzu UFLC system (Kyoto, Japan) consisting of a quaternary pump, an autosampler, a diode array detector and a temperature-controlled column oven. The chromatographic separations were performed on an Atlantis dC18 column using the isocratic mixture of 10 mM aqueous formic acid solution and acetonitrile (90:10, v/v) at a flow rate of 1.0 mL/min at 25°C. Data acquisition was performed by recording chromatograms at 285 nm. Concentration of HMF was calculated by means of a calibration curve built in the range between 1 and 10 mg/L (1, 2, 5, 10 mg/L). The LOD and LOQ values for HMF were 10 mg/L and 30 μg/L, respectively.

3.2.5. Analysis of α-dicarbonyl compounds

Derivatization of α-dicarbonyl compounds was carried out with o-phenylenediamine. Five hundred µL of supernatant was mixed with 150 µL of 0.2% o-phenylenediamine solution containing 11 mM diethylenetriaminepenta acetic acid and 150 µL of 0.5 M sodium phosphate buffer (pH 7). The mixture was immediately filtered through 0.45 µm syringe filter into an auto sampler vial. It was kept at room temperature, at dark for 2 h prior to measurement.

α-Dicarbonyl compounds were determined by using an Agilent 1200 series HPLC system coupled with an Agilent 6130 single quadrupole mass spectrometer. The chromatographic separation was performed on a Merck Purospher Star RP-18e column (150 mm x 4.6 mm, 5 µm) using a gradient mixture of (A) 1% formic acid in water and (B) 1% formic acid in methanol as the mobile phase at a flow rate of 0.7 mL/min at 30 °C. The gradient mixture was started from 30% B and increased to 60% B in 12 min, then it was decreased to 30% B in 3 min. The chromatographic run was completed in 15 min. The injection volume was 10 µL. The electrospray source had the following settings: drying gas (N_2) flow of 11.0 L/min at 320°C, nebulizer pressure of 400 psig and capillary voltage of 4000 V. The fragmentor voltage was set to 100 V. MS data were acquired in positive mode and α-dicarbonyl compounds were identified by selected ion monitoring (SIM) mode. The SIM ions $[M+H]^+$ were as follows for the quinoxaline derivatives of glucosone: 251; 1- or 3-DG: 235; MGO: 145; and GO: 131; threosone: 191. A dwell time was set at 97 ms for each. The SIM ions of the quinoxaline derivatives of α-dicarbonyl compounds were used for quantitation. The concentrations of quinoxaline, 2-methylquinoxaline and 2,3-dimethylquinoaxaline were calculated by means of external calibration curves in the range between 0.1 and 1.0 mg/L (0.1, 0.25, 0.5, 0.75 and 1.0 mg/L). Working solutions of 3-DG and glucosone in the concentration range between 0.1 and 1 mg/L were derivatised and analyzed as described above to build their external calibration curves. Also, the calibration curve of glucosone was used for semi-quantitation of threosone derivatives, since both have same proton-accepting groups. All working solutions were prepared in acetonitrile-water (50:50, v/v). The LOD and LOQ values for α-dicarbonyl compounds ranged from 2.5 to 15 µg/L and from 8.3 to 50 µg/L, respectively.

3.2.6. Analysis of free amino acids

One mL of clear supernatant from juice samples was immediately filtered through 0.45 µm syringe filter and put into an auto sampler vial. The samples were analyzed by a Waters Acquity UPLC system coupled to a triple quadrupole detector operated in positive electrospray

ionization mode. Chromatographic separations were performed on an Atlantis HILIC column (150 x 2.1 mm i.d., 3 μm) by using a gradient mixture of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 0.4 mL/min. The eluent composition starting with 15% A linearly increased to 40% in 4 min and held for 5 min. Then, it was decreased to the initial conditions (15% A) in 3 min. The column was at 30°C and the Waters ACQUITY FTN auto sampler was at 10 °C during the analysis. The electrospray source had the following settings: capillary voltage of 3.5 kV; cone voltage of 20 V; extractor voltage of 3 V; source temperature of 120 °C; desolvation temperature of 350 °C; and desolvation gas (nitrogen) flow of 900 L/h. Quantifications were performed by means of external calibration curves built for all amino acids in a range between 0.1 and 5.0 mg/L (0.1, 0.25, 0.5, 1, 2.5 and 5.0 mg/L). The LOD and LOQ values for amino acids ranged from 0.2 to 5 μg/L and from 0.7 to 16.7 μg/L, respectively.

3.2.7. Analysis of pH and Brix

The pH of the juice samples was measured using a PHM210 model pH meter (MeterLab, France) and the brix of the juice samples was measured using a Pocket Pal-3 model refractometer (Atago, Japan).

3.2.8. Kinetic and statistical data analysis

Data used for modelling was expressed in mmol/L. All individual analytical measurements were used to estimate model parameters. The mass balance of reactants and reaction products are given in the **Figure 3.2**.

A kinetic model was derived from the reaction network comprising α-dicarbonyl compounds formation pathways in caramelisation. For each reaction step of the kinetic model which was characterized by a reaction rate constant (k), differential equations were set up and solved by numerical integration. Numerical integration and estimation of the model parameters (k) were performed by non-linear regression using determinant criterion [248] with Athena Visual Studio software (v.14.2) (www.athenavisual.com). For each storage temperature, parameter estimation was performed separately. Model discrimination was used for evaluation of fitting of experimentally obtained data and mathematical model. The goodness of fit of the models to the experimental data as well as the highest posterior density (HPD) intervals of the estimated parameters were used to criticize the kinetic models. Arrhenius equation was used to

determinate the temperature dependence of the reaction rate constants by means of activation energies (E_a, kJ/mol).

Free amino acid data were subjected to analysis of variance (one-way ANOVA). The SPSS 17.0 statistical package was used for the evaluation of statistical significance of the differences between mean values by Tukey test. $P<0.05$ was considered to be statistically significant for the results.

3.3. RESULTS AND DISCUSSION

3.3.1. Changes in reactants and reaction products

Changes in the concentrations of reactants (sucrose, glucose, fructose, and free amino acids) and reaction products (3-DG, glucosone, GO, threosone, MGO and HMF) were monitored in apple juice, orange juice, and peach nectar during storage at 4, 27 and 37 °C for 24 weeks. The results showed that there were no significant changes in concentrations of reactants and reaction products in the samples stored at 4 °C (**Table 3.1**). Therefore, kinetic analysis was limited with the data observed for 27 and 37 °C. In addition, the concentrations of free amino acids remained relatively stable in all samples during storage at all temperatures (**Table 3.2**). However, it was previously reported that some of free amino acids like tryptophan decreased during storage of peach and orange juices [30, 242]. Additionally, Wang, et al. [249] reported total amino acid contents significantly decreased in parallel with the decrease in pH due to the condensation between free amino and carbonyl groups in Maillard reaction during storage of carrot juice. On the contrary, no change was observed in the pH values of samples during storage in our study (**Table 3.3**).

Table 3.1. Changes in products and reactants (mmol/L) during storage of apple juices, orange juices and peach nectars at 4 °C.

	Time (week)	Sucrose	Glucose	Fructose	HMF	3-DG	Glucosone	Glyoxal	Threosone	Methylglyoxal
Apple Juice	0	135.82 ± 0.66	86.28 ± 0.39	419.12 ± 0.04	nd*	0.10 ± 0.00	0.06 ± 0.00	nd	nd	nd
	2	129.68 ± 6.51	80.16 ± 4.10	418.82 ± 0.82	nd	0.08 ± 0.01	0.05 ± 0.01	nd	nd	nd
	4	121.81 ± 4.99	79.43 ± 4.10	414.73 ± 0.03	nd	0.08 ± 0.02	0.05 ± 0.02	nd	nd	nd
	6	120.62 ± 4.99	82.03 ± 0.72	407.31 ± 5.38	nd	0.12 ± 0.04	0.06 ± 0.01	nd	nd	nd
	8	127.54 ± 3.37	77.56 ± 4.77	415.24 ± 3.33	nd	0.13 ± 0.03	0.06 ± 0.00	nd	nd	nd
	10	122.07 ± 1.69	78.30 ± 4.08	414.49 ± 3.13	nd	0.11 ± 0.01	0.07 ± 0.02	nd	nd	nd
	12	119.42 ± 3.42	81.29 ± 0.71	410.55 ± 0.09	nd	0.11 ± 0.02	0.07 ± 0.01	nd	nd	nd
	14	123.27 ± 1.71	79.03 ± 4.08	417.41 ± 1.32	nd	0.12 ± 0.02	0.06 ± 0.01	nd	nd	nd
	16	124.46 ± 1.72	79.77 ± 4.07	421.84 ± 2.45	nd	0.11 ± 0.02	0.07 ± 0.00	nd	nd	nd
	18	121.56 ± 3.43	77.22 ± 4.12	415.80 ± 2.54	nd	0.12 ± 0.01	0.06 ± 0.01	nd	nd	nd
	20	121.54 ± 5.25	79.82 ± 0.70	415.04 ± 2.55	nd	0.11 ± 0.01	0.07 ± 0.01	nd	nd	nd
	22	121.22 ± 3.40	79.06 ± 4.10	416.02 ± 2.51	nd	0.13 ± 0.02	0.08 ± 0.00	nd	nd	nd
	24	120.59 ± 4.38	76.79 ± 4.24	415.68 ± 2.39	nd	0.13 ± 0.03	0.09 ± 0.01	nd	nd	nd
Orange Juice	0	117.01 ± 0.19	193.87 ± 0.17	179.62 ± 1.08	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	2	112.79 ± 0.90	196.88 ± 4.00	194.58 ± 2.26	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	4	111.76 ± 0.89	194.91 ± 3.96	192.63 ± 2.24	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	6	107.40 ± 5.50	192.95 ± 3.92	190.69 ± 2.21	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	8	111.25 ± 0.89	193.93 ± 3.94	191.66 ± 2.23	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	10	110.22 ± 0.88	191.96 ± 3.90	189.71 ± 2.20	nd	0.01 ± 0.01	0.00 ± 0.00	nd	nd	nd
	12	109.71 ± 0.87	190.98 ± 3.88	188.74 ± 2.19	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	14	110.28 ± 0.27	201.15 ± 5.36	199.80 ± 6.49	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	16	109.28 ± 0.27	199.14 ± 5.30	197.80 ± 6.43	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	18	108.78 ± 0.27	198.13 ± 5.28	196.80 ± 6.40	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	20	108.28 ± 0.27	197.13 ± 5.25	195.80 ± 6.36	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd
	22	107.78 ± 0.27	196.12 ± 5.22	194.80 ± 6.33	nd	0.01 ± 0.00	0.01 ± 0.01	nd	nd	nd
	24	109.15 ± 0.72	193.62 ± 1.49	193.56 ± 0.68	nd	0.01 ± 0.00	0.00 ± 0.00	nd	nd	nd

*nd : not determined.

Table 3.1 continue.

	Time (week)	Sucrose	Glucose	Fructose	HMF	3-DG	Glucosone	Glyoxal	Threosone	Methylglyoxal
Peach Nectar	0	366.89 ± 4.65	50.12 ± 0.07	45.12 ± 0.75	nd	0.01 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	nd
	2	367.65 ± 4.15	58.73 ± 0.47	50.94 ± 3.46	nd	0.01 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	nd
	4	363.91 ± 11.14	44.61 ± 0.65	48.42 ± 2.56	nd	0.01 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	nd
	6	364.43 ± 5.31	53.58 ± 0.73	52.29 ± 0.32	nd	0.01 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	nd
	8	384.57 ± 0.55	55.39 ± 2.50	51.18 ± 0.10	nd	0.01 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	nd
	10	369.23 ± 7.38	59.12 ± 3.91	50.39 ± 0.42	nd	0.01 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	nd
	12	345.81 ± 0.59	60.58 ± 8.94	49.94 ± 1.24	nd	0.01 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	nd
	14	356.86 ± 2.73	59.85 ± 4.54	53.39 ± 1.19	nd	0.01 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	nd
	16	372.34 ± 11.02	55.70 ± 2.85	49.53 ± 1.64	nd	0.01 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	nd
	18	388.80 ± 2.06	59.42 ± 1.40	46.36 ± 0.40	nd	0.00 ± 0.00	0.04 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	nd
	20	371.45 ± 13.95	56.01 ± 2.14	49.07 ± 2.27	nd	0.01 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	nd
	22	370.71 ± 9.71	61.71 ± 1.61	51.13 ± 3.24	nd	0.01 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	nd
	24	368.20 ± 2.68	58.96 ± 5.57	52.92 ± 2.87	nd	0.01 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	nd

*nd : not determined.

Table 3.2. Changes in individual free amino acids (mg/L) during storage of apple juices, orange juices and peach nectars at different temperatures and times.

	Storage Temperature & Time	Ala	Arg	Asn	Asp	Cys	Gaba	Gln	Glu	Gly	His	Leu	Ile	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Apple Juice	0	14.95 ±2.33^{a}	1.23 ±0.53^{a}	122.31 ±15.01^{a}	24.77 ±3.27^{a}	nd	6.95 ±0.81^{a}	1.86 ±0.19^{a}	18.45 ±2.49^{a}	1.17 ±0.02^{a}	0.67 ±0.07^{a}	2.01 ±0.30^{a}	3.82 ±0.51^{a}	1.62 ±0.15^{a}	nd	1.29 ±0.17^{a}	0.24 ±0.09^{a}	5.38 ±0.80^{a}	3.79 ±0.62^{a}	nd	0.27 ±0.04^{a}	0.59 ±0.01^{a}
	27°C 24 week	15.93 ±0.87^{a}	1.02 ±0.09^{a}	129.37 ±10.39^{a}	28.40 ±2.23^{a}	nd	7.16 ±0.17^{a}	2.22 ±0.31^{a}	19.33 ±1.65^{a}	1.36 ±0.28^{a}	0.61 ±0.04^{a}	2.18 ±0.11^{a}	4.04 ±0.15^{a}	1.66 ±0.13^{a}	nd	1.33 ±0.08^{a}	0.22 ±0.01^{a}	6.03 ±0.46^{a}	3.84 ±0.25^{a}	nd	0.32 ±0.02^{a}	0.79 ±0.02^{a}
	37°C 24 week	13.72 ±0.84^{a}	0.95 ±0.04^{a}	116.08 ±6.09^{a}	31.59 ±2.26^{a}	nd	5.49 ±0.23^{a}	2.28 ±1.02^{a}	16.79 ±0.81^{a}	1.54 ±0.07^{a}	0.60 ±0.01^{a}	1.82 ±0.12^{a}	3.50 ±0.18^{a}	1.65 ±0.09^{a}	nd	1.29 ±0.23^{a}	0.81 ±0.03^{bc}	4.94 ±0.34^{a}	3.13 ±0.47^{a}	nd	0.35 ±0.03^{a}	0.66 ±0.05^{a}
Orange Juice	0	99.5 ±8.4^{a}	179.1 ±18.7^{a}	401.7 ±36.0^{a}	202.6 ±17.6^{a}	nd	96.0 ±5.5^{a}	29.1 ±2.5^{a}	194.3 ±16.5^{abc}	12.4 ±0.9^{a}	4.7 ±0.4^{abc}	5.4 ±0.6^{a}	10.3 ±0.9^{a}	26.6 ±2.1^{a}	nd	8.0 ±0.8^{a}	69.9 ±5.1^{ab}	123.6 ±10.4^{ab}	16.1 ±1.5^{a}	nd	1.9 ±0.3^{a}	4.6 ±0.3^{a}
	27°C 24 week	86.4 ±4.4^{a}	169.3 ±5.7^{a}	308.6 ±12.8^{a}	203.2 ±8.8^{a}	nd	85.8 ±1.6^{a}	32.7 ±10.8^{a}	159.9 ±10.9^{a}	10.3 ±0.6^{a}	3.6 ±0.2^{a}	5.3 ±0.0^{a}	8.2 ±0.7^{a}	24.0 ±0.8^{a}	nd	7.1 ±0.3^{a}	54.8 ±3.6^{a}	86.7 ±5.1^{a}	13.1 ±0.8^{a}	nd	1.9 ±0.1^{a}	3.9 ±0.1^{a}
	37°C 24 week	96.3 ±3.7^{a}	178.9 ±6.0^{a}	402.1 ±16.3^{a}	233.7 ±7.6^{a}	nd	95.8 ±0.2^{a}	33.7 ±6.5^{a}	136.1 ±4.3^{a}	13.4 ±1.0^{a}	3.0 ±0.1^{a}	6.6 ±0.3^{a}	9.3 ±0.0^{a}	26.8 ±0.4^{a}	nd	7.5 ±0.1^{a}	106.1 ±7.1^{c}	107.3 ±1.8^{ab}	14.0 ±0.1^{ab}	nd	2.3 ±0.1^{a}	4.3 ±0.1^{a}
Peach Nectar	0	86.10 ±3.78^{a}	7.57 ±0.39^{a}	287.06 ±2.90^{abc}	65.41 ±0.51^{a}	29.42 ±2.41^{a}	nd	54.44 ±2.76^{a}	46.93 ±1.08^{ab}	10.59 ±1.58^{a}	16.70 ±0.86^{a}	18.46 ±0.74^{ab}	18.92 ±0.14^{a}	8.51 ±0.48^{ab}	2.84 ±0.01^{a}	31.60 ±1.05^{ab}	12.16 ±0.59^{a}	118.13 ±6.41^{a}	41.06 ±1.15^{a}	5.39 ±0.14^{a}	nd	18.52 ±0.28^{ab}
	27°C 24 week	98.12 ±5.42^{a}	7.82 ±0.46^{a}	318.98 ±16.80^{bcd}	138.28 ±5.10^{bc}	37.55 ±1.58^{a}	nd	53.40 ±0.24^{a}	56.90 ±0.41^{b}	11.22 ±0.08^{a}	16.78 ±0.22^{a}	23.07 ±0.35^{b}	21.67 ±0.04^{a}	9.74 ±0.17^{a}	2.57 ±0.00^{a}	36.50 ±0.29^{a}	12.54 ±0.88^{a}	130.37 ±5.47^{a}	48.13 ±0.67^{a}	4.05 ±0.04^{a}	nd	21.20 ±0.57^{b}
	37°C 24 week	98.40 ±6.95^{a}	7.86 ±0.28^{a}	289.14 ±19.98^{a}	237.24 ±3.12^{ef}	43.16 ±3.50^{a}	nd	53.17 ±0.18^{a}	44.83 ±2.90^{a}	11.75 ±0.49^{a}	15.10 ±0.33^{a}	21.47 ±1.36^{a}	20.76 ±2.07^{a}	10.48 ±0.35^{b}	2.53 ±0.00^{a}	32.95 ±2.08^{b}	12.56 ±0.54^{a}	127.62 ±7.37^{a}	45.09 ±2.96^{a}	4.15 ±0.99^{a}	nd	20.68 ±1.00^{b}

Table 3.3. Changes in Brix and pH values during storage of apple juices, orange juices and peach nectars at different temperatures and times.

Temp.	Time	**Apple Juice**		**Orange Juice**		**Peach Nectar**	
(°C)	(week)	Brix (°Bx)	pH	Brix (°Bx)	pH	Brix (°Bx)	pH
27	0	15.00 ± 0.20	3.53 ± 0.02	11.75 ± 0.25	3.36 ± 0.00	16.17 ± 0.45	3.52 ± 0.02
27	2	14.85 ± 0.25	3.49 ± 0.01	11.05 ± 0.15	3.39 ± 0.02	16.13 ± 0.34	3.51 ± 0.02
27	4	14.95 ± 0.05	3.51 ± 0.01	11.65 ± 0.35	3.38 ± 0.04	15.87 ± 0.25	3.53 ± 0.01
27	6	14.85 ± 0.25	3.54 ± 0.01	11.00 ± 0.20	3.37 ± 0.02	16.60 ± 0.16	3.51 ± 0.02
27	8	15.05 ± 0.15	3.51 ± 0.01	11.85 ± 0.25	3.39 ± 0.01	16.03 ± 0.12	3.51 ± 0.02
27	10	14.75 ± 0.25	3.54 ± 0.01	11.20 ± 0.30	3.38 ± 0.02	16.83 ± 0.09	3.52 ± 0.02
27	12	15.00 ± 0.20	3.53 ± 0.02	11.05 ± 0.15	3.39 ± 0.03	16.03 ± 0.12	3.49 ± 0.01
27	14	15.10 ± 0.30	3.52 ± 0.02	11.65 ± 0.35	3.35 ± 0.02	16.03 ± 0.12	3.52 ± 0.02
27	16	15.00 ± 0.10	3.53 ± 0.02	11.00 ± 0.20	3.41 ± 0.01	16.83 ± 0.09	3.51 ± 0.02
27	18	14.85 ± 0.35	3.50 ± 0.00	11.85 ± 0.25	3.38 ± 0.02	16.03 ± 0.12	3.52 ± 0.02
27	20	15.05 ± 0.15	3.50 ± 0.02	11.20 ± 0.30	3.39 ± 0.01	15.90 ± 0.08	3.52 ± 0.02
27	22	14.90 ± 0.30	3.52 ± 0.02	11.35 ± 0.05	3.34 ± 0.02	16.00 ± 0.43	3.51 ± 0.02
27	24	15.00 ± 0.10	3.52 ± 0.02	10.55 ± 0.35	3.36 ± 0.02	16.27 ± 0.41	3.52 ± 0.02
37	0	15.00 ± 0.20	3.53 ± 0.02	11.75 ± 0.25	3.36 ± 0.00	16.17 ± 0.45	3.52 ± 0.02
37	2	14.85 ± 0.35	3.53 ± 0.02	11.70 ± 0.10	3.38 ± 0.02	16.20 ± 0.36	3.53 ± 0.01
37	4	15.05 ± 0.15	3.51 ± 0.01	11.85 ± 0.25	3.39 ± 0.03	16.23 ± 0.29	3.51 ± 0.02
37	6	15.55 ± 0.25	3.54 ± 0.01	11.20 ± 0.30	3.35 ± 0.02	16.20 ± 0.16	3.53 ± 0.01
37	8	14.90 ± 0.30	3.51 ± 0.01	11.35 ± 0.05	3.41 ± 0.01	16.27 ± 0.33	3.51 ± 0.02
37	10	15.35 ± 0.55	3.51 ± 0.02	10.55 ± 0.35	3.38 ± 0.02	15.93 ± 0.05	3.51 ± 0.02
37	12	15.00 ± 0.10	3.53 ± 0.02	11.70 ± 0.10	3.39 ± 0.01	16.27 ± 0.33	3.50 ± 0.02
37	14	14.90 ± 0.30	3.52 ± 0.02	10.75 ± 0.55	3.34 ± 0.02	16.40 ± 0.16	3.51 ± 0.02
37	16	14.75 ± 0.25	3.53 ± 0.00	11.35 ± 0.45	3.34 ± 0.00	16.20 ± 0.36	3.53 ± 0.01
37	18	15.00 ± 0.20	3.52 ± 0.02	11.85 ± 0.25	3.41 ± 0.01	15.97 ± 0.17	3.51 ± 0.02
37	20	15.10 ± 0.30	3.53 ± 0.00	11.25 ± 0.35	3.38 ± 0.02	16.13 ± 0.34	3.52 ± 0.02
37	22	15.00 ± 0.10	3.52 ± 0.02	11.45 ± 0.05	3.39 ± 0.01	15.87 ± 0.25	3.52 ± 0.02
37	24	14.90 ± 0.20	3.53 ± 0.00	10.75 ± 0.55	3.40 ± 0.04	16.60 ± 0.16	3.52 ± 0.01

Our findings do not support that Maillard reaction takes place in apple juice, orange juice and peach nectar during storage. Comparing to acid conditions, Maillard reaction occurs more quickly under neutral conditions. In addition, it significantly accelerates at water activities of 0.6 – 0.7 [250]. As acidic and high-water activity systems, fruit juices are not considered suitable for the Maillard reaction to take place during storage. Therefore, formation of α-dicarbonyl compounds in juices and nectars during storage would be due to sugar dehydration reactions. The total sugar contents of juices were expressed as °Brix for fruit juices (**Table 3.3**). Initial °Brix values of apple juice, orange juice and peach nectar were 15°, 12°, and 16°, respectively. Individual concentrations of glucose, fructose and sucrose were monitored in the samples during storage. In general, the concentrations of glucose and fructose increased at the end of storage for all samples (**Figure 3.4-3.6**). Meanwhile, concentration sucrose decreased significantly. After storage for 24 weeks, total loss of sucrose was found 50%, 62% and 54% at

27°C, and 93%, 89% and 96% at 37°C in apple juice, orange juice and peach nectar, respectively. However, the decrease of sucrose content was not in a linear relationship with the increase of fructose and glucose contents. A similar trend was also reported by Wibowo, et al. [251] and Wibowo, et al. [64] during storage of mango and orange juices, respectively, for 32 weeks. A possible explanation is that the complex carbohydrates which were not analyzed in this study decomposes to monosaccharides.

Under acidic conditions, HMF can be formed by enolisation and dehydration of glucose or fructose [182]. As shown in **Figure 3.4-3.6**, formation of HMF followed a typical kinetic pattern in juices stored at 37°C, while there was no accumulation of HMF at 27°C. The maximum levels of HMF were 16.2 ± 0.7, 3.8 ± 0.2 and 12.2 ± 0.5 mg/L in apple juice, orange juice and peach nectar, respectively. The Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union (AIJN) has declared a maximum HMF level of 10 mg/L for fruit juices [198]. The present findings seem to be consistent with other researches which found almost no or in small quantities of HMF formation at lower temperatures between 20 and 28°C, and increase in the accumulation of HMF at higher temperatures between 30 and 40°C, during storage [55, 64, 242]. The type and concentration of sugars, pH, and temperature impact HMF formation in fruit juices during storage [64].

In general, the total concentration of α-dicarbonyl compounds was found higher in apple and orange juices than peach nectar. Predominant α- dicarbonyl compound was glucosone in apple and orange juices, while 3-DG was the main one in peach nectar. The concentration of glucosone exponentially increased to an apparent maximum, and tended to decrease afterwards in apple and orange juices at 37 °C (**Figure 3.4 and 3.5**). The maximum concentration reached 3.5 mmol/L in apple juice after 16 weeks of storage, while it reached to 1.7 mmol/L in orange juice after 14 weeks of storage. Paravisini and Peterson [65] and Paravisini and Peterson [29] reported that 3-DG was the predominant α- dicarbonyl compound in apple and orange juices stored for 10 weeks at 4 and 35°C. However, Smejkal, et al. [163] reported that glucosone was the major α- dicarbonyl compound formed in model sucrose solutions heated at temperatures below 100 °C. It is known that glucosone is formed from the oxidation of sugars [3]. Although there is no evidence of the relationship between oxygen levels in fruit juice and the formation of reactive carbonyl species, deoxyosone compounds are known to oxidatively decompose into α-dicarbonyl compounds in the presence of molecular oxygen [29, 252]. Initial concentration of 3-DG was lower than that of glucosone in peach nectar, however, its concentration started to increase dramatically with time after 4 weeks of storage at 37°C and 8 weeks of storage at

27°C (**Figure 3.6**). The maximum concentration of 3-DG reached 0.4 mmol/L in peach nectar after 14 weeks of storage at 37°C. Previous studies have reported 3-DG as the major α-dicarbonyl compound in different fruit juices [9, 29, 65].

The concentrations of shorter chain α-dicarbonyl compounds, GO, MGO and threosone were comparably lower than the concentrations of C_6- skeletal α-dicarbonyl compounds, glucosone and 3-DG. GO was the only shorter chain α-dicarbonyl compounds that was dedected in all samples during storage. However, MGO was detected only in apple and orange juices, while threosone was detected only in peach nectar. From a quantitative point of view, MGO and threosone were of only minor importance for stored fruit juices, concordant with the literature [29, 65]. At 37°C, the maximum concentration of GO was found as 0.4 mmol/L in apple juice, 0.2 mmol/L in orange juice and 0.08 mmol/L in peach nectar, stored for 24 weeks. Previous studies reported that the concentration of GO was 0.5 mmol/L and 0.09 mmol/L in apple and orange juices stored at 35°C for 10 weeks [29, 30]. Others reported very low concentrations of GO in different fruit juices sold in supermarkets ranging from not detected to 0.005 mmol/L [236, 237].

3.3.2. Kinetic Modelling

The mass balance of reactants and products calculating as the relative ratio of each compound (%) for apple juice, orange juice and peach nectar stored for 24 weeks at 27 and 37°C is presented in **Figure 3.2**. In general, the recovery values were approximately 100% or slightly higher through the storage period for all samples. These results are consistent with those of Nguyen, et al. [215] who studied the multiresponse kinetic modelling of *N*-carboxymethyllysine formation in aqueous model systems of sugars and casein. To the authors, the measurement variations might cause the deficiencies in the mass balance. In our fruit juice samples as real food matrices, carbohydrates like fibers found naturally in fruit juices, which were not included in mass balance, might hydrolyze to sugars, since the decrease of sucrose content was lower than the increase of fructose and glucose content.

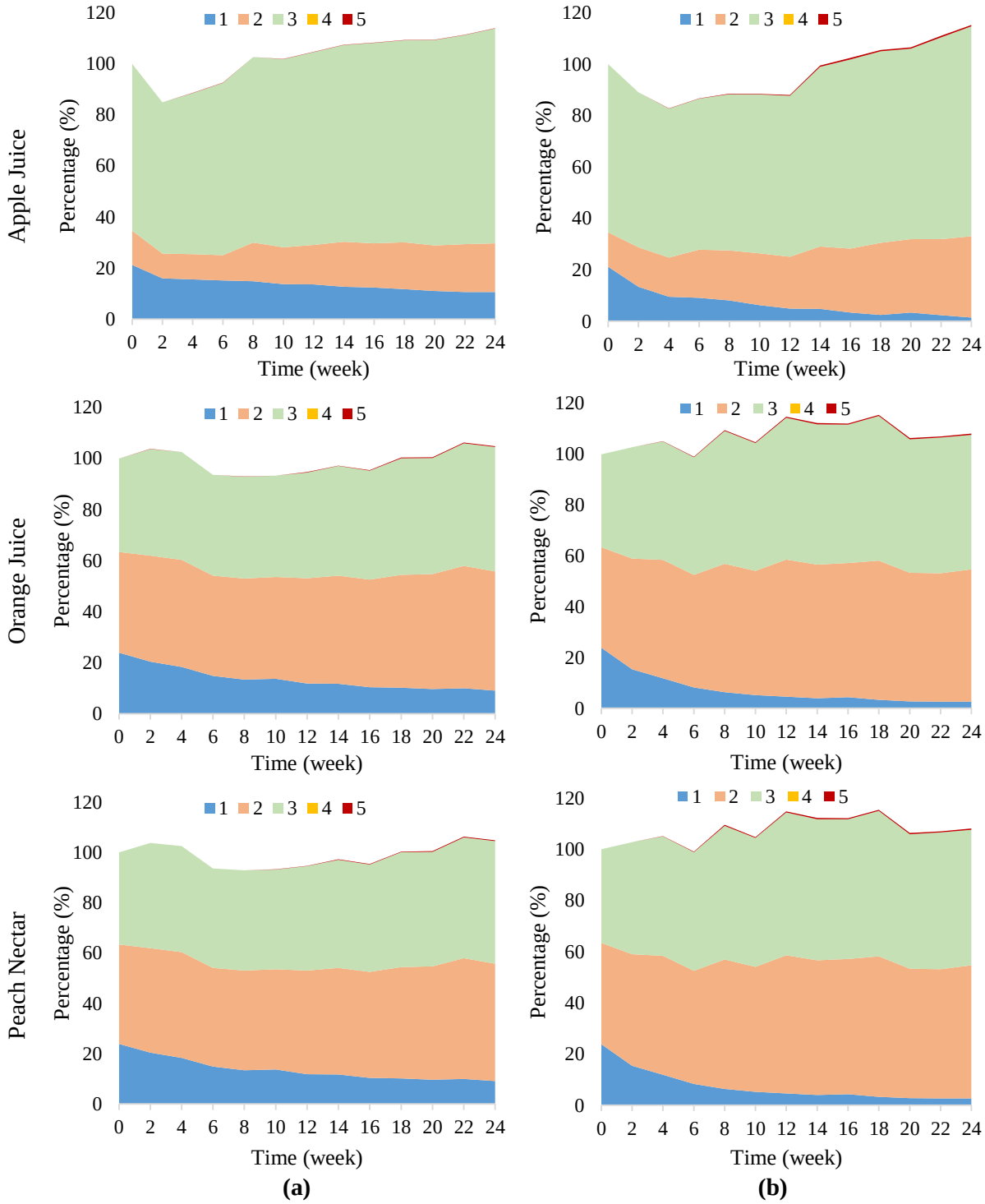

Figure 3.2. Mass balance of reactants and reaction products stored at 27 °C (a) and 37 °C (b). (1): sucrose, (2): glucose, (3): fructose, (4): 5-Hydroxymethylfurfural, (5): total dicarbonyl compounds.

A comprehensive reaction mechanism was initially built comprising the formation pathways of α-dicarbonyl compounds and HMF as given in **Figure 3.3**. However, the confidence intervals of rate constants were not well estimated for comprehensive model (**Table 3.4** and **Figures 3.4-3.6**).

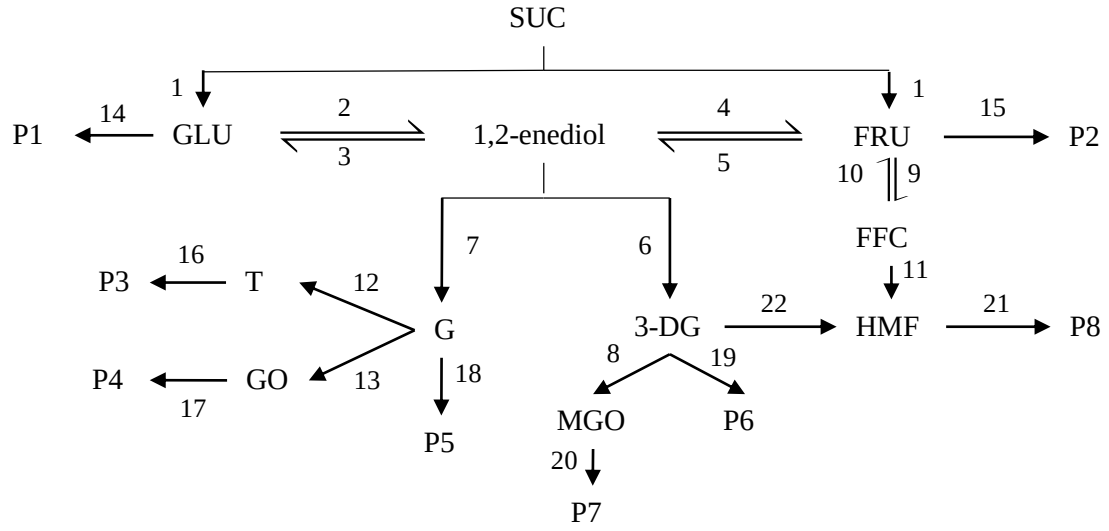

Figure 3.3. Comprehensive mechanistic model for sugar degradation reactions during storage of apple juice, orange juice and peach nectar. SUC, sucrose; GLU, glucose; FRU, fructose; FFC, fructofuranosyl cation; 3-DG, 3-deoxyglucosone; G, glucosone; MGO, methylglyoxal; GO, glyoxal; T, threosone; HMF, 5-hydroxymethyl-2-furfural; P, products.

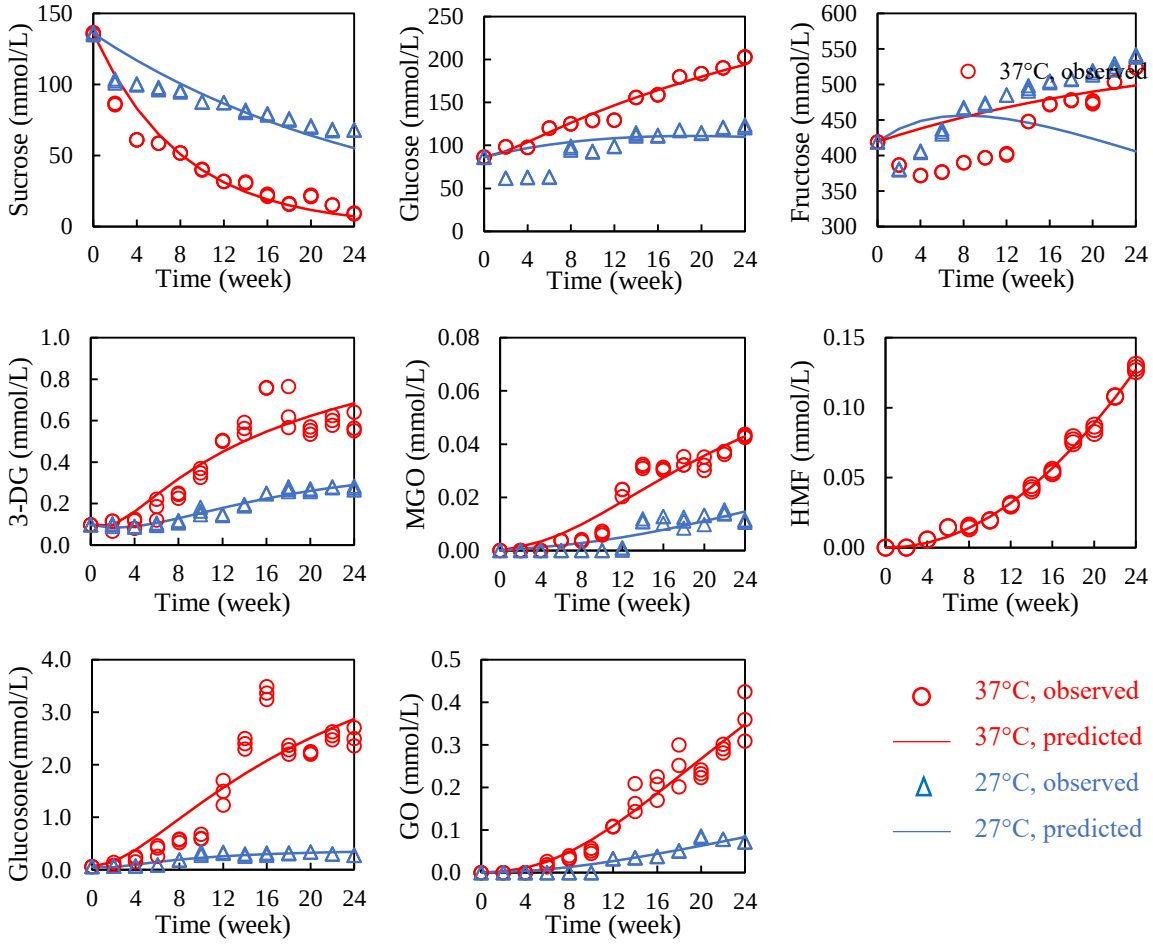

Figure 3.4. Kinetic model fit (lines) to the experimental data (symbols) of reactants and products according to the compherensive mechanistic model during storage of apple juice.

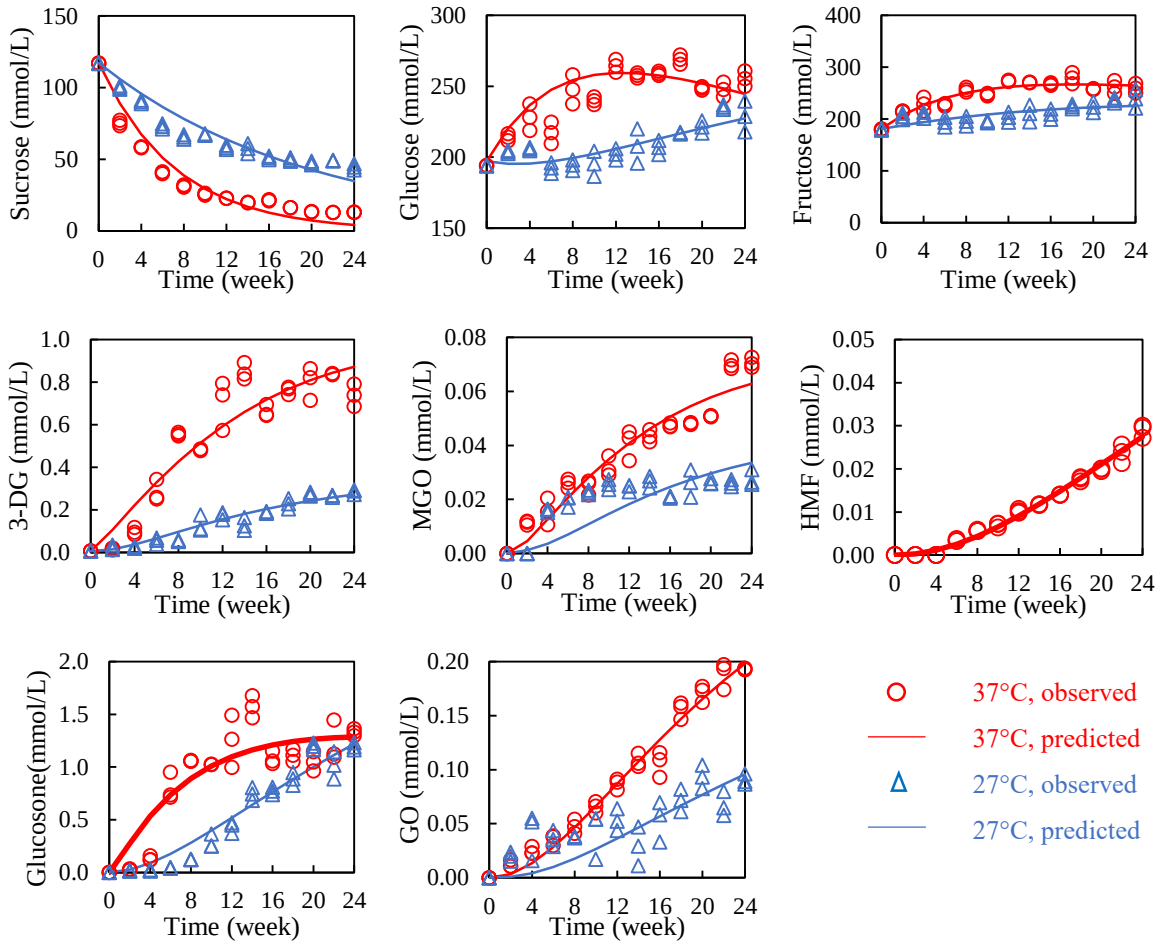

Figure 3.5. Kinetic model fit (lines) to the experimental data (symbols) of reactants and products according to the compherensive mechanistic model during storage of orange juice.

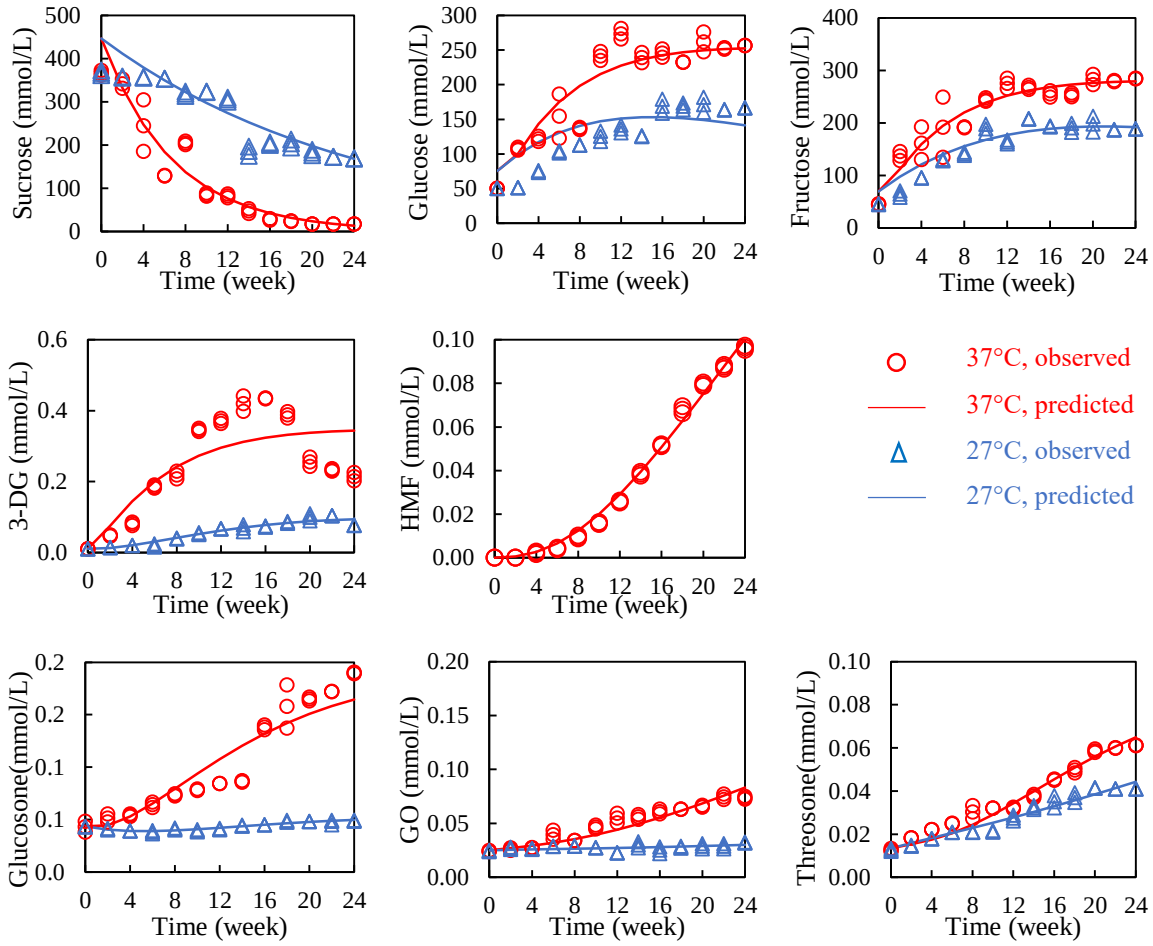

Figure 3.6. Kinetic model fit (lines) to the experimental data (symbols) of reactants and products according to the compherensive mechanistic model during storage of peach nectar.

Table 3.4. Estimated reaction rate constants (k, week^{-1} x 10^3) with 95% highest posterior density (HPD) intervals at different temperatures according to the comprehensive kinetic model in Fig. 2 for sugar degradation during storage of apple juices, orange juices and peach nectars.

		Apple Juice		Orange Juice		Peach Nectar	
Elementary Reaction Steps		37°C		37°C		37°C	
		k	HPD	k	HPD	k	HPD
1	SUC→FUR + GLU	122.77	±9.796	138.47	±9.644	147.77	±14.63
2	GLU→1,2-ED	174.35	±58.71	0.40	ind*	3509.45	ind*
3	1,2-ED→GLU	237.45	±100.4	3279.00	ind*	2053.58	±399.9
4	1,2-ED→FRU	0.00	LB	0.00	LB	1613.91	ind*
5	FRU→1,2-ED	12.53	±3.872	4.60	ind*	2500.60	±457
6	1,2-ED→3-DG	1.74	±1.337	218.33	±28.59	0.56	±0.5605
7	1,2-ED→G	3.30	±1.818	645.16	±214.5	0.05	±0.01058
8	3-DG→MGO	9.65	±4.168	85.42	±19.88	0.00	fixed
9	FRU→FFC	1.00	±0.0294	0.50	ind*	0.90	ind*
10	FFC→FRU	0.00	LB	39.57	±17.93	70.10	ind*
11	FFC→HMF	1.00	ind*	1.62	ind*	4.67	±0.5938
12	G→T	0.00	fixed	0.00	fixed	80.23	±28.79
13	G→GO	17.42	±8.925	13.21	±3.364	23.33	±1.708
14	GLU→P1	0.00	LB	15.59	±1.322	9.13	±20.83
15	FRU→P2	0.00	LB	0.02	±0.9873	0.37	ind*
16	T→P3	0.00	fixed	0.00	fixed	172.33	±84.93
17	GO→P4	86.95	±93.81	47.32	±33.12	0.00	LB
18	G→P5	129.44	±130.1	186.60	±85.56	0.00	LB
19	3-DG→P6	359.01	±340.1	0.00	LB	699.09	±742.7
20	MGO→P7	113.72	±85.8	1171.07	±294.3	0.00	fixed
21	HMF→P8	0.00	LB	58.20	ind*	53.90	±20.04
22	3-DG→HMF	0.00	LB	0.06	±0.3547	0.00	LB

Table 3.4 continue

	Elementary Reaction Steps	Apple Juice 27°C		Orange Juice 27°C		Peach Nectar 27°C	
		k	HPD	k	HPD	k	HPD
1	SUC→FUR + GLU	37.49	±3.198	50.57	±3.297	40.70	±3.513
2	GLU→1,2-ED	0.00	LB	37.68	±9.852	0.90	ind*
3	1,2-ED→GLU	31.66	±45.06	94.25	±32.11	215.45	±230.8
4	1,2-ED→FRU	113.40	ind*	18.83	±44.09	203.38	ind*
5	FRU→1,2-ED	0.30	ind*	12.94	±11.72	0.00	LB
6	1,2-ED→3-DG	48.09	±19.37	0.64	±0.4568	39.32	±13.58
7	1,2-ED→G	227.25	±220.8	1.38	±0.5055	9.23	±6.787
8	3-DG→MGO	3.82	±2.683	189.71	±177.9	0.00	fixed
9	FRU→FFC	0.00	fixed	0.00	fixed	0.00	fixed
10	FFC→FRU	0.00	fixed	0.00	fixed	0.00	fixed
11	FFC→HMF	0.00	fixed	0.00	fixed	0.00	fixed
12	G→T	0.00	fixed	0.00	fixed	29.81	±1.171
13	G→GO	15.14	±1.427	59.29	±51.71	20.61	±16.6
14	G→P1	22.78	±5.007	0.00	LB	63.98	±8.777
15	FRU→P2	0.00	LB	0.00	LB	40.41	±5.597
16	T→P3	0.00	fixed	0.00	fixed	0.00	LB
17	GO→P4	0.00	LB	706.67	±696.9	24.64	±26.02
18	G→P5	612.73	±735.1	0.00	LB	0.00	LB
19	3-DG→P6	135.57	±90.58	0.00	LB	122.48	±66.97
20	MGO→P7	11.21	±90.98	1511.32	±1487	0.00	fixed
21	HMF→P8	0.00	fixed	0.00	fixed	0.00	fixed
22	3-DG→HMF	0.00	fixed	0.00	fixed	0.00	fixed

**ind*, indeterminate, which means a large uncertainty in the estimated parameter within 95% confidence interval. *LB, lower bound.*

The simplification of reaction mechanism was performed through excluding some of the reaction steps by model discrimination discussed hereinafter. As shown in **Figure 3.7**, the numbers in the network indicate each elementary reaction step which is characterized by a reaction rate constant (k) as parameters. The predicted data and rate constants of each reaction were obtained by solving the differential equations simultaneously. Model discrimination was performed in order to obtain the best model fitting to the experimental data and reduce unquantified parameters. Differential equations for each reaction step which were built from the kinetic model shown in **Figure 3.7** as given below:

$$\frac{d[SUC]}{dt} = -k_1[SUC]$$
$$\frac{d[GLU]}{dt} = k_1[SUC] + k_3[1,2-enediol] - k_2[GLU]$$
$$\frac{d[FRU]}{dt} = k_1[SUC] + k_4[1,2-enediol] - (k_5 + k_{10})[FRU]$$
$$\frac{d[HMF]}{dt} = k_{11}[FFC] + k_8[3-DG]$$
$$\frac{d[3-DG]}{dt} = k_6[1,2-enediol] - (k_8 + k_9 + k_{15})[3-DG]$$
$$\frac{d[G]}{dt} = k_7[1,2-enediol] - (k_{12} + k_{13} + k_{14})[G]$$
$$\frac{d[GO]}{dt} = k_{12}[G]$$
$$\frac{d[T]}{dt} = k_{13}[G]$$
$$\frac{d[MGO]}{dt} = k_9[3-DG]$$
$$\frac{d[1,2-enediol]}{dt} = k_2[GLU] + k_5[FRU] - (k_3 + k_4 + k_6 + k_7)[1,2-enediol]$$
$$\frac{d[FFC]}{dt} = k_{10}[FRU] - k_{11}[FFC]$$
$$\frac{d[P_1]}{dt} = k_{14}[G]$$
$$\frac{d[P_2]}{dt} = k_{15}[3-DG]$$

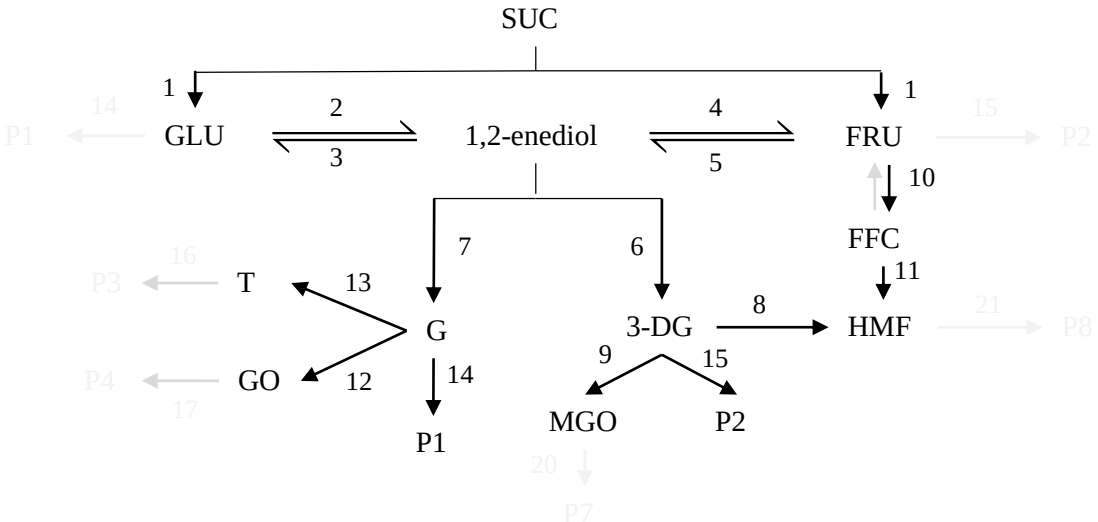

Figure 3.7. Proposed mechanistic model for sugar degradation during storage of apple juice, orange juice and peach nectar. SUC, sucrose; GLU, glucose; FRU, fructose; FFC, fructofuranosyl cation; 3-DG, 3-deoxyglucosone; G, glucosone; MGO, methylglyoxal; GO, glyoxal; T, threosone; HMF, 5-hydroxymethyl-2-furfural; P, products.

A sequence of sugar degradation reactions is characterized by the initial enolisation known as "Lobry de Bruyn-Alberda van Ekenstein rearrangement" and is followed by further reactions like dehydration, oxidation, retro-aldol condensation. As it is well known from the literature, the formation of isomeric carbohydrates starts with the opening of hemiacetal ring followed by enolization via acid- and base- catalyzed mechanisms. Moreover, the interconversion of sugars increases with increasing pH [4]. Under acidic conditions, dehydration, which lead to furaldehyde compounds is favored rather than the isomerization [253]. The kinetics of isomerization of glucose and fructose under alkaline and low moisture conditions were investigated before [6, 225]. However, this has not defined for acidic and aqueous media such as juices until now. In this respect, the importance of enolisation in fruit juices was tested by including and excluding the 1,2-enediol intermediate (unquantified) from the comprehensive reaction network. When 1,2-enediol intermediate was eliminated from the mathematical model, the reaction rate of each step, especially those of sucrose hydrolysis could not be well estimated and the model fittings to the experimental data were not well obtained. The results indicated that the enolisation of glucose and fructose was a significant step under the stated conditions. Other model practices were also performed in order to test whether further reaction steps of certain products (quantitively not important compounds such as MGO and threosone) can be omitted from the kinetic model. After several possible chemical mechanisms has been practiced as summarized above, the proposed sugar degradation reaction mechanism shown in **Figure 3.7** was found to be the best fit to the experimental data with acceptable interval parameters (**Table 3.5**). Since it is known that sugar degradation reactions obey first degree reaction kinetics, these elementary reaction steps in the proposed model were defined by differential equations in accordance with first degree kinetics [51].

Table 3.5. Estimated reaction rate constants (k, week^{-1} x 10^3) with 95% highest posterior density (HPD) intervals at different temperatures according to the proposed kinetic model in **Figure 3.7** for sugar degradation reactions during storage of apple, orange juices and peach nectars.

		Apple Juice				Orange Juice				Peach Nectar			
	Elementary Reaction	**37°C**		**27°C**		**37°C**		**27°C**		**37°C**		**27°C**	
	Steps	**k**	**HPD**	**k**	**HPD**	**k**	**HPD**	**k**	**HPD**	**k**	**HPD**	**k**	**HPD**
1	SUC→FUR + GLU	123.7	±10.03	36.3	±3.06	147.4	±9.68	50.7	±3.29	147.2	±13.1	44.8	±3.79
2	GLU→1,2-ED	598.4	ind*	33.1	±22.02	58.3	±29.84	37.5	±8.48	3690.7	ind*	595.5	±556.3
3	1,2-ED→GLU	781.8	±111.70	66.6	±104.20	495.4	±277.1	99.3	±29.90	2047.3	±161.5	441.6	±531.4
4	1,2-ED→FRU	544.3	±390.00	90.5	±83.33	28.5	±30.53	39.2	±34.08	407.8	±282	65.0	±68.81
5	FRU→1,2-ED	143.1	±98.33	0.0	±0.0	0.9	fixed	17.8	±9.34	684.2	±463.6	105.0	±66.6
6	1,2-ED→3-DG	0.7	±0.08	2.1	±1.22	6.7	±2.14	0.4	±0.05	0.3	±0.037	36.4	±33.03
7	1,2-ED→G	2.0	±0.27	9.6	±5.73	20.4	±6.59	1.2	±0.14	0.0001	±0.002	0.00002	±0.009
8	3-DG→HMF	0.7	±1.98	0.0	fixed	2.0	±0.68	0.0	fixed	11.8	±2.79	0.0	fixed
9	3-DG→MGO	4.6	±0.41	3.5	±0.43	6.0	±0.60	11.7	±2.51	0.0	fixed	0.0	fixed
10	FRU→FFC	0.2	±0.04	0.0	±0	7.1	±2.26	0.0	±0.0	22.6	±12.69	0.0	fixed
11	FFC→HMF	5.3	ind*	0.0	fixed	0.0	±0.02	0.0	fixed	0.4	±0.22	0.0	fixed
12	G→GO	10.0	±1.02	5.1	±1.36	9.2	±0.74	8.7	±1.49	23.2	±1.57	4.8	±1.25
13	G→T	0.0	fixed	0.0	fixed	0.0	fixed	0.0	fixed	22.1	±1.19	29.4	±1.29
14	G→P1	56.1	fixed	562.0	fixed	453.2	fixed	21.1	fixed	0.0	fixed	29.7	±17.9
15	3-DG→P2	107.6	fixed	123.3	fixed	228.2	fixed	71.7	fixed	427.2	fixed	94365.5	±58130

*_ind_, indeterminate, which means a large uncertainty in the estimated parameter within 95% confidence interval.

In order to determine the temperature dependence of each reactions during storage, Arrhenius equation was used with evaluation of all data (**Table 3.6**). The activation energies of most of the chemical reactions were reported as at a level of 120 kJ/mol [254]. According to the results, the activation energies for each reaction step was found to be in the range of -230 and 122 kJ/mol. It is clearly seen that, especially hydrolysis of sucrose into glucose and fructose was fairly temperature dependent in juices (E_a; 48 - 56 kJ/mol). The calculated negative activation energy values might indicate that no energy barriers were presented in these reaction steps due to the accumulation of intermediate compounds [255].

Table 3.6. Activation energies (Ea, kJ/mol) according to the proposed kinetic model in Figure 3 for sugar degradation during storage of apple, orange juices and peach nectars.

	Elementary Reaction Steps	Apple Juice		Orange Juice		Peach Nectar	
		Ea	R^2	Ea	R^2	Ea	R^2
1	SUC→FUR + GLU	54.16	0.96	55.51	0.98	48.23	0.97
2	GLU→1,2-ED	11.10	0.03	48.19	0.99	34.95	0.43
3	1,2-ED→GLU	-5.94	0.01	56.29	0.90	79.16	0.86
4	1,2-ED→FRU	2.41	0.00	-81.09	0.96	-18.61	0.51
5	FRU→1,2-ED	65.09	0.53	-145.77	0.97	19.99	0.18
6	1,2-ED→3-DG	-20.02	0.52	112.67	0.94	-141.35	0.90
7	1,2-ED→G	10.12	0.07	94.62	0.88	23.43	0.72
8	3-DG→HMF	-127.02	0.51	-107.71	0.51	-77.04	0.51
9	3-DG→MGO	18.43	1.00	-24.21	0.90	-	
10	FRU→FFC	-30.71	0.03	65.16	0.21	-65.77	0.51
11	FFC→HMF	-90.94	0.51	-230.31	0.51	-188.99	0.51
12	G→GO	-9.82	0.72	-0.15	0.02	-97.29	0.71
13	G→T	-		-		26.54	0.79
14	G→P1	-36.53	0.46	15.13	0.06	54.27	0.34
15	3-DG→P2	-26.52	0.97	-41.38	0.56	121.55	0.41

The proposed reaction mechanism shown in **Figure 3.7** contains the steps of (i) sucrose hydrolysis and isomerization of glucose and fructose, (ii) formation of HMF, (iii) formation and elimination of α-carbonyl compounds.

(i) Sucrose hydrolysis and isomerization of glucose and fructose

The degradation of sucrose in the absence of amino groups consists of a complex reaction mechanism, which depends on several environmental conditions as pH, water activity, and temperature. It is known that the reaction occurs very rapidly in acidic conditions and low moisture systems [235]. Sucrose hydrolysis takes place by protonation of the glycosidic linkage.

The H^+ used in this step can be derived from water dissociation at high temperatures while it can be formed from acid hydrolysis in aqueous systems [256]. From this point of view, acid hydrolysis of sucrose was determined as the main chemical reaction responsible for the formation of α-dicarbonyl compounds in juices (pH 3.4). The hydrolysis rates of sucrose to glucose and fructose (k_1) were 0.04, 0.05 and 0.05 $week^{-1}$ at 27°C; 0.1, 0.2 and 0.2 $week^{-1}$ at 37°C for apple juice, orange juice and peach nectar, respectively (**Table 3.5**).

The concentrations of glucose and fructose increased with the decrease of sucrose in a nonlinear relation. Fructose is known to be the main product of glucose and fructose isomerization in the 1,2-enolization reaction called the Lobry De Bruyn-Alberda Van Ekenstein transformation [213]. Epimerization of glucose to mannose also occurs through this transformation, but it is reported that the glucose – mannose transformation is not as significant as the glucose-fructose interconversion [87]. Indeed, mannose formation could not be detected in furtherance. Thereby, this isomerization was not included in the proposed model. Considering the reaction rate constants, the transformation of glucose – 1,2-enediol (k_2, k_3) was faster than the epimerization of fructose to 1,2-enediol (k_4, k_5) during storage of all juices at both temperatures (**Table 3.5**). This indicated that fructose underwent the further reactions like degradation to HMF in parallel with the enolisation reactions. On the other hand, the reaction rate constants of glucose to 1,2-enediol (k_2) transformation steps were estimated as indeterminate in apple juice and peach nectar as given in **Table 3.5**. A possible explanation for this observation is that 1,2-enediol intermediate could not be quantified. Hence, an unquantified compound causes the highest intervals or indetermination of parameters in a mechanistic model. But as mentioned in model discrimination, 1,2-enolisation reaction was crucial to create the mechanistic model of the sugar degradation reactions during storage of juices. Contrarily, Kocadagli and Gokmen [6] indicated that 1,2-enediol formation in interconversion of glucose-fructose was unnecessary, since glucose to fructose proceeded faster with very high initial rate during caramelisation reaction of glucose under the heating conditions of 160 – 200 °C up to 20 min. Since the reactants were in solid state in that study, their melting became important for the reaction to proceed, and in the absence of amino compounds, the open chain form of glucose increased and it rapidly isomerized to fructose after melting. This suggests that the physical form of the reactants in food systems is a strong determinant in whether the enolisation step was important or not.

(ii) Formation of HMF

As noted before, HMF accumulated only at 37°C in the samples during storage. HMF forms from sugars through 2 possible pathways: (i) dehydration of 3-DG and (ii) dehydration of fructose. However, it has been previously reported that the pathway of 3-DG dehydration to HMF is less efficient in comparison to the fructose dehydration [5]. Comparing to 3-DG, HMF formation from fructose were found almost 7, 4 and 2 times higher in apple juices , orange juices and peach nectars, respectively. Similarly, for dry conditions, Kocadagli and Gokmen [6] reported that HMF formation from fructose was kinetically predominant pathway in comparison to the 3-DG pathway in a heated glucose/wheat flour model systems. According to the proposed model, HMF formed through the fructofuranosyl cation (FFC) which was generated by dehydration of fructose under acidic conditions of juices. The rate constants of fructose dehydration to FFC (k_{10}) were found significantly higher than that of FFC dehydration to HMF (k_{11}) in orange juice and peach nectar (**Table 3.5**). For apple juice, the rate constant of HMF formation from FFC (k_{11}) showed a large uncertainty in the estimated parameter within 95 % confidence interval. The cyclic forms of fructose may lead to FFC without the requirement of thermodinamically controlled ring opening process [5]. In HMF formation through fructose pathway, formation of FFC from fructose was found to be the fast step and the rate determining step was the HMF formation from FFC.

(iii) Formation and elimination of α-dicarbonyl compounds

Many sugar dehydration products may form through enediol intermediates in fruit juices during processing and storage periods [4]. Dehydration and oxidation reactions of 1,2-enediol intermediate results in the formation of 3-DG and glucosone as shown in **Figure 3.8-3.10**. Kocadagli and Gokmen [6] reported that heating at elevated temperatures accelerates sugar dehydration reaction which results in the formation of 3-DG especially under low moisture conditions.

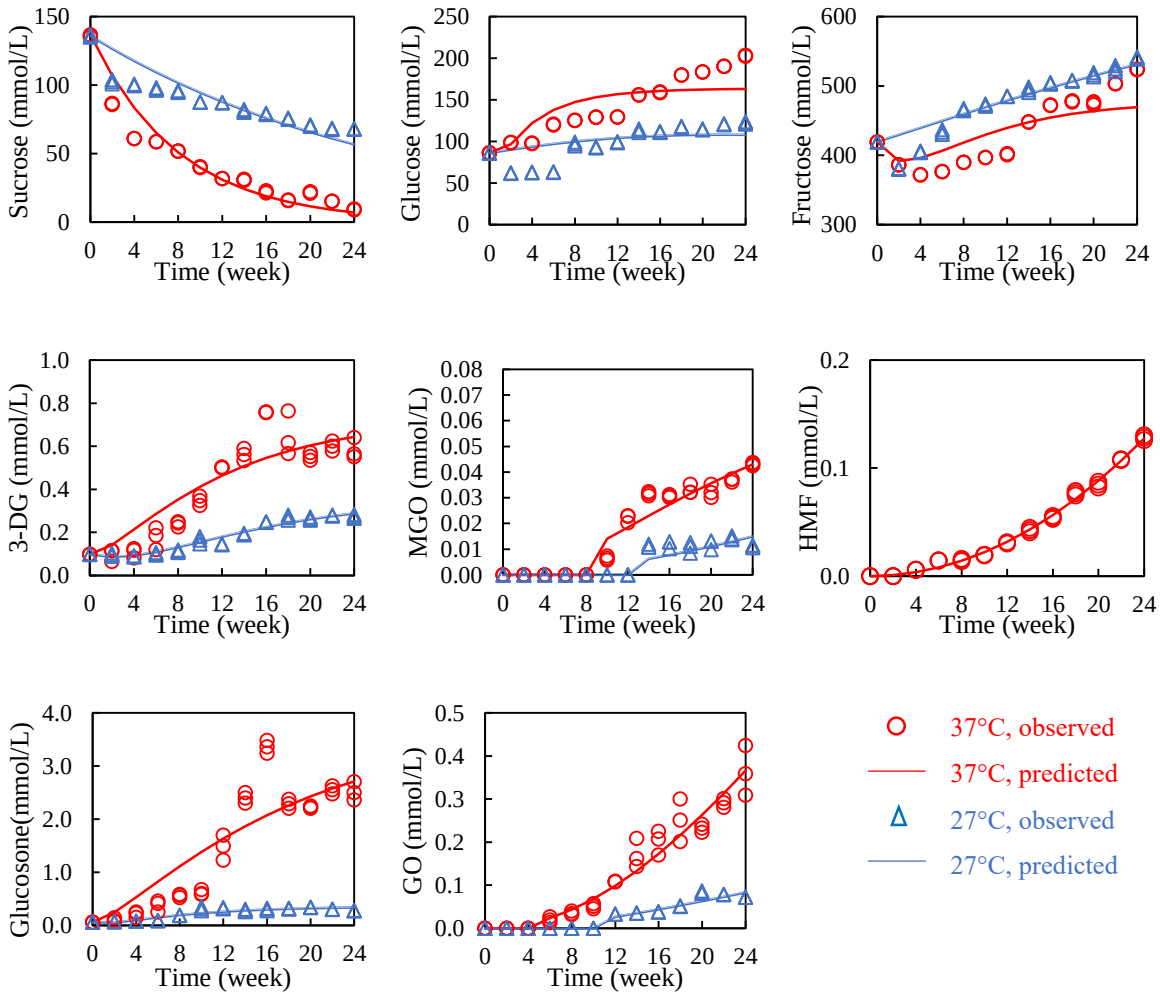

Figure 3.8. Kinetic model fit (lines) to the experimental data (symbols) of reactants and products during storage of apple juice.

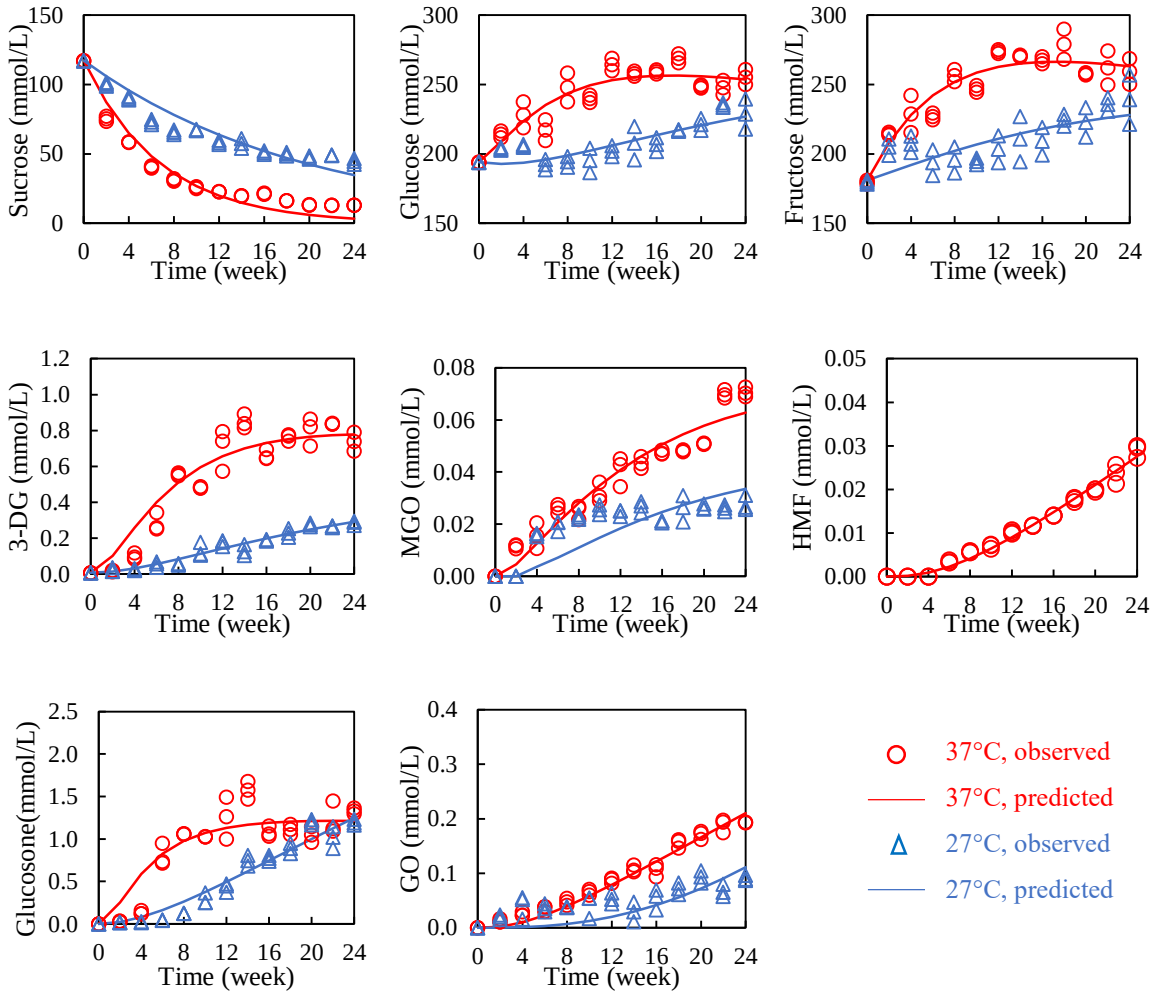

Figure 3.9. Kinetic model fit (lines) to the experimental data (symbols) of reactants and products during storage of orange juice.

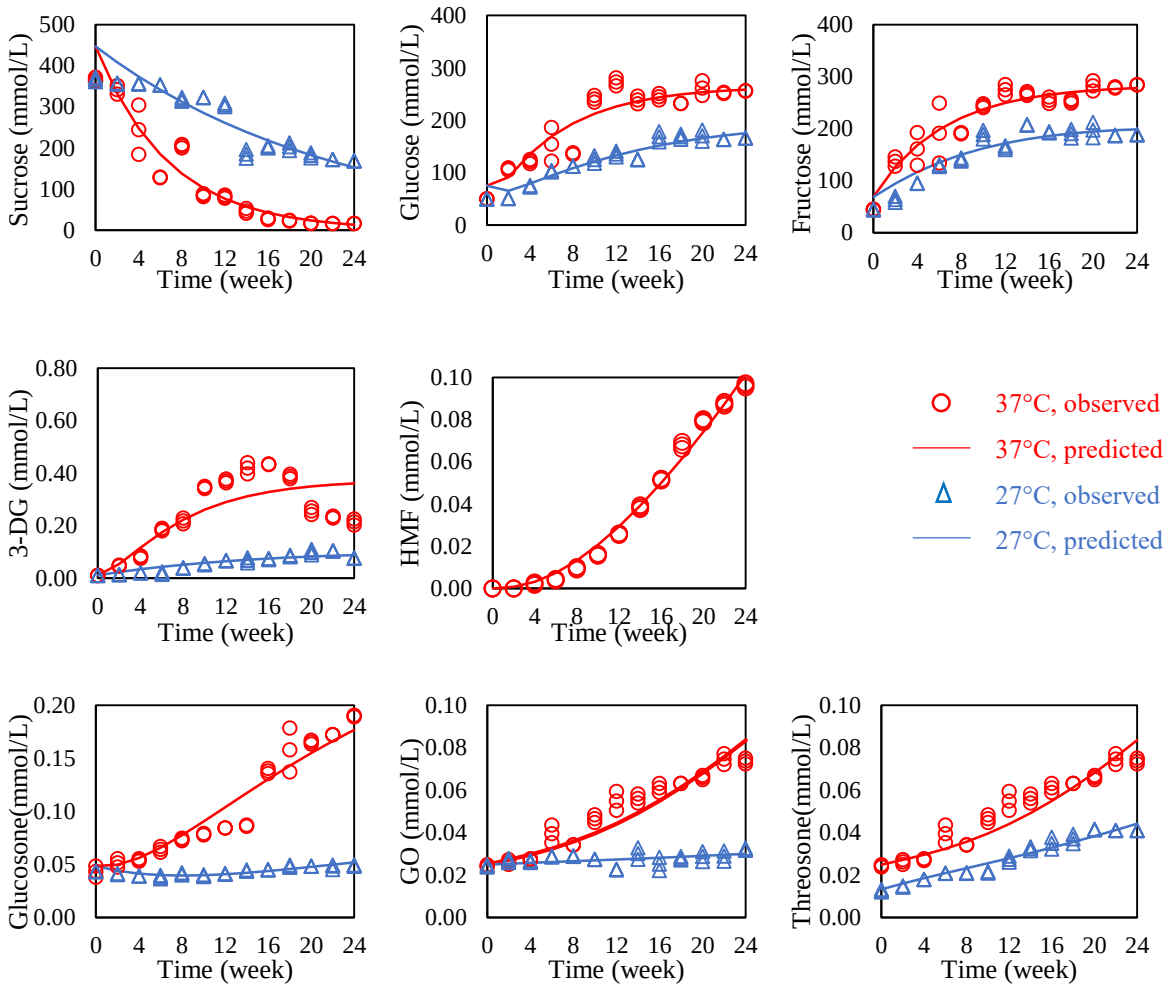

Figure 3.10. Kinetic model fit (lines) to the experimental data (symbols) of reactants and products during storage of peach nectar.

Among the samples, 3-DG formation was the fastest in orange juice. It should be noted here that orange juice contained approximately 2.4 times and 3.2 times more glucose than apple juice and peach nectar, respectively. The rate constants of 3-DG formation (k_6) were estimated as 6.7×10^{-3} week^{-1}, 0.7×10^{-3} week^{-1}, and 0.3×10^{-3} week^{-1} for orange juice, apple juice, and peach nectar, respectively (**Table 3.5**). Hollnagel and Kroh [90] reported that glucose forms more dicarbonyl fragments than fructose, since fructose tends to yield cyclic products rather than the fragmentation products. Although much less has been published on the chemistry of glucosone formation in aqueous and acidic food systems, removal of 2 protons, especially by transition metal catalysis in the presence of molecular oxygen may lead to glucosone [2]. The rate of glucosone formation through 1,2-enediol intermediate (k_7) increased (±95% HPD) with increasing storage temperature in all samples except apple juice (**Table 3.5**). The rate constants

of glucosone formation (k_7) were estimated as 0.020 $week^{-1}$, 0.002 $week^{-1}$, and $0.03x10^{-3}$ $week^{-1}$ for orange juice, apple juice, and peach nectar, respectively. A possible explanation for lower glucosone concentrations in peach nectar could be due to its decomposition to GO and threosone. GO and threosone can be formed by *retro*-aldolisation of glucosone [4]. Paravisini and Peterson [29] and Paravisini and Peterson [30] reported that threosone was highly quantified in orange and apple juices during storage. At 37 °C, the rate constants of GO formation (k_{12}) were estimated as 0.010 $week^{-1}$, 0.009 $week^{-1}$, and 0.023 $week^{-1}$ for apple juice, orange juice, and peach nectar, respectively. Threosone formation (k_{13}) could only be detected in peach nectar with a rate constant of 0.022 $week^{-1}$ and 0.029 $week^{-1}$ at 37 and 27 °C, respectively.

MGO can be formed by *retro*-aldolization of 3-DG or 1-deoxyglucosone [4]. Since it was not detected in peach nectar, the rate constant of MGO formation was fixed to zero. In general, the reaction rate constants of MGO formation (k_9) were not well estimated within the 95 % confidence interval. The low concentrations of MGO might cause high standard deviations on the estimated rate constants. In this regard, the importance of MGO was tested. When MGO was excluded from the comprehensive reaction network, the model fit and the reaction rates were not given well estimated. Therefore, estimated rate constants together with the model fit imprecisely were acceptable when MGO was included in the model. On the other hand, it is possible to say that degradation of 3-DG to MGO (k_9) was kinetically more important than its degradation to HMF (k_8) as understood from the estimated rate constants. 1-DG is formed by 2,3-enolization of fructose under alkaline conditions [6]. Expectedly, 1-DG was not detected in the acidic samples in agreement with the study of Hellwig, et al. [59]. Thereby, this pathway was not included in the mechanistic model.

3.4. CONCLUSION

In this study, we built a multi-response kinetic model to provide a deep understanding of the most possible pathway of sugar degradations leading to α-dicarbonyl compounds in fruit juices during storage. The proposed model described well the fate of sugars and major α-dicarbonyl compounds in apple juice, orange juice, and peach nectar. It was possible to unravel complicated reaction routes taking place in fruit juice using this model. Isomerization of glucose and fructose via 1,2-enolization, formation of HMF from fructose rather than 3-DG pathway, MGO formation through degradation of 3-DG and GO formation through retro-aldolization of glucosone were kinetically important reaction steps in stored juice samples. On the other hand, the results clearly indicated that the formation rates of α-dicarbonyl compounds

in peach nectar as a sucrose added beverage were lower than in apple and orange juices as no added-sugar juices due to the full acetal structure of sucrose compared to the hemi-acetal structure of monosaccharides. Another striking result from the study is that the main α-dicarbonyl compound was glucosone in apple and orange juice contrary to the literature reported that 3-DG is the major. Identifying and quantifying other intermediate compounds could contribute to further knowledge about sugar degradation reactions taking place in juices as sugar rich and acidic systems.

CHAPTER 4

CHANGES IN α-DICARBONYL COMPOUNDS AND 5-HYDROXYMETHYLFURFURAL DURING STORAGE OF FRUIT JUICE CONCENTRATES AND DRIED FRUITS: EFFECT OF INITIAL CONCENTRATES AND pH

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4.1. INTRODUCTION

The concentration operation of fruit juices and drying of fruits are the improtant ways to preserve the fruit products and extend their shelf-lives. Thus, they can be stored at ambient temperatures for a long time by decreasing the water content. However, prolonged storage and thermal process cause deteriorative reactions resulted in the formation of α-dicarbonyl compounds and 5-Hydroxymethylfurfural (HMF) through Maillard reaction and caramelization as mentioned detailed in Chapter 1 [2]. Historically, most of the studies on the deteriorative reactions have focused on the quantitative changes in the initial reactants and changes in the color degree during the storage of fruit products [30, 50]. Less has been reported the contribution of the chemical markers such as α-dicarbonyl compounds and HMF to such reactions. Among them Paravisini and Peterson [30] have indicated that the Maillard reaction have a significant role in non-enzymatic browning reactions due to the increase in reactive carbonyl species and the losses of amino acids in orange juice during storage. However, the contribution of Maillard reaction has not been confirmed by showing adduct formation in this study [30]. On the other hand, Olano, et al. [257] have stated that caramelisation is favored in comparison with Maillard reaction on non-enzymatic reactions in aqueous acidic sugary systems such as dessert wine. In support, Kroh [4] have showed that caramelisation is favored rather than Maillard reaction in the aqeous solutions containing fructose and glucose which were adjusted pH 3.5 with different combinations of amino acids. Since the Maillard reaction and caramelization occurs simultaneously in foods, it is not an easy task to clarify these complex reaction networks which were strongly influenced by many factors such as pH, initial reactant concentrations, water activity, temperature, and storage conditions in the complex food systems. Therefore, we first studied on the fruit juices as aqueous real fruit products during storage and we suggested that only sugar degradation reactions were mainly responsible for the formation of α-dicarbonyl compounds and HMF in fruit juices by using multiresponse kinetic modelling approach in this thesis study (Chapter 3). In this section of the study, the first aim is to investigate the formation of α-dicarbonyl compounds and HMF in mid-/low-moisture fruit products (fruit juice concentrates) with different Brix levels and in dried fruit products with different pH values in order to understand the effect of initial reactant concentrations and the effect of pH. In this respect, apple juice concentrate was selected as the most consumed juice type in the world, whereas pomegranate juice concentrate was representative for the ingredient used directly in highly concentrated form in foods. Date, grape, and blueberry were selected as

the representative for nötr, acidic and high-acidic fruits, respectively. The second aim is to investigate the role of Maillard reaction in fruit products during storage in depth by means of high-resolution mass spectrometry scan analysis.

4.2. EXPERIMENTAL

4.2.1. Chemicals and consumables

Formic acid (98%) was purchased from JT Baker (Deventer, The Netherlands). HMF (98%) was purchased from Acros (Geel, Belgium). 3-Deoxyglucosone (3-DG) (75%), glucosone (≥98%), quinoxaline (99%), 2-methylquinoxaline (97%), 2,3-dimethylquinoxaline (97%), o-phenylenediamine (98%), diethylenetriaminepentaacetic acid (98%), 5-methylquinoxaline (98%), L-theanine (≥98%), methanol, and acetonitrile were purchased from Sigma-Aldrich (Steinheim, Germany). Disodium hydrogen phosphate anhydrous and sodium dihydrogen phosphate dihydrate were purchased from Merck (Darmstadt, Germany). The Carrez I and Carrez II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 mL of water, respectively. Ultra-pure water was used throughout the experiments (Milli Q-System, Millipore, Milford, MA). Syringe filters (nylon, 0.45 lm) and Oasis HLB cartridges (30 mg, 1mL) were supplied by Waters (Milford, MA).

4.2.2. Sample Preparation and Storage

Apple juice concentrate was selected as the most consumed juice type in the world, whereas pomegranate juice concentrate was representative for the ingredient used directly in highly concentrated form in foods like salad dressings, starters. They were analyzed to investigate the effect of different concentration levels (30, 50, 65/ 70 °Bx) on the formation of α-dicarbonyl compounds and HMF. The apple juice concentrate (70 °Bx) and pomegranate juice concentrate (65 °Bx) samples were supplied immediately after the production from a universal fruit juice company in Turkey. The apple and pomegranate juice concentrates with 50°Bx and 30°Bx were prepared from 70 °Bx apple juice concentrate and 65 °Bx pomegranate juice concentrates, respectively, with sterile deionized water under aseptic conditions. Then, all concentrates were divided into sterile glass test tubes and pasteurized in a water-bath (85 °C - 10 min) in threes at a batch. Non-pasteurized apple and pomegranate juice concentrates were kept as control samples. The pasteurized samples were stored at 37 °C for 20 weeks and the sub-samples were taken from the stored ones 3 parallels in every 2 weeks. All samples were kept frozen at -18 °C prior to analysis.

Date, grape, and blueberry were selected as the representative for nötr, acidic and high-acidic fruits, respectively, to investigate the effect of pH. The blueberry (fresh), grape (sultana type - fresh), and date palm fruit (mid-fresh) samples were obtained from local markets in Turkey. Fresh grape and blueberry samples were pretreated by immersing samples into hot water for 15 minutes before drying. All samples were dried in an oven (Memmert UNE 400; Memmert GmbH + Co.KG, Schwabach Germany) at a temperature of 70 °C until the aw of the samples were approximately 0.6 (approximately for 17- 18 h). Dried samples were vacuum packaged with heat seal as 10 g portions using commercially available packaging materials, which were 9 x 18 cm packages made from polyethylene terephthalate (PET), aluminum (AL), and polyethylene (PE) barrier films. The samples were stored at 37 °C for a period of 6 months and the sampling was performed in triplicate and biomonthly. All samples were kept frozen at -18 °C prior to analysis.

4.2.3. Extraction

Five hundred milligrams of apple and pomegranate juice concentrates (70/65 and 50 °Bx) and 500 μL 30 °Bx concentrates were diluted with 1 mL water, vortexed for 1 min, and then centrifuged at 12,000 xg for 3 min. The diluted supernatants were used for analysis. Raisins, dried dates, and dried blueberries (2 g) were triple extracted with 40 mL of water (20-10-10 mL) by using firstly ultra-turrax homogenizing and then vortexing for 3 min. After centrifugation at 10,000 xg for 5 min, combined supernatants were used for analysis. The clear supernatant-A (supernatants of the concentrates and dried fruits) was used for the determination of α-dicarbonyl compounds, free amino acids, and the adducts and Schiff bases of α-dicarbonyl compounds, HMF with amino acids in the samples.

The diluted supernatants of the concentrates and the combined supernatants of the dried fruits were cleaned up for HMF and sugar analysis. For Carrez clarification, 1 mL of the supernatant was mixed with 50 μL of Carrez I and 50 μL of Carrez II solutions. The mixture was centrifuged at 10,000 xg for 5 min. The clear supernatant-B was used for the determination of HMF and sugar in the samples.

4.2.4. Analysis of sugars

Sugars were determined using an analytical method described elsewhere with minor modifications [223]. One mL of the clear supernatant-B was passed through a preconditioned (by passing 1 mL methanol and 1 mL water) OASIS HLB cartridge. The first 8 drops of the

eluent were discarded and the rest was collected into a vial for analysis. The analysis was performed on an Agilent 1200 HPLC system (Waldbronn, Germany) equipped with a quaternary pump, and autosampler coupled with an Agilent 1100 refractive index detector and temperature-controlled column oven. The chromatographic separations were performed on a Shodex Sugar SH-1011 column (300 mm x 8 mm, 6 μm) conditioned at 50 °C. The concentrations of sucrose, glucose, and fructose were calculated from the calibration curves built for each compound in the range between 0.25 and 2.5 g/L (0.25, 0.5, 0.75, 1, and 2.5 g/L).

4.2.5. Analysis of free amino acids

Free amino acids were determined using an analytical method described elsewhere with some modifications [223]. The clear supernatant-A was diluted with water prior to analysis and then centrifuged at 12,000 xg for 3 min. The supernatant was immediately filtered through a 0.45 μm syringe filter and put into an autosampler vial. The samples were analyzed by an Agilent 1260 Infinity II system coupled to a triple quadrupole detector operated in positive electrospray ionization mode. Chromatographic separations were performed on a Merck ZIC®-HILIC column (150 x 4.6 mm, 3.5 μm, 200Å) by using a gradient mixture of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 1 mL/min. L-theanine was used as an internal standard (0.5 mg/L). Quantifications were performed using the calibration curves built for all amino acids in a range between 0.1 and 5.0 mg/L (0.1, 0.25, 0.5, 1, 2.5 and 5.0 mg/L).

4.2.6. Analysis of α-dicarbonyl compounds

Derivatization. α-Dicarbonyl compounds were determined using an analytical method based on the derivatization with o-phenylenediamine described elsewhere [8]. The mixture was kept at room temperature, at dark for 2 h prior to measurement.

UPLC-ESI-MS Measurement. α-Dicarbonyl compounds were analyzed by an Agilent 1260 Infinity II system coupled to a triple quadrupole detector operated in positive electrospray ionization mode. Chromatographic separations were α-dicarbonyl compounds performed on a Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 μm) by using a gradient mixture of 1% formic acid in water (A) and 1% formic acid in methanol (B) at a flow rate of 1 mL/min. The eluent composition starting with 20% B and then linearly increased to 60% in 8 min. Then, it was decreased to the initial conditions (20% B) in 2 min and held for 3 min. The column was at 40 °C and the autosampler was at 10 °C during the analysis. The electrospray source had the

following settings: gas temperature 250 °C; the gas flow of 10 L/min; nebulizer 60 psi; capillary voltage of 1.5 kV; sheat gas temperature 400 °C; sheat gas flow 12 L/min; nozzle voltage 500 V.

α-Dicarbonyl compounds were identified by selected ion monitoring (SIM) mode and the SIM ions [$M+H^+$] were as follows for the quinoxaline derivatives of glucosone; 251, 3-DG; 235, (E)-3,4-DGE and (Z)-3,4-DGE; 217, threosone; 191, 3-DT; 175.1, DA; 159, MGO; 145, GO; 131. A dwell time was set at 90 ms for each. Working solutions of glucosone and 3-DG were derivatized and then the concentrations of glucosone, 3-DG, quinoxaline, and 2-methylquinoxaline were calculated using calibration curves built in the range between 0.1 and 5 mg/L (0.1, 0.5, 1, 2, 5 mg/L). 5-Methylquinoxaline was used as an internal standard (0.5 mg/L). Also, the calibration curve of glucosone was used for semi-quantitation of threosone derivatives and the 3-DG calibration curve was used for semi-quantitation of 3-DT, (E)-3,4-DGE, and (Z)-3,4-DGE derivatives since both have the same proton-accepting groups. All working solutions were prepared in water.

4.2.7. Analysis of HMF

HMF was determined using an analytical method described elsewhere with some minor modifications [160]. One mL of clear supernatant-B was filtered through a 0.45 μm syringe filter and put into an autosampler vial. The filtered sample was injected onto an Agilent 1200 series HPLC system consisting of a quaternary pump, an autosampler, a diode array detector, and a temperature-controlled column oven. The chromatographic separations were performed on an Atlantis dC18 column (250 × 4.6 mm, 5 μm) using a gradient mixture of (A) 10 mM formic acid in water and (B) acetonitrile as the mobile phase at a flow rate of 1 mL/min at 30 °C. The concentration of HMF was calculated using a calibration curve built in the range between 0.1 and 20 mg/L (0.1, 1, 2, 5, 10, 20 mg/L).

4.2.8. Analysis of the adducts and Schiff bases of α-dicarbonyl compounds and 5-hydroxymethylfurfural with amino acids by high-resolution mass spectrometry

The adducts were determined using an analytical method described elsewhere with minor modifications [258]. The clear supernatant-A was diluted with water prior to analysis and then centrifuged at 12,000 xg for 3 min. The supernatant was immediately filtered through a 0.45 μm syringe filter and put into an autosampler vial. The samples were analyzed by a Thermo Scientific Dionex Ultimate Rapid Separation RSLC system coupled to a Thermo Scientific Q Exactive Orbitrap HRMS. The HRMS system was operated in both positive and negative

modes. The chromatographic separations were performed on a Thermo Scientific Accucore aQ C18 column (100 mm x 2.1 mm i.d., 2.6 μm) by using a gradient mixture of 0.1% formic acid in water (A) and methanol (B) at a flow rate of 0.25 mL/min (30 °C). The corresponding ions were extracted from the total ion chromatograms to confirm the presence of the reaction intermediates between amino acids and α-dicarbonyl compounds, HMF in the apple juice concentrates, and raisins during storage.

4.2.9. Determination of the levels of pH, Brix, and aw

The pH of the clear Supernatant-A was measured using a PHM210 model pH meter (MeterLab, France) and the brix of the apple and pomegranate juice concentrate samples was measured using a Pocket Pal-3 model refractometer (Atago, Japan). The aw of the dried date, raisin and dried blueberry samples was measured at 25 °C using a Novasina LabTouch-aw model water activity meter (Lachen, Switzerland).

4.2.10. Statistical analysis

All data of the 70, 65, 50, and 30 °Bx of the apple and pomegranate juice concentrates were adjusted to 11.2 °Bx according to the reference levels of directives of the European Parliament and the Council [259]. The elimination of the variation of the analyte concentrations due to the different Brix levels was required to compare the results with each other. The data were subjected to analysis of variance (one-way ANOVA). The Statistical Package for the Social Sciences (SPSS 17.0) statistical package was used for the evaluation of statistical significance of the differences between mean values by the Duncan test. $P<0.05$ was considered to be statistically significant for the results.

4.3. RESULTS AND DISCUSSION

4.3.1. Changes in pH, Brix and aw values

The pH, the concentration of initial reactants, and aw are well known as the important factors affecting the reactions occurred in foods during storage. The pH and Brix value, as shown in **Table 4.1**, showed no significant change ($p > 0.05$) in all types (30, 50, 70/65 °Bx) of apple and pomegranate juice concentrates during the storage period of 20 weeks, concordantly with our previous study on the formation of α-dicarbonyl compounds in fruit juices during storage [260]. Similarly, there was no significant change ($p > 0.05$) in the pH value of raisin and dried blueberry samples during the 6 months storage (**Table 4.2**). The result was consistent with the earlier study reported by Adiamo, et al. [261], where pH showed no significant change in dried

tomato slices during storage (6 months). However, the decrease in pH value from 6.61 to 5.02 was observed in dried date samples during the storage (**Table 4.2**). It is difficult to explain this result, but there are several reasons for the decrease in pH of dried date samples. A possible explanation could be attributed to the reaction of amines and carbonyl groups to form acidic compounds as a result of the degradation of sugars to carboxylic acids [262]. In addition, Swales and Wedzicha [263] indicated that different amino acids might lead to a great variety of different compounds resulting in a different pH value during Maillard reaction. Nevertheless, the explanation of Maillard reaction causing the decrease in pH value in dried date is doubtful, since the pH levels in raisin and dried blueberry were constant during storage. The strongest reason for this might be the mid-fresh date samples containing sulfur for the preservation that causes the decrease in pH during storage. This result was in accordance with the previous report in which the pH of dried apricots decreased with the increase in SO_2 concentration during the storage, due to the increase in hydrogen ion concentration [167].

Table 4.1. The pH, and Brix values in apple, pomegranate juice concentrates during storage.

Time	**Apple Juice Concentrate**		**Pomegranate Juice Concentrate**	
Week	**pH**	**Brix**	**pH**	**Brix**
0	3.7±0.02[a]	30.6±0.30[a]	3.2±0.03[a]	30.7±0.25[a]
10	3.7±0.02[a]	30.7±0.10[a]	3.2±0.02[a]	30.1±0.20[a]
20	3.7±0.02[a]	30.8±0.15[a]	3.2±0.01[a]	30.4±0.60[a]
0	3.7±0.01[a]	50.1±0.40[a]	3.2±0.01[a]	50.1±0.15[a]
10	3.7±0.02[a]	50.1±0.20[a]	3.2±0.01[a]	49.9±0.25[a]
20	3.7±0.01[a]	50.3±0.15[a]	3.2±0.02[a]	50.2±0.25[a]
0	3.7±0.01[a]	70.0±0.10[a]	3.2±0.05[a]	64.4±0.30[a]
10	3.7±0.01[a]	70.3±0.05[a]	3.2±0.02[a]	64.6±0.25[a]
20	3.7±0.01[a]	70.3±0.05[a]	3.2±0.01[a]	64.8±0.10[a]

Mean values in the same column with different letters are significantly different at the 5% confidence level.

The aw value (approximately 0.6) was used as a reference parameter for drying of date, grape, and bluberry, in order to inhibit the microbial growth of microorganisms during the long storage at 37 °C. No significant change ($p > 0.05$) in the aw value (**Table 4.2**) of dried date, raisin, and dried blueberry samples was observed during storage as expected through the impermeable vacuum packaging material, in accordance with the literature [261].

Table 4.2. The pH and aw values in dried date, raisin and dried blueberry during storage.

	Date		Raisin		Blueberry	
Month	**pH**	**aw**	**pH**	**aw**	**pH**	**aw**
0	6.61 ± 0.04^{a}	0.57 ± 0^{a}	3.79 ± 0.01^{a}	0.62 ± 0.02^{a}	2.64 ± 0.03^{a}	0.58 ± 0.01^{a}
1	6.11 ± 0.05^{b}	0.58 ± 0.01^{a}	3.78 ± 0^{a}	0.60 ± 0.01^{a}	2.65 ± 0.05^{a}	0.57 ± 0.01^{a}
2	5.69 ± 0.04^{c}	0.57 ± 0^{a}	3.8 ± 0.03^{a}	0.60 ± 0.01^{a}	2.62 ± 0.05^{a}	0.57 ± 0.00^{a}
3	5.52 ± 0.04^{d}	0.57 ± 0^{a}	3.79 ± 0.03^{a}	0.58 ± 0.01^{a}	2.68 ± 0.07^{a}	0.58 ± 0.00^{a}
4	5.42 ± 0.05^{e}	0.57 ± 0^{a}	3.76 ± 0.03^{a}	0.59 ± 0.01^{a}	2.67 ± 0.07^{a}	0.58 ± 0.01^{a}
5	5.17 ± 0.03^{f}	0.57 ± 0^{a}	3.76 ± 0.04^{a}	0.61 ± 0.00^{a}	2.66 ± 0.04^{a}	0.57 ± 0.00^{a}
6	5.02 ± 0.09^{g}	0.57 ± 0^{a}	3.73 ± 0.02^{ab}	0.62 ± 0.02^{a}	2.67 ± 0.04^{a}	0.57 ± 0.00^{a}

Mean values in the same column with different letters are significantly different at the 5% confidence level.

Hereafter, all evaluations of the analysis of sugars, free amino acids, α-dicarbonyl compounds, and HMF were made considering the reference Brix levels (11.2 °Bx) described in section 4.2.10 for apple and pomegranate juice concentrates.

4.3.2. Changes in the concentrations of sugars and free amino acids

Sugars and amino acids play a crucial role in the Maillard reaction to form α-dicarbonyl compounds and HMF which are responsible for the formation of toxic compounds such as AGEs [1, 5]. The individual concentrations of fructose, glucose, and sucrose were monitored in the apple and pomegranate juice concentrates during the storage for 20 weeks at 37 °C. As shown in **Table 4.3**, the highest sugar type in both apple and pomegranate samples was detected as fructose, glucose, and sucrose, respectively, concordant with the literature [260]. In general, the concentrations of fructose and glucose increased slightly with the decrease of sucrose concentrations in a nonlinear relation during storage. Gürsul Aktağ and Gökmen [260] reported a similar trend in the concentrations of fructose, glucose, and sucrose in apple, orange, and peach juice during storage. The nonlinear relationship between the formation of reducing sugars and the degradation of sucrose was explained by Akhavan and Wrolstad [54] that fructose and glucose formed from the hydrolysis of sucrose might be used in the Maillard reaction. Thus, only slight increase or no changes in the concentrations of fructose and glucose was observed during storage. On the other hand, the sugar concentrations in fruit juice concentrates were not affected by the changes in Brix levels during storage. For the dried fruits (date, raisin, and blueberry), fructose and glucose concentrations were quantified with no significant changes ($p > 0.05$) during the storage for 6 months at 37 °C, as seen in **Table 4.4**. Similarly, Pragati, et al. [264] also reported that no significant change in total sugar content of dehydrated aonla fruit was observed during storage for 3 months.

Table 4.3. The concentrations of sugars (g/100g) and total free aminoacids (mg/kg) in apple and pomegranate juice concentrates during storage. All data were adjusted to 11.2 °Bx.

		Apple Juice Concentrate				Pomegranate Juice Concentrate			
Brix	**Week**	**Sucrose**	**Glucose**	**Fructose**	**Total Free Amino Acid**	**Sucrose**	**Glucose**	**Fructose**	**Total Free Amino Acid**
30	0	1.7 ± 0.0^{j}	2.5 ± 0.0^{a}	6.5 ± 0.1^{a}	895.2 ± 42.6^{de}	1.6 ± 0.1^{c}	3.3 ± 0.1^{a}	3.8 ± 0.1^{a}	748.7 ± 52.9^{d}
30	2	1.6 ± 0.0^{i}	2.6 ± 0.0^{bc}	6.8 ± 0.1^{abcd}	916.5 ± 5.3^{e}	1.5 ± 0.0^{c}	3.5 ± 0.0^{abc}	4.5 ± 0.0^{b}	588.0 ± 61.3^{c}
30	4	1.5 ± 0.0^{h}	2.6 ± 0.0^{bc}	6.8 ± 0.1^{abc}	861.8 ± 15.8^{cd}	1.5 ± 0.0^{bc}	3.7 ± 0.1^{bcde}	4.3 ± 0.2^{b}	584.7 ± 33.7^{bc}
30	6	1.2 ± 0.0^{g}	2.6 ± 0.1^{ab}	6.8 ± 0.2^{ab}	832.9 ± 5.1^{c}	1.4 ± 0.0^{bc}	3.6 ± 0.0^{bcd}	4.5 ± 0.0^{b}	550.0 ± 44.3^{bc}
30	8	1.1 ± 0.0^{f}	2.7 ± 0.1^{cde}	7.2 ± 0.1^{def}	827.0 ± 7.8^{c}	1.3 ± 0.1^{ab}	3.7 ± 0.1^{cde}	4.4 ± 0.1^{b}	555.4 ± 51.4^{bc}
30	10	1.0 ± 0.0^{e}	2.8 ± 0.1^{de}	7.0 ± 0.1^{bcde}	709.5 ± 8.2^{b}	1.2 ± 0.0^{a}	3.8 ± 0.1^{de}	4.4 ± 0.1^{b}	494.6 ± 28.8^{abc}
30	12	0.9 ± 0.0^{d}	2.9 ± 0.0^{e}	7.4 ± 0.1^{f}	734.7 ± 5.1^{b}	1.2 ± 0.0^{a}	3.9 ± 0.1^{e}	4.4 ± 0.0^{b}	576.5 ± 13.4^{bc}
30	14	0.7 ± 0.1^{c}	2.8 ± 0.0^{de}	7.2 ± 0.1^{ef}	746.7 ± 6.4^{b}	1.2 ± 0.0^{a}	3.9 ± 0.1^{de}	4.5 ± 0.0^{b}	516.8 ± 9.2^{abc}
30	16	0.7 ± 0.0^{bc}	2.8 ± 0.0^{de}	7.3 ± 0.0^{ef}	636.8 ± 4.3^{a}	1.2 ± 0.0^{a}	3.8 ± 0.1^{de}	4.4 ± 0.1^{b}	523.4 ± 29.2^{abc}
30	18	0.6 ± 0.0^{ab}	2.9 ± 0.0^{e}	7.4 ± 0.0^{ef}	624.1 ± 10.7^{a}	1.2 ± 0.1^{a}	3.7 ± 0.1^{de}	4.5 ± 0.0^{b}	408.9 ± 21.8^{a}
30	20	0.6 ± 0.0^{a}	2.7 ± 0.0^{bcd}	7.1 ± 0.1^{cdef}	593.2 ± 10.7^{a}	1.2 ± 0.0^{a}	3.5 ± 0.0^{ab}	4.5 ± 0.1^{b}	471.0 ± 43.8^{ab}
50	0	1.9 ± 0.0^{h}	2.7 ± 0.0^{a}	6.9 ± 0.0^{a}	1102.5 ± 52.5^{g}	2.0 ± 0.1^{c}	3.4 ± 0.1^{a}	4.4 ± 0.1^{a}	855.9 ± 73.2^{e}
50	2	1.5 ± 0.0^{g}	2.7 ± 0.0^{ab}	7.0 ± 0.1^{ab}	987.5 ± 5.4^{f}	1.8 ± 0.0^{b}	3.8 ± 0.0^{b}	4.8 ± 0.0^{b}	621.7 ± 15.5^{d}
50	4	1.4 ± 0.0^{f}	2.8 ± 0.0^{cd}	7.1 ± 0.1^{abc}	852.7 ± 15.4^{e}	1.7 ± 0.1^{b}	3.9 ± 0.1^{bcd}	4.9 ± 0.0^{bc}	513.6 ± 12.9^{cd}
50	6	1.2 ± 0.0^{e}	2.8 ± 0.0^{abc}	7.1 ± 0.0^{abc}	730.3 ± 4.6^{d}	1.6 ± 0.0^{b}	3.8 ± 0.1^{b}	4.9 ± 0.1^{b}	450.1 ± 28.1^{bc}
50	8	1.1 ± 0.0^{d}	2.8 ± 0.0^{bcd}	7.2 ± 0.1^{bcd}	711.8 ± 6.6^{d}	1.8 ± 0.1^{b}	3.8 ± 0.1^{b}	4.8 ± 0.1^{b}	383.7 ± 5.8^{ab}
50	10	0.9 ± 0.0^{c}	3.0 ± 0.1^{de}	7.3 ± 0.1^{de}	568.5 ± 6.5^{bc}	1.7 ± 0.0^{b}	4.4 ± 0.0^{e}	4.9 ± 0.1^{bc}	392.7 ± 11.1^{ab}
50	12	0.9 ± 0.0^{c}	2.9 ± 0.0^{cde}	7.5 ± 0.0^{e}	529.8 ± 3.7^{b}	1.7 ± 0.0^{b}	4.0 ± 0.0^{bcd}	4.9 ± 0.0^{bc}	367.2 ± 9.5^{ab}
50	14	0.8 ± 0.0^{b}	3.0 ± 0.0^{ef}	7.5 ± 0.0^{e}	606.3 ± 5.4^{c}	1.7 ± 0.1^{b}	3.9 ± 0.2^{bc}	4.7 ± 0.2^{b}	329.4 ± 5.3^{a}
50	16	0.8 ± 0.0^{b}	3.0 ± 0.0^{ef}	7.4 ± 0.1^{de}	554.0 ± 3.6^{bc}	1.7 ± 0.1^{b}	4.2 ± 0.0^{cde}	5.0 ± 0.1^{bcd}	305.8 ± 5.4^{a}
50	18	0.7 ± 0.0^{b}	3.2 ± 0.1^{fg}	7.3 ± 0.0^{cde}	471.1 ± 7.9^{a}	1.4 ± 0.0^{a}	4.3 ± 0.0^{de}	5.2 ± 0.0^{cd}	293.6 ± 7.6^{a}
50	20	0.6 ± 0.0^{a}	3.2 ± 0.1^{g}	7.4 ± 0.1^{de}	468.2 ± 8.6^{a}	1.4 ± 0.0^{a}	4.2 ± 0.0^{de}	5.3 ± 0.1^{d}	293.5 ± 1.1^{a}
70/65	0	2.2 ± 0.0^{j}	2.2 ± 0.0^{a}	6.2 ± 0.2^{a}	995.5 ± 10.7^{g}	1.8 ± 0.1^{b}	3.5 ± 0.1^{a}	4.5 ± 0.0^{a}	1230.4 ± 96.3^{f}
70/65	2	1.9 ± 0.0^{i}	2.5 ± 0.0^{b}	6.4 ± 0.0^{bc}	579.6 ± 14.2^{f}	1.7 ± 0.0^{ab}	3.8 ± 0.2^{ab}	4.9 ± 0.2^{b}	683.3 ± 13.2^{e}
70/65	4	1.8 ± 0.0^{h}	2.6 ± 0.1^{cdef}	6.4 ± 0.1^{ab}	552.8 ± 45.5^{ef}	1.7 ± 0.1^{ab}	3.9 ± 0.1^{b}	4.9 ± 0.0^{ab}	385.7 ± 10.5^{d}
70/65	6	1.7 ± 0.0^{h}	2.6 ± 0.0^{bcde}	6.5 ± 0.0^{bc}	512.8 ± 19.8^{e}	1.5 ± 0.1^{a}	3.5 ± 0.0^{a}	4.7 ± 0.2^{ab}	418.3 ± 10.7^{d}
70/65	8	1.6 ± 0.1^{g}	2.5 ± 0.0^{bc}	6.7 ± 0.1^{c}	399.2 ± 3.9^{d}	1.5 ± 0.1^{a}	4.0 ± 0.2^{b}	4.7 ± 0.1^{ab}	339.4 ± 13.4^{cd}
70/65	10	1.4 ± 0.0^{f}	2.6 ± 0.1^{bcd}	6.9 ± 0.0^{d}	343.8 ± 4.1^{c}	1.5 ± 0.1^{a}	3.8 ± 0.1^{ab}	4.8 ± 0.1^{ab}	268.9 ± 4.6^{bc}
70/65	12	1.3 ± 0.0^{e}	2.5 ± 0.0^{bc}	7.1 ± 0.0^{de}	344.5 ± 2.3^{c}	1.5 ± 0.1^{a}	3.9 ± 0.0^{ab}	4.9 ± 0.1^{ab}	233.2 ± 17.0^{ab}
70/65	14	1.2 ± 0.0^{d}	2.6 ± 0.0^{bcd}	7.3 ± 0.1^{e}	310.2 ± 2.6^{bc}	1.5 ± 0.0^{a}	3.9 ± 0.1^{b}	5.0 ± 0.1^{b}	214.8 ± 10.7^{ab}
70/65	16	1.1 ± 0.0^{c}	2.8 ± 0.0^{ef}	7.3 ± 0.0^{e}	267.3 ± 1.8^{ab}	1.5 ± 0.0^{a}	4.1 ± 0.1^{b}	5.0 ± 0.2^{b}	202.9 ± 7.5^{ab}
70/65	18	1.0 ± 0.0^{b}	2.8 ± 0.0^{f}	7.3 ± 0.1^{e}	263.9 ± 4.5^{ab}	1.5 ± 0.1^{a}	4.0 ± 0.1^{b}	4.8 ± 0.1^{ab}	202.6 ± 17.2^{ab}
70/65	20	0.9 ± 0.0^{a}	2.7 ± 0.0^{def}	7.3 ± 0.0^{e}	232.0 ± 4.1^{a}	1.4 ± 0.1^{a}	4.1 ± 0.0^{b}	4.9 ± 0.1^{ab}	165.2 ± 5.9^{a}

Mean values in the same column with different letters are significantly different at the 5 % confidence level.

Table 4.4. The concentrations of sugars (g/100g) and total free aminoacids (mg/kg) in dried dates, raisins and dried blueberries during storage.

	Month	Glucose	Fructose	Total Reducing Sugar	Total Free AA
DATE	0	24.1 ± 1.1^{a}	24.4 ± 1.3^{a}	48.5 ± 2.4^{a}	3799.2±75.5^{e}
	1	23.3 ± 1.6^{a}	26.8 ± 1.2^{ab}	50.0 ± 2.8^{a}	2420.9±12.2d
	2	22.7 ± 1.4^{a}	27.4 ± 1.3^{ab}	50.1 ± 2.8^{a}	1303.4±119.3^{c}
	3	22.8 ± 0.4^{a}	29.0 ± 0.7^{b}	51.8 ± 1.0^{a}	1227.9±11.3^{c}
	4	22.1 ± 0.5^{a}	28.4 ± 0.6^{ab}	50.6 ± 1.0^{a}	1160.6±53.8^{bc}
	5	24.4 ± 1.2^{a}	29.6 ± 1.1^{b}	54.0 ± 2.4^{a}	971.7±23.8^{ab}
	6	24.5 ± 0.4^{a}	27.8 ± 1.5^{ab}	52.3 ± 1.1^{a}	946.7±36.1^{a}
RAISIN	0	27.7 ± 0.7^{ab}	39.1 ± 2.9^{a}	66.8 ± 3.7^{a}	8117.1 ± 91.9^{c}
	1	27.2 ± 0.5^{ab}	38.5 ± 0.1^{a}	65.7 ± 0.6^{a}	3732.6 ± 757.9^{b}
	2	27.2 ± 0.3^{ab}	38.7 ± 0.4^{a}	65.9 ± 0.1^{a}	2318.3 ± 117.3^{a}
	3	27.8 ± 1.2^{ab}	37.4 ± 0.1^{a}	65.2 ± 1.3^{a}	2071.7 ± 190.7^{a}
	4	26.5 ± 0.5^{a}	40.4 ± 0.2^{a}	66.9 ± 0.7^{a}	1809.8 ± 44.9^{a}
	5	31.4 ± 0.5^{b}	39.9 ± 1.0^{a}	71.3 ± 0.4^{a}	1853.6 ± 40.1^{a}
	6	27.2 ± 2.8^{ab}	40.2 ± 4.3^{a}	67.4 ± 7.1^{a}	1397.6 ± 22.5^{a}
BLUEBERRY	0	25.0 ± 0.7^{a}	32.1 ± 1.3^{a}	57.1 ± 2.1^{a}	3099.1±520.1^{b}
	1	25.0 ± 1.2^{a}	32.3 ± 0.3^{a}	57.4 ± 1.6^{a}	1724.0±308.5^{a}
	2	24.7 ± 4.0^{a}	30.2 ± 5.0^{a}	54.9 ± 9.0^{a}	1543.7±192.2^{a}
	3	25.6 ± 0.7^{a}	28.7 ± 2.4^{a}	54.3 ± 3.2^{a}	1504.8±441.2^{a}
	4	25.9 ± 0.5^{a}	28.6 ± 0.7^{a}	54.6 ± 1.2^{a}	893.2±207.3^{a}
	5	25.6 ± 0.9^{a}	28.4 ± 0.1^{a}	54.0 ± 0.8^{a}	968.7±294.7^{a}
	6	24.8 ± 0.3^{a}	28.8 ± 0.2^{a}	53.6 ± 0.5^{a}	891.4±220.2^{a}

Mean values in the same column with different letters are significantly different at the 5 % confidence level.

Twenty free amino acids including γ-aminobutyric acid (GABA) were detected during the storage of both juice concentrates (apple, pomegranates) and dried fruits (date, raisin, blueberry) (**Table A1, A2 and A3 in Annex 1**). The major amino acids found in apple and pomegranate juice concentrates were asparagine and glutamic acid, respectively. On the other hand, the main amino acid found in dried date was GABA while arginine was the dominant in raisin and dried blueberry. These results match those observed earlier, except for the dried date [265-268]. The previous study indicated that asparagine and proline were the main amino acids found in dates, however, there was no report about the GABA level in dates in the literature [269]. The present study showed that the concentrations of total free amino acids decreased in all samples (apple, pomegranate juice concentrates, raisin, dried date, and blueberry) during the storage (**Table 4.3, 4.4**). The decrease percentages of 75 % for the dried date, 83 % for raisin, and 71 % for the dried blueberry were calculated at the end of the storage for 6 months. The results accord with the study reported by Pu, et al. [270] that the decrease in total free amino acids was observed in dried jujube fruit stored at ambient temperature for 6 months. For apple and pomegranate juice

concentrates, the decrease ratios in total free amino acids were noted as the percentages of 34 - 37% for 30 °Bx, 58 - 66% for 50 °Bx, 77 - 87% for 70 / 65 °Bx, respectively. As seen from the results, the loss of total free amino acids increased with the increase in brix levels of apple and pomegranate juice concentrations. These findings differed from the previous results where total free amino acids remained stable during the same storage conditions (6 months at 37 °C) of apple, orange and peach juices [260]. It was not surprising that the Maillard reaction did not occur in fruit juices which have high acidic and high aw conditions. As it is well known from the literature, the Maillard reaction favors the neutral and/or alkaline conditions and the aw of 0.5 – 0.8 [271]. Despite their acidic natures, dried fruits and juice concentrates could be convenient for the Maillard reaction to take place. A possible explanation for this might be that the aw levels of dried fruits (date, raisin, blueberry) and juice concentrates (apple and pomegranate) varying from 0.6 to 0.8 could provide a suitable environment for the Maillard reaction. Another research supported these findings that the increase in the loss of free amino acids with the increase in brix levels of the juice concentrates during storage might be strongly connected with the Maillard reaction [272].

4.3.3. Changes in the concentrations of α-dicarbonyl compounds during storage

Effect of concentration. α-Dicarbonyl compounds are the precursors causing the formation of both undesired (AGEs) and desired (volatile aroma compounds) products during the Maillard reaction and/or caramelization. Although several studies on the formation of α-dicarbonyl compounds in model systems (especially neutral or alkaline) have been presented in the literature, only very little information was found for real acidic foods. Changes in each α-dicarbonyl compounds including 3-DG, glucosone, threosone, DA, MGO and GO during the storage of apple and pomegranate juice concentrates were monitored as given in **Table 4.5**. The main α-dicarbonyl compounds found in both apple and pomegranate juice concentrates were 3-DG and glucosone depending on the brix levels. The concentration of 3-DG increased with the increase in the brix levels of apple and pomegranate juice concentrates, whereas glucosone level decreased in the high brix level (70 / 65 °Bx) during the storage. Glucosone was quantified in the concentration of 425.6±23.9, 138.8±9.8 and 11.8±0.5 mg/kg in 30, 50, and 70 °Bx of apple juice concentrates, respectively, at the end of the storage (**Table 4.5**). For the pomegranate juice concentration, the concentration of glucosone was found as 161±8.2, 45.5±3.1, and 8.5±0.3 mg/kg in 30, 50, and 65 °Bx levels, respectively (**Table 4.5**). It is also noteworthy that glucosone concentration was increasing in the juice concentrates at 30 and 50 °Bx levels, while it was decreasing at 70/65 °Bx during storage. Moreover, the concentration of glucosone was

found much more than that of 3-DG at 30 °Bx of apple and pomegranate juice concentrates in contrast to 50 and 70/65 °Bx. Contrarily, earlier studies reported that 3-DG was the main α-dicarbonyl compound found in foods such as fruit juices and concentrates [9, 30]. However, the present finding is in agreement with our previous studies which indicated that the increase in the concentration of glucosone was much more than that of 3-DG in apple juice stored for 6 months at 37 °C and in apple juice concentrate produced industrially [260, 265]. Ruiz-Matute, et al. [31] reported that glucosone displayed a drastically decrease during storage of honey for 12 months at 40 °C. Although there is no evidence for the relationship between the levels of oxygen, reactant concentration, and the formation of glucosone in foods, in the presence of molecular oxygen and under the aqueous conditions, glucosone easily forms from the oxidation of sugars catalyzed by transition metal ions and/or oxidation of Amadori product by hydrolysis [2, 3]. As mentioned above, the main α-dicarbonyl compound profile changed from glucosone to 3-DG when the brix level increased from 30 to 70/65 °Bx during the storage. This understandable result can be explained by the formation of 3-DG from Amadori products and/or monosaccharides through 1,2-enolization with the removal of water during Maillard reaction and/or caramelization [2]. Moreover, the accumulation of 3-DG is independent from the presence of oxygen and accelerates at low aw levels [3, 271]. The maximum levels of 3-DG in 30, 50 and 70 °Bx of apple juice concentrates were determined as 202.1±8.8, 311.1±10.5 and 362±10.9 mg/kg, respectively, at the end of storage, while it was 143.2±5.4, 318.4±4.2 and 408.5±2.4 mg/kg in 30, 50 and 65 °Bx of pomegranate juice concentrates, respectively (**Table 4.5**). In support, a previous study surveyed that the concentration of 3-DG was also found as the dominant α-dicarbonyl compound ranging between 3.4 – 198.4 mg/L in various juice concentrates with high brix levels [159]. Despite the lower levels comparing to the 3-DG and glucosone, the breakdown products of α-dicarbonyl compounds as threosone, DA, MGO, and GO were also detected. In general, changes in the concentration of threosone and GO which are formed from the *retro*-aldolisation of glucosone showed a similar trend with glucosone during storage [4]. Namely, the concentration of threosone and GO was reached at a maximum in 30 °Bx of both apple and pomegranate juice concentrates when compare to the 50 and 70/65 °Bx. On the other hand, DA and MGO, which are the breakdown products of 3-DG by *retro*-aldolisation, showed a non-linear correlation with the 3-DG. As mentioned in the literature, the degradation of 3-DG to DA and MGO was well known to accelerate under alkaline conditions, thus the accumulation of DA and MGO was lower than those and non-correlative with 3-DG during storage [2].

Table 4.5. The concentrations of α-dicarbonyl compounds and HMF (mg/kg) in apple and pomegranate juice concentrates during storage. All data were adjusted to 11.2 °Bx.

Apple Juice Concentrate								
Brix	**Week**	**3-DG**	**Glucosone**	**T**	**DA**	**MGO**	**GO**	**HMF**
30	0	71.1±2.7^{a}	72.1±3.0^{a}	0.8±0.0^{a}	0.2±0.0^{a}	3.0±0.0^{a}	0.5±0.1^{a}	0.6±0.0^{a}
30	2	91.1±5.7^{b}	202.1±19.0^{b}	2.1±0.3^{bc}	0.2±0.0^{ab}	2.8±0.1^{a}	2.0±0.3^{b}	2.8±0.0^{b}
30	4	107.3±2.4^{bc}	260.8±10.2^{c}	2.6±0.2^{bcd}	0.3±0.0^{abc}	3.0±0.1^{a}	3.3±0.2^{b}	5.3±0.0^{c}
30	6	115.7±6.0^{c}	319.9±16.1^{d}	3.5±0.6^{e}	0.3±0.0^{abc}	3.7±0.2^{b}	6.5±1.0^{cd}	8.1±0.1^{d}
30	8	143.8±3.7^{d}	311.0±11.0^{cd}	2.2±0.1^{bcd}	0.3±0.0^{bc}	3.5±0.1^{b}	5.1±0.2^{c}	14.1±0.2^{e}
30	10	151.8±0.6^{de}	335.3±11.1^{de}	2.0±0.0^{b}	0.3±0.0^{c}	3.6±0.0^{b}	5.9±0.3^{cd}	17.9±0.1^{f}
30	12	179.1±7.7^{fg}	392.5±24.9^{f}	2.8±0.2^{bcde}	0.5±0.0^{d}	3.8±0.1^{b}	6.8±0.6^{d}	24.1±0.2^{g}
30	14	172.0±6.6^{f}	378.6±7.5^{ef}	2.9±0.0^{cde}	0.5±0.0^{d}	3.6±0.0^{b}	7.3±0.1^{d}	27.0±0.2^{h}
30	16	166.0±7.6^{ef}	396.5±21.0^{f}	2.9±0.3^{cde}	0.5±0.0^{d}	3.9±0.1^{b}	8.8±0.6^{e}	30.3±0.2^{i}
30	18	196.4±3.5^{gh}	403.8±16.8^{f}	2.5±0.3^{bcd}	0.5±0.1^{d}	4.2±0.2^{c}	9.4±0.5^{e}	36.5±0.3^{j}
30	20	202.1±8.8^{h}	425.6±23.9^{f}	3.0±0.1^{de}	0.5±0.0^{d}	4.7±0.1^{d}	12.2±0.5^{f}	30.6±0.3^{i}
50	0	65.5±3.5^{a}	58.1±4.0^{a}	0.6±0.0^{a}	0.2±0.0^{a}	3.5±0.1^{a}	0.4±0.1^{a}	0.8±0.0^{a}
50	2	109.1±15.4^{b}	105.6±13.5^{bc}	0.6±0.1^{a}	0.5±0.0^{b}	4.4±0.5^{b}	0.8±0.2^{ab}	4.5±0.0^{ab}
50	4	117.7±16.8^{b}	90.5±17.0^{b}	0.5±0.0^{a}	0.5±0.0^{b}	3.4±0.4^{a}	0.7±0.2^{ab}	9.1±0.2^{b}
50	6	174.8±3.3^{c}	145.6±3.1^{e}	0.5±0.0^{a}	0.5±0.1^{b}	4.5±0.2^{b}	1.2±0.1^{bc}	15.8±0.1^{c}
50	8	203.9±11.2^{cd}	120.3±1.7^{cde}	0.4±0.0^{a}	0.5±0.0^{b}	4.4±0.1^{b}	1.1±0.1^{bc}	23.8±0.3^{d}
50	10	208.9±8.3^{d}	117.6±2.9^{cd}	0.4±0.0^{a}	0.5±0.0^{b}	4.1±0.2^{ab}	1.1±0.1^{bc}	31.7±0.5^{e}
50	12	253.8±9.8^{e}	123.6±7.9^{cde}	0.6±0.1^{a}	1.0±0.0^{c}	4.5±0.3^{b}	1.6±0.2^{cd}	42.3±0.1^{f}
50	14	273.3±12.7^{e}	142.2±3.9^{de}	0.5±0.0^{a}	1.0±0.1^{c}	4.7±0.3^{b}	1.7±0.1^{d}	47.9±1.2^{g}
50	16	285.7±2.4e^{f}	129.1±2.7^{cde}	0.4±0.2^{a}	1.0±0.1^{c}	4.6±0.2^{b}	1.3±0.2^{cd}	48.3±2.0^{g}
50	18	286.1±3.9e^{f}	125.2±2.8^{cde}	0.5±0.0^{a}	1.0±0.0^{c}	4.2±0.0^{ab}	0.6±0.1^{a}	67.5±4.1^{h}
50	20	311.1±10.5^{f}	138.8±9.8^{de}	0.4±0.0^{a}	1.3±0.0^{d}	4.9±0.2^{b}	0.3±0.1^{a}	74.2±2.6^{i}
70	0	74.2±2.3^{a}	24.0±1.6^{d}	0.6±0.0^{b}	1.0±0.0^{a}	4.0±0.2^{a}	2.0±0.1^{a}	0.9±0.1^{a}
70	2	109.0±9.0^{b}	19.6±0.7^{c}	0.3±0.0^{a}	1.2±0.1^{a}	5.0±0.3^{b}	1.8±0.2^{a}	4.5±0.1^{ab}
70	4	129.8±2.8^{c}	15.7±1.3^{b}	0.3±0.0^{a}	1.0±0.1^{a}	5.6±0.3^{bc}	1.7±0.1^{a}	8.8±0.1^{b}
70	6	175.6±3.0^{d}	17.4±0.1^{b}	0.3±0.0^{a}	1.3±0.1^{a}	6.1±0.0^{c}	2.1±0.1^{a}	17.5±0.3^{c}
70	8	207.9±2.3^{e}	12.1±0.4^{a}	0.3±0.1^{a}	1.1±0.1^{a}	5.9±0.1^{bc}	1.8±0.1^{a}	26.6±0.9^{d}
70	10	233.5±7.3^{f}	10.8±0.1^{a}	0.3±0.0^{a}	1.2±0.1^{a}	5.6±0.7^{bc}	1.7±0.0^{a}	34.1±1.8^{e}
70	12	282.1±5.3^{g}	11.9±0.6^{a}	0.4±0.0^{ab}	2.0±0.3^{b}	5.9±0.1^{bc}	2.3±0.3^{a}	47.4±1.9^{f}
70	14	315.6±4.7^{h}	12.2±0.1^{a}	0.5±0.0^{b}	2.5±0.2^{c}	6.3±0.1^{c}	2.7±0.7^{a}	57.3±1.1^{g}
70	16	332.4±4.1^{h}	11.7±0.2^{a}	0.6±0.1^{b}	2.2±0.0^{b}	5.9±0.4^{bc}	2.6±0.2^{a}	67.3±2.0^{h}
70	18	359.6±3.1^{i}	12.3±0.2^{a}	0.5±0.1^{b}	2.5±0.0^{c}	6.0±0.2^{bc}	2.7±0.6^{a}	75.4±3.0^{i}
70	20	362.0±10.9^{i}	11.8±0.5^{a}	0.6±0.1^{b}	2.3±0.0^{bc}	5.5±0.1^{bc}	2.2±0.2^{a}	88.1±4.1^{j}

Table 4.5 continue.

Pomegranate Juice Concentrate								
Brix	**Week**	**3-DG**	**Glucosone**	**T**	**DA**	**MGO**	**GO**	**HMF**
30	0	52.8±0.2^{a}	38.7±0.1^{a}	4.6±0.0^{a}	0.1±0.0^{a}	0.9±0.0^{ab}	6.6±0.7^{a}	1.1±0.0^{a}
30	2	64.8±0.4^{b}	89.4±4.3^{b}	4.6±0.0^{a}	0.2±0.0^{ab}	0.9±0.0^{a}	7.2±0.2^{a}	5.5±0.1^{b}
30	4	81.5±2.4^{c}	132.7±0.7^{c}	4.7±0.0^{a}	0.2±0.0^{b}	1.1±0.1^{bc}	7.3±1.2^{a}	10.0±0.5^{c}
30	6	84.8±0.9^{c}	141.5±0.6^{cd}	4.7±0.0^{a}	0.2±0.0^{ab}	1.2±0.0^{c}	6.2±0.7^{a}	16.7±0.2^{d}
30	8	103.5±0.6^{d}	135.0±4.9^{c}	5.1±0.4^{a}	0.2±0.0^{b}	1.4±0.0^{d}	6.4±0.5^{a}	23.3±1.3^{e}
30	10	114.9±0.7^{e}	145.5±4.1^{cde}	6.6±0.4^{b}	0.2±0.0^{b}	1.6±0.0^{de}	8.4±1.0^{a}	33.8±1.1^{f}
30	12	140.7±4.0^{f}	151.7±3.1^{def}	8.2±0.1^{c}	0.3±0.0^{c}	1.8±0.1^{f}	12.0±0.8^{b}	43.7±0.5^{g}
30	14	139.7±7.4^{f}	155.8±6.0^{ef}	9.0±0.3^{d}	0.3±0.0^{c}	1.7±0.1^{ef}	13.2±1.8^{b}	49.6±1.1^{h}
30	16	140.9±0.6^{f}	159.5±0.8^{f}	9.3±0.0^{d}	0.3±0.0^{c}	1.8±0.0^{f}	15.0±0.7^{b}	61.1±0.2^{i}
30	18	140.2±1.2^{f}	155.7±1.9^{ef}	9.3±0.1^{d}	0.3±0.0^{c}	1.8±0.0^{f}	15.3±1.6^{b}	72.7±0.0^{j}
30	20	143.2±5.4^{f}	161.0±8.2^{f}	9.5±0.0^{d}	0.3±0.0^{c}	1.9±0.1^{f}	14.8±1.5^{b}	75.4±0.3^{k}
50	0	46.7±2.4^{a}	23.8±1.5^{a}	0.3±0.0^{a}	0.3±0.1^{a}	1.0±0.0^{a}	1.0±0.0^{a}	1.8±0.2^{a}
50	2	84.5±1.8^{b}	27.6±0.9^{a}	0.3±0.0^{a}	0.4±0.1^{a}	1.2±0.1^{ab}	1.1±0.1^{a}	6.3±0.1^{a}
50	4	124.4±6.2^{c}	32.6±1.5^{b}	0.3±0.0^{a}	0.5±0.0^{a}	1.3±0.1^{abc}	1.2±0.1^{a}	17.3±0.5^{ab}
50	6	160.9±4.9^{d}	36.0±1.5^{bc}	0.4±0.0^{b}	0.5±0.0^{ab}	1.5±0.1^{bc}	1.1±0.1^{a}	28.2±3.8^{bc}
50	8	185.5±0.7^{e}	35.0±0.4^{bc}	0.5±0.0^{b}	0.5±0.0^{abc}	1.5±0.0^{cd}	1.1±0.1^{a}	37.4±0.8^{cd}
50	10	213.2±3.5^{f}	36.8±1.6^{bc}	0.6±0.0^{c}	0.9±0.3^{bcd}	1.8±0.1^{de}	1.1±0.1^{a}	51.5±2.3^{d}
50	12	242.4±5.8^{g}	36.7±0.8^{bc}	0.7±0.0^{d}	0.9±0.1^{bcd}	2.0±0.1^{e}	2.0±0.2^{b}	81.6±0.8^{e}
50	14	261.1±5.6^{gh}	42.1±0.3^{de}	0.7±0.0^{de}	0.9±0.1^{cd}	2.3±0.0^{f}	2.0±0.2^{b}	100.6±8.9^{f}
50	16	272.8±10.9^{h}	38.8±1.4^{cd}	0.8±0.0^{ef}	0.9±0.1^{cd}	2.4±0.2^{fg}	2.0±0.2^{b}	116.5±10.3^{g}
50	18	278.1±12.2^{h}	39.1±0.3^{cd}	0.8±0.1^{f}	0.9±0.1^{cd}	2.6±0.2^{g}	2.1±0.1^{b}	132.2±6.9^{h}
50	20	318.4±4.2^{i}	45.5±3.1^{e}	1.0±0.0^{g}	1.0±0.1^{d}	3.0±0.1^{h}	2.2±0.4^{b}	147.4±1.1^{i}
65	0	53.0±0.4^{a}	15.2±0.3^{f}	0.3±0.0^{bc}	0.9±0.0^{a}	1.2±0.0^{a}	0.4±0.0^{a}	2.6±0.1^{a}
65	2	90.9±4.9^{b}	10.6±0.7^{e}	0.2±0.0^{a}	1.0±0.1^{ab}	1.5±0.0^{a}	0.5±0.0^{b}	13.5±0.0^{ab}
65	4	143.6±1.1^{c}	8.9±0.2^{d}	0.2±0.0^{ab}	1.1±0.0^{b}	1.5±0.1^{a}	0.7±0.0^{c}	20.6±0.1^{b}
65	6	182.3±2.2^{d}	7.4±0.1^{ab}	0.3±0.0^{bc}	1.0±0.0^{ab}	1.4±0.0^{a}	0.7±0.0^{de}	27.0±1.3^{b}
65	8	229.2±2.7^{e}	7.1±0.1^{a}	0.4±0.0^{cd}	1.0±0.0^{ab}	1.5±0.1^{a}	0.7±0.0^{cd}	52.1±3.7^{c}
65	10	268.3±8.9^{f}	7.2±0.0^{ab}	0.4±0.0^{d}	1.0±0.0^{b}	1.6±0.0^{a}	0.8±0.0^{e}	78.1±3.5^{d}
65	12	321.6±0.9^{g}	7.9±0.2^{abc}	0.5±0.0^{e}	2.0±0.0^{c}	2.3±0.3^{b}	1.0±0.0^{g}	109.1±0.2^{e}
65	14	341.9±5.2^{h}	8.0±0.2^{abc}	0.6±0.0^{f}	2.2±0.1^{d}	2.4±0.1^{b}	0.9±0.0^{f}	134.2±0.1^{f}
65	16	376.4±0.7^{i}	8.1±0.0^{bcd}	0.6±0.0^{f}	2.0±0.1^{c}	2.3±0.1^{b}	0.9±0.0^{fg}	157.3±1.7^{g}
65	18	380.9±1.6^{i}	8.0±0.1^{abc}	0.6±0.0^{f}	2.0±0.0^{c}	2.7±0.1^{b}	1.0±0.0^{fg}	188.1±1.9^{h}
65	20	408.5±2.4^{j}	8.5±0.3^{cd}	0.7±0.0^{g}	2.1±0.0^{d}	3.1±0.2^{c}	1.0±0.0^{h}	231.5±14.6^{i}

Mean values in the same column with different letters are significantly different at the 5 % confidence level.

Effect of pH. The concentrations of 3-DG, glucosone, 3-DT, (E)-3,4-DGE, (Z)-3,4-DGE, GO, MGO and DA were observed during storage, as shown in **Table 4.6.** The maximum levels of 3-DG which was the dominant α-dicarbonyl compound found in dried date, raisin, and dried blueberry were 7251±896.5, 4438.2±237.3, and 3644±642.2 mg/kg, respectively, at the end of the storage of 6 months at 37 °C. These results are consistent with the findings of Aktağ and Gökmen [159] which indicated that the highest median level of 3-DG was found in dried dates among various dried fruits. Similarly, Maasen, et al. [10] reported that 3-DG was the most

abundant dicarbonyl in most food and drinks, and dried fruits were one of the foods which have the highest total dicarbonyl concentrations. These results might be explained with the effect of pH on Maillard reaction in dried fruits which have similar aw levels (0.6) in the same storage conditions since sugar - amino acid reaction accelerates in the neutral and/or alkaline conditions as mentioned above [271]. As seen in **Table 4.2**, the pH of dried date, raisin and dried blueberry was 6.61, 3.79, and 2.64, respectively, at the beginning of the storage. Considering the maximum levels of dominant dicarbonyl were found in the dried fruit (date) with the highest pH value, it is possible to say that alkaline conditions trigger the formation of α-dicarbonyl compounds through Maillard reaction [2]. Moreover, it is well known that HMF formation is increased under acidic conditions [4]. Therefore, the degradation of 3-DG to HMF in raisin and dried blueberry might be another explanation for the lower levels of 3-DG in these fruit samples. The second major dicarbonyl at the beginning of storage, glucosone, was decreasing while 3-DG level was increasing in dried fruits during storage. This behavior was similar to the decrease in glucosone in highly concentrated apple and pomegranate juice as mentioned above. The level of glucosone decreased dramatically in the first month of the storage and then continued relatively slow. Firstly, the main reason for this is that the vacuum packaging might inhibit the formation of glucosone which is formed via oxidation, by preventing the air transition [3]. Second, the formation of glucosone is performed by the hydrolysis from the first carbon of α-amino ketone during the oxidation of Amadori products [97], therefore the results provided strong evidence for the high concentrations of glucosone observed at low-concentrated solutions such as fruit juices which have low brix levels. Third, glucosone could degrade to form the breakdown products such as GO and 3-DT during storage of dried fruits. As seen in **Table 4.6**, GO and 3-DT increased whereas glucosone decreased during storage in all dried fruits. In this study, both *trans* (E) and *cis* (Z) form of 3,4-DGE which is formed by elimination of water from 3-DG [273] was detected first in dried date, raisin, and dried blueberry with an increasing trend similar to 3-DG during storage (**Table 4.6**). In the earlier studies, 3-DG was found to convert to *cis* and *trans* forms of 3,4-DGE under mild acid conditions [181]. When compare the concentrations of *E* and *Z* derivatives of 3,4-DGE during storage, *E* form of 3,4-DGE was much higher than the *Z* form, in all samples. A plausible explanation for this finding was stated that 3,4-DGE only in the *Z* form, will rearrange to the heterocyclic HMF, and therefore (E)-3,4-DGE accumulated during the storage whereas (Z)-3,4-DGE formed and transformed at the same time [96, 101, 102, 181]. Furthermore, (E)-3,4-DGE was found as the second dominant α-dicarbonyl compound in dried date, raisin and dried blueberry in the concentration of 324.7±60.3, 273.9±47.3, and 112.4±8 mg/kg, respectively, at the end of the

storage. The concentrations of shorter chain α-dicarbonyl compounds, DA and MGO, which forms from 1-DG and 3-DG preferably under alkaline conditions, were comparably lower than the concentrations of C_6-skeletal α-dicarbonyl compounds and GO as explained before.

Table 4.6. The concentrations of α-dicarbonyl compounds and HMF (mg/kg) in dried dates, raisins and dried blueberries during storage.

Month	3-DG	Glucosone	3-DT	(E)-3,4-DGE	(Z)-3,4-DGE	GO	MGO	DA	HMF
				DATE					
0	1074.6±210.8^{a}	400.7±30.8^{e}	1.5±0.3^{a}	3.6±1.3^{a}	8.0±2.0^{a}	12.3±0.6^{a}	7.7±1.7^{a}	1.0±0.1^{a}	nd
1	1944.0±147.8^{a}	50.0±0.9^{d}	1.7±0.0^{a}	12.7±1.3^{a}	14.6±0.4^{a}	11.4±1.0^{a}	3.7±0.5^{a}	0.8±0.0^{a}	11.7±0.3^{a}
2	3727.6±176.5^{b}	38.8±1.9^{c}	2.5±0.3^{a}	60.7±2.5^{ab}	38.8±2.8^{b}	21.6±1.3^{b}	6.7±1.0^{a}	1.3±0.3^{a}	41.9±3.5^{a}
3	4275.8±162.4^{b}	22.1±0.2^{ab}	2.5±0.2^{a}	96.1±5.3^{b}	50.2±1.6^{b}	27.1±0.8^{c}	5.8±1.9^{a}	1.4±0.3^{a}	91.9±0.9^{b}
4	4555.7±4.9^{b}	29.5±0.9^{bc}	6.6±1.2^{b}	134.8±5.0^{b}	68.2±0.3^{c}	27.9±2.0^{c}	6.9±1.8^{a}	1.4±0.2^{a}	149.6±9.0^{c}
5	6687.6±88.8^{c}	23.4±2.0^{ab}	1.8±0.1^{a}	236.8±10.5^{c}	91.1±1.9^{d}	11.9±1.8^{a}	7.8±2.6^{a}	2.4±0.3^{b}	243.7±2.2^{d}
6	7251.0±896.6^{c}	15.5±1.3^{a}	4.9±1.2^{b}	324.7±60.3^{d}	110.8±12.9^{e}	21.1±0.2^{b}	7.6±2.8^{a}	2.4±0.5^{b}	514.4±27.2^{e}
				RAISIN					
0	791.4±42.9^{a}	383.5±22.9^{c}	10.3±1.0^{a}	13.0±1.6^{a}	21.7±1.9^{a}	11.8±1.2^{a}	5.2±0.5^{a}	1.3±0.1^{cd}	189.5±7.9^{a}
1	1494.7±64.1^{b}	95.0±16.6^{b}	17.3±2.3^{b}	70.1±13.9^{ab}	32.9±4.1^{b}	10.7±1.5^{a}	6.4±1.2^{a}	1.4±0.0^{d}	418.9±52.5^{a}
2	1675.9±45.0^{b}	30.5±1.9^{a}	24.1±0.3^{c}	125.4±3.5^{bc}	47.0±5.7^{c}	8.9 ± 0.3^{a}	8.9±2.2^{ab}	1.6±0.3^{d}	1553.7±89.7^{b}
3	2469.1±10.0^{c}	33.1±0.7^{a}	17.4±1.8^{b}	124.0±20.9^{bc}	50.5±0.7^{c}	15.3±0.4^{b}	6.1±0.6^{a}	0.9±0.0^{bc}	1663.3±61.2^{b}
4	2878.2±29.4^{d}	23.8±4.5^{a}	25.8±1.2^{cd}	183.6±20.2^{c}	62.4±0.0^{d}	18.7±0.1^{c}	11.0±0.7^{bc}	0.9±0.0^{bc}	2747.0±271.6^{c}
5	4377.9±45.8^{e}	16.1±1.3^{a}	24.3±0.9^{c}	191.9±20.9^{c}	66.2±0.7^{d}	32.0±1.1^{d}	12.4±0.6^{bc}	0.5±0.0^{ab}	2858.6±120.8^{c}
6	4438.2±237.3^{e}	16.6±1.6^{a}	29.7±0.0^{d}	273.9±47.3^{d}	66.7±0.1^{d}	29.0±0.8^{d}	14.1±0.5^{c}	0.4±0.0^{a}	4151.1±310.7^{d}
				BLUEBERRY					
0	144.0± 8.1^{a}	157.4±35.0^{c}	0.4±0.1^{a}	2.3±0.6^{a}	0.5±0.1^{a}	6.1±0.6^{bc}	1.6±0.5^{a}	0.9±0.0^{a}	40.2±5.5^{a}
1	1144.4±270.6^{ab}	89.8±13.1^{b}	0.5±0.1^{a}	15.1±0.6^{a}	2.4±1.4^{a}	3.5±0.4^{a}	2.4±1.1^{ab}	1.0±0.0^{a}	546.6±84.8^{a}
2	2311.9±770.6^{bc}	29.5±2.2^{a}	0.9±0.1^{a}	33.2±8.1^{ab}	4.4±1.9^{ab}	4.2±0.7^{ab}	3.3±1.9^{ab}	1.4±0.2^{ab}	1440.0±200.2^{b}
3	2410.6±775.8^{bc}	18.4±2.0^{a}	1.6±0.3^{a}	56.9±2.3^{bc}	8.7±1.6^{b}	5.4±0.8^{abc}	3.6±1.8^{ab}	1.6±0.2^{b}	1553.7±66.6^{bc}
4	2643.2±456.3^{bc}	13.0±1.7^{a}	5.2±2.0^{b}	77.9±10.2^{cd}	8.8±2.2^{b}	6.8±0.4^{c}	4.2±1.4^{b}	1.6±0.2^{b}	1655.6±91.6^{bc}
5	3397.1±590.6^{c}	17.1±6.1^{a}	3.0±0.3^{ab}	101.7±22^{d}	1.8±0.1^{a}	12.7±0.8^{d}	4.3±1.0^{b}	1.5±0.1^{c}	1937.1±403.9^{bc}
6	3644.0±642.2^{c}	12.0±3.0^{a}	2.5±0.2^{ab}	112.4±8.0^{d}	1.6±0.4^{a}	17.0±0.8^{e}	4.9±1.1^{b}	1.5±0.1^{c}	2105.3±93.7^{c}

Mean values in the same column with different letters are significantly different at the 5 % confidence level.

4.3.4. Changes in the concentrations of 5-hydroxymethylfurfural during storage

HMF can be easily formed by enolisation and dehydration of sugars during Maillard reaction or caramelization reactions under acidic and low moisture conditions [4, 8]. The concentration of HMF increased in all samples during the storage and reached a maximum as 4151.1±310.7 mg/kg in raisin at the end of the storage. For apple and pomegranate juice concentration, HMF level increased parallel with the brix level as shown in **Table 4.5**. The possible explanation why HMF accumulates more in the high-concentrated juices is the formation of HMF from sugars by the removal of 3 molecules of water [5]. The levels of HMF were 30.6±0.3, 74.2±2.6 and 88.1±4.1 mg/kg in 30, 50 and 70 °Bx of apple juice concentrates, respectively and 75.4±0.3,

147.4±1.1 and 231.5±14.6 mg/kg in 30, 50 and 65 °Bx of pomegranate juice concentrates, respectively, at the end of storage. Similarly, Wang, et al. [249] reported that HMF concentration in carrot juice concentrate increased with the increase in brix level from 20 °Bx to 60 °Bx during storage for 5 months at 37 °C. What is surprising from the results is that the concentrations of HMF in pomegranate juice concentrate were higher than that of in apple juice concentrate, despite the lower fructose level in pomegranate juice concentrates than that in apple juice concentrates. In the meantime, the concentration of 3-DG was also high in pomegranate juice concentrates especially in 65 °Bx comparing to 70 °Bx of apple juice concentrates. The possible pathways for the formation of HMF were proposed by Gürsul Aktağ and Gökmen [260] as dehydration of fructose or dehydration of 3-DG in the responsibility of only caramelization during the storage of various fruit juices. Conversely, it was suggested that caramelization and Maillard reaction occurred concurrently in the present study. This discrepancy could be attributed to the lower pH level of pomegranate juice concentrate than those of the apple juice concentrate taking into consideration that HMF prefers the more acidic conditions. Indeed, the effect of pH on the formation of HMF was clearly seen in the dried fruits. To the results, the HMF level in raisin and dried blueberry which had pH levels of 3.79 and 2.64 was almost 9 and 4 times more than in dried dates with the pH level of 6.61. But surprisingly, the maximum level of HMF (4151.1±310.7 mg/kg) was detected in raisin (**Table 4.6**), although dried blueberry had the lowest pH. Aktağ and Gökmen [159] reported the highest amount of HMF reaching up to 2400.9 mg/kg in dried blueberry samples among various dried fruits. A possible reason is related to the initial amount of total free amino acids found in raisin (8117.1±91.9 mg/kg) and dried blueberry (3099.1±520.1 mg/kg) considering the Maillard reaction taking place during storage.

4.3.5. Confirmation of the adducts and Schiff bases of α-dicarbonyl compounds and 5-hydroxymethylfurfural with amino acids

It is well known that sugar decomposition and Maillard reaction simultaneously occur during thermal processing or storage of foods. To the results, when the concentration of juices changes from aqueous to highly concentrated, the Maillard reaction seems to become prominent in this complex reaction network due to the loss of free amino acids. Amino acids bear several reactive sites such as nucleophilic and/or sulfhydryl groups to react with α-dicarbonyl compounds and HMF during the Maillard reaction. Michael adduct and Schiff base might be the possible adducts among the numerous adducts of the reactive α-dicarbonyl compounds and HMF. The adducts of α-dicarbonyl compounds and HMF with all free amino acids were analyzed in full

scan mode of HRMS in 70 °Bx of apple juice concentrate and raisin both at the beginning (control) and at the end of the storage (**Table 4.7 and Tables A4, A5 in Annex 1)**. Apple juice concentrate was selected as representative for mass scan rather than pomegranate juice concentrates, since it contains a high amount of asparagine which is the precursor of acrylamide and also it is the most consumed fruit juice product. Raisin was selected among the dried fruits, because the highest ratio of amino acid decrease was observed in it, and also it represents the acidic fruit in comparison with the dried date. First, dicarbonyl and HMF adducts with amino acids could not be detected in fresh grapes and also not detected or detected in very low signal intensity in control apple juice concentrates (data not shown). Hereafter, the evaluation of the confirmation of amino acid adducts of α-dicarbonyl compounds was performed for 3-DG which was the major dicarbonyl both in 70 °Bx of apple juice concentrate and raisin. Other dicarbonyl adducts with amino acids can be seen in the supplementary material. For the confirmation, experimental masses of the adducts were compared with the corresponding exact masses of the adducts detected in the samples. The adducts and Schiff bases of 3-DG in apple juice concentrate and raisin were confirmed generally with very high mass accuracy ($\Delta < 2$ ppm) (**Table 4.7**). Similarly, Michael adducts and Schiff bases of HMF with amino acids were confirmed with very high mass accuracy ($\Delta < 2$ ppm) in general (**Table 4.7**). As illustrated in **Figure 4.1.A**, the presence of $[M+H]^+$ ion having *m/z* of 295.11359 ($C_{10}H_{18}O_8N_2$) with Δ= -0.02 ppm confirming the formation of 3-DG –Asn adduct in apple juice concentrate had the relative abundance of signal response of 3.10^5 whereas the $[M+H]^+$ ion having *m/z* of 277.10324 ($C_{10}H_{16}O_7N_2$) with Δ= 0.11 ppm confirming the Schiff base of 3DG-Asn had the signal intensity of 2.10^6 in **Figure 4.1.B**. Similarly, the Schiff base of 3-DG – Arg which was confirmed by the presence of $[M+H]^+$ ion having *m/z* of 337.17178 ($C_{12}H_{24}O_7N_4$) with Δ= 0.02 ppm had the higher signal response of 6.10^6 than the adduct of 3-DG – Arg which was confirmed by the presence of $[M+H]^+$ ion having *m/z* of 319.16122 ($C_{12}H_{22}O_6N_4$) with Δ= 0.14 ppm had the signal response of 5.10^4, in raisin (**Figure 4.2.A and B**). Signal responses of the Schiff bases were seemed remarkably higher than that of the adducts of 3-DG with Asn and Arg in apple juice concentrate and raisin, respectively. Contrarily, the signal intensity (9.10^7) of Michael adduct of HMF with Asn in apple juice concentrate, having the $[M+H]^+$ ion with *m/z* of 259.09201 ($C_{10}H_{14}O_6N_2$, Δ= -1.75 ppm) was higher than the signal response (7.10^5) of the Schiff base HMF with Asn, having the $[M+H]^+$ ion, *m/z* of 241.08182 ($C_{10}H_{12}O_5N_2$, Δ= -0.33 ppm). Likewise, the signal response of the Michael adduct of HMF with Arg, having the $[M+H]^+$ ion, *m/z* of 301.15051 ($C_{12}H_{20}O_5N_4$, Δ= -0.44 ppm) was more intense than the Schiff base of HMF with Arg, having the $[M+H]^+$ ion, *m/z* of 283.14001 ($C_{12}H_{18}O_4N_4$, Δ= -0.24 ppm).

Table 4.7. High-resolution mass spectrometry (HRMS) performances of the adducts and the Schiff bases of 3-DG and HMF with free amino acids possibly formed in 70°Bx of apple juice concentrates and raisins at the end of the storage.

Amino Acid	Formula	Exact Mass [M+H$^+$]	Experimental Mass [M+H$^+$]	Δ (ppm)	Formula	Exact Mass [M+H$^+$]	Experimental Mass [M+H$^+$]	Δ (ppm)
	Apple Juice Concentrate							
	Adducts				**Schiff Bases**			
	3-DG							
Ala	$C_9H_{17}O_7N$	252.10778	252.10779	0.04	$C_9H_{15}O_6N$	234.09722	234.09723	0.07
Arg	$C_{12}H_{24}O_7N_4$	337.17178	337.17154	-0.70	$C_{12}H_{22}O_6N_4$	319.16122	-	
Asn	$C_{10}H_{18}O_8N_2$	295.11359	295.11359	-0.02	$C_{10}H_{16}O_7N_2$	277.10303	277.10306	0.11
Asp	$C_{10}H_{17}O_9N$	296.09761	296.09732	-0.97	$C_{10}H_{15}O_8N$	278.08705	278.0871	0.20
GABA	$C_{10}H_{19}O_7N$	266.12343	266.12341	-0.06	$C_{10}H_{17}O_6N$	248.11287	248.11288	0.08
Gln	$C_{11}H_{20}O_8N_2$	309.12942	309.12946	0.69	$C_{11}H_{18}O_7N_2$	291.11886	291.11795	-2.50
Glu	$C_{11}H_{19}O_9N$	310.11326	310.11325	-0.01	$C_{11}H_{17}O_8N$	292.10270	292.10251	-0.63
Gly	$C_8H_{15}O_7N$	238.09213	238.09213	0.02	$C_8H_{13}O_6N$	220.08157	220.08153	-0.16
His	$C_{12}H_{19}O_7N_3$	318.12958	318.12961	0.10	$C_{12}H_{17}O_6N_3$	300.11902	300.11792	-3.64
Leu/Ile	$C_{12}H_{23}O_7N$	294.15473	294.15454	-0.64	$C_{12}H_{21}O_6N$	276.14417	276.14423	0.23
Lys	$C_{12}H_{24}O_7N_2$	309.16563	309.16577	0.47	$C_{12}H_{22}O_6N_2$	291.15507	291.15509	0.09
Met	$C_{11}H_{21}O_7NS$	312.11115	312.11105	-0.31	$C_{11}H_{19}O_6NS$	294.10059	294.10056	-0.10
Phe	$C_{15}H_{21}O_7N$	328.13908	328.13907	-0.03	$C_{15}H_{19}O_6N$	310.12852	310.12845	-0.21
Pro	$C_{11}H_{19}O_7N$	278.12343	278.12344	0.05	$C_{11}H_{17}O_6N$	260.11287	260.11288	0.08
Ser	$C_9H_{17}O_8N$	268.10269	268.10263	-0.23	$C_9H_{15}O_7N$	250.09213	250.09213	0.02
Thr	$C_{10}H_{19}O_8N$	282.11834	282.11838	0.12	$C_{10}H_{17}O_7N$	264.10778	264.10779	0.04
Trp	$C_{17}H_{22}O_7N_2$	367.14998	367.14963	-0.95	$C_{17}H_{20}O_6N_2$	349.13942	-	
Tyr	$C_{15}H_{21}O_8N$	344.13399	344.13382	-0.50	$C_{15}H_{19}O_7N$	326.12343	326.12338	-0.14
Val	$C_{11}H_{21}O_7N$	280.13908	280.13907	-0.04	$C_{11}H_{19}O_6N$	262.12852	262.12854	0.10
	HMF							
Ala	$C_9H_{13}O_5N$	216.08665	216.08664	-0.04	$C_9H_{11}O_4N$	198.07609	198.07605	-0.17
Arg	$C_{12}H_{20}O_5N_4$	301.15065	-		$C_{12}H_{18}O_4N_4$	283.14009	-	
Asn	$C_{10}H_{14}O_6N_2$	259.09246	259.09201	-1.75	$C_{10}H_{12}O_5N_2$	241.08190	241.08182	-0.33
Asp	$C_{10}H_{13}O_7N$	260.07648	260.07648	0.00	$C_{10}H_{11}O_6N$	242.06592	242.06593	0.08
GABA	$C_{10}H_{15}O_5N$	230.10230	230.10231	0.05	$C_{10}H_{13}O_4N$	212.09174	212.09174	0.01
Gln	$C_{11}H_{16}O_6N_2$	273.10811	273.10843	1.16	$C_{11}H_{14}O_5N_2$	255.09755	255.09857	4.01
Glu	$C_{11}H_{15}O_7N$	274.09213	274.09259	1.69	$C_{11}H_{13}O_6N$	256.08157	256.08154	-0.08
Gly	$C_8H_{11}O_5N$	202.07100	202.07115	0.76	$C_8H_9O_4N$	184.06044	184.06044	0.03
His	$C_{12}H_{15}O_5N_3$	282.10845	282.10693	-5.36	$C_{12}H_{13}O_4N_3$	264.09789	264.09891	3.88
Leu/Ile	$C_{12}H_{19}O_5N$	258.13360	258.13364	0.14	$C_{12}H_{17}O_4N$	240.12304	240.12304	-0.08
Lys	$C_{12}H_{20}O_5N_2$	273.14450	273.14471	0.79	$C_{12}H_{18}O_4N_2$	255.13394	255.13356	-1.46
Met	$C_{11}H_{17}O_5NS$	276.09002	-		$C_{11}H_{15}O_4NS$	258.07946	-	
Phe	$C_{15}H_{17}O_5N$	292.11795	292.11786	-0.31	$C_{15}H_{15}O_4N$	274.10739	274.10657	-2.98
Pro	$C_{11}H_{15}O_5N$	242.10230	242.10226	-0.14	$C_{11}H_{13}O_4N$	224.09174	224.09172	-0.06
Ser	$C_9H_{13}O_6N$	232.08156	232.08154	-0.09	$C_9H_{11}O_5N$	214.07100	214.07101	0.07
Thr	$C_{10}H_{15}O_6N$	246.09721	246.09721	0.00	$C_{10}H_{13}O_5N$	228.08665	228.08669	0.16
Trp	$C_{17}H_{18}O_5N_2$	331.12885	-		$C_{17}H_{16}O_4N_2$	313.11829	-	
Tyr	$C_{15}H_{17}O_6N$	308.11286	308.11499	6.90	$C_{15}H_{15}O_5N$	290.10230	290.10233	0.09
Val	$C_{11}H_{17}O_5N$	244.11795	244.11797	0.07	$C_{11}H_{15}O_4N$	226.10739	226.10739	0.03

Table 4.7 continue.

Amino Acid	Formula	Exact Mass [M+H$^+$]	Experimental Mass [M+H$^+$]	Δ (ppm)	Formula	Exact Mass [M+H$^+$]	Experimental Mass [M+H$^+$]	Δ (ppm)
	Raisin							
	Adducts				**Schiff Bases**			
	3-DG							
Ala	$C_9H_{17}O_7N$	252.10778	252.10779	0.04	$C_9H_{15}O_6N$	234.09722	234.09723	0.07
Arg	$C_{12}H_{24}O_7N_4$	337.17178	337.17178	0.02	$C_{12}H_{22}O_6N_4$	319.16122	319.16125	0.14
Asn	$C_{10}H_{18}O_8N_2$	295.11359	295.11359	-0.02	$C_{10}H_{16}O_7N_2$	277.10303	277.10324	0.77
Asp	$C_{10}H_{17}O_9N$	296.09761	296.09756	-1.45	$C_{10}H_{15}O_8N$	278.08705	278.08755	1.84
GABA	$C_{10}H_{19}O_7N$	266.12343	266.12344	0.06	$C_{10}H_{17}O_6N$	248.11287	248.11288	0.08
Gln	$C_{11}H_{20}O_8N_2$	309.12942	309.12943	0.59	$C_{11}H_{18}O_7N_2$	291.11886	291.11792	-2.60
Glu	$C_{11}H_{19}O_9N$	310.11326	310.11307	-0.61	$C_{11}H_{17}O_8N$	292.10270	292.10236	-1.15
Gly	$C_8H_{15}O_7N$	238.09213	238.0921	-0.11	$C_8H_{13}O_6N$	220.08157	220.08157	0.04
His	$C_{12}H_{19}O_7N_3$	318.12958	318.12958	0.00	$C_{12}H_{17}O_6N_3$	300.11902	300.1192	0.63
Leu/Ile	$C_{12}H_{23}O_7N$	294.15473	294.15472	-0.01	$C_{12}H_{21}O_6N$	276.14417	276.1442	0.11
Lys	$C_{12}H_{24}O_7N_2$	309.16563	309.16574	0.37	$C_{12}H_{22}O_6N_2$	291.15507	291.15533	0.93
Met	$C_{11}H_{21}O_7NS$	312.11115	-		$C_{11}H_{19}O_6NS$	294.10059	294.10043	-0.51
Phe	$C_{15}H_{21}O_7N$	328.13908	328.13907	-0.03	$C_{15}H_{19}O_6N$	310.12852	310.12854	0.08
Pro	$C_{11}H_{19}O_7N$	278.12343	278.12344	0.05	$C_{11}H_{17}O_6N$	260.11287	260.11288	0.08
Ser	$C_9H_{17}O_8N$	268.10269	268.10269	0.00	$C_9H_{15}O_7N$	250.09213	250.09213	0.02
Thr	$C_{10}H_{19}O_8N$	282.11834	282.11835	0.01	$C_{10}H_{17}O_7N$	264.10778	264.10776	-0.08
Trp	$C_{17}H_{22}O_7N_2$	367.14998	367.14996	-0.04	$C_{17}H_{20}O_6N_2$	349.13942	349.13947	0.15
Tyr	$C_{15}H_{21}O_8N$	344.13399	344.13385	-0.42	$C_{15}H_{19}O_7N$	326.12343	326.12341	-0.05
Val	$C_{11}H_{21}O_7N$	280.13908	280.1391	0.07	$C_{11}H_{19}O_6N$	262.12852	262.12854	0.10
	HMF							
Ala	$C_9H_{13}O_5N$	216.08665	216.08665	0.03	$C_9H_{11}O_4N$	198.07609	198.07608	-0.02
Arg	$C_{12}H_{20}O_5N_4$	301.15065	301.15051	-0.44	$C_{12}H_{18}O_4N_4$	283.14009	283.14001	-0.24
Asn	$C_{10}H_{14}O_6N_2$	259.09246	259.09247	0.02	$C_{10}H_{12}O_5N_2$	241.08190	241.08186	-0.14
Asp	$C_{10}H_{13}O_7N$	260.07648	260.07626	-0.83	$C_{10}H_{11}O_6N$	242.06592	242.06589	-0.11
GABA	$C_{10}H_{15}O_5N$	230.10230	230.10231	0.05	$C_{10}H_{13}O_4N$	212.09174	212.09174	0.01
Gln	$C_{11}H_{16}O_6N_2$	273.10811	273.10806	-0.18	$C_{11}H_{14}O_5N_2$	255.09755	255.0988	4.91
Glu	$C_{11}H_{15}O_7N$	274.09213	274.09201	-0.43	$C_{11}H_{13}O_6N$	256.08157	256.08151	-0.20
Gly	$C_8H_{11}O_5N$	202.07100	202.07101	0.08	$C_8H_9O_4N$	184.06044	184.06044	0.03
His	$C_{12}H_{15}O_5N_3$	282.10845	282.10831	-0.50	$C_{12}H_{13}O_4N_3$	264.09789	264.09882	3.53
Leu/Ile	$C_{12}H_{19}O_5N$	258.13360	258.13361	0.03	$C_{12}H_{17}O_4N$	240.12304	240.12303	-0.01
Lys	$C_{12}H_{20}O_5N_2$	273.14450	273.14453	0.12	$C_{12}H_{18}O_4N_2$	255.13394	255.13394	0.03
Met	$C_{11}H_{17}O_5NS$	276.09002	276.09033	1.13	$C_{11}H_{15}O_4NS$	258.07946	-	
Phe	$C_{15}H_{17}O_5N$	292.11795	292.11795	0.00	$C_{15}H_{15}O_4N$	274.10739	274.10739	0.03
Pro	$C_{11}H_{15}O_5N$	242.10230	242.10231	0.05	$C_{11}H_{13}O_4N$	224.09174	224.09174	0.01
Ser	$C_9H_{13}O_6N$	232.08156	232.08157	0.04	$C_9H_{11}O_5N$	214.07100	214.07103	0.14
Thr	$C_{10}H_{15}O_6N$	246.09721	246.09727	0.25	$C_{10}H_{13}O_5N$	228.08665	228.08665	0.02
Trp	$C_{17}H_{18}O_5N_2$	331.12885	331.12875	-0.29	$C_{17}H_{16}O_4N_2$	313.11829	313.11844	0.50
Tyr	$C_{15}H_{17}O_6N$	308.11286	308.11288	0.07	$C_{15}H_{15}O_5N$	290.10230	290.10226	-0.12
Val	$C_{11}H_{17}O_5N$	244.11795	244.11797	0.07	$C_{11}H_{15}O_4N$	226.10739	226.10738	-0.04

To sum up, the Schiff bases of 3-DG gave a more intense signal response than the 3-DG – amino acid adducts, whereas the Michael adducts of HMF were more intense than the Schiff bases of it in both apple juice concentrate and raisin at the same conditions. It is difficult to explain this contradictory result, but it might be related to the reactivity of amino acids and the stability of 3-DG and HMF. As it is well known that the easy addition of nucleophilic groups (-SH, -NH_2) of amino acids to the carbonyl group of 3-DG or to the β-carbon of HMF leads to the formation of the amino acid adducts of 3-DG and HMF, whereas Schiff bases formed through the vinylogous β-elimination of water from 3-DG or HMF [274, 275]. Another remarkable result is that 3DG - Met adduct had the second intense signal response following the HMF-Asn Michael adduct in apple juice concentrate despite the lower amount of methionine. The reactive sulfur-containing side chains of methionine, although less reactive than several amino acids such as lysine, arginine, might lead to involve preferably in Maillard reaction in this study. It should be noted here that it is not possible to predict the kinetics or reaction mechanism from these results because of the countless possibility of adduct formation and complexity of reaction networks in the real food system. Moreover, the MS/MS experiments are needed to elucidate the formation mechanism of adducts. Nevertheless, the confirmation of the adducts of the dicarbonyl compounds and HMF with amino acids proved the Maillard reaction occurring in fruit products during storage.

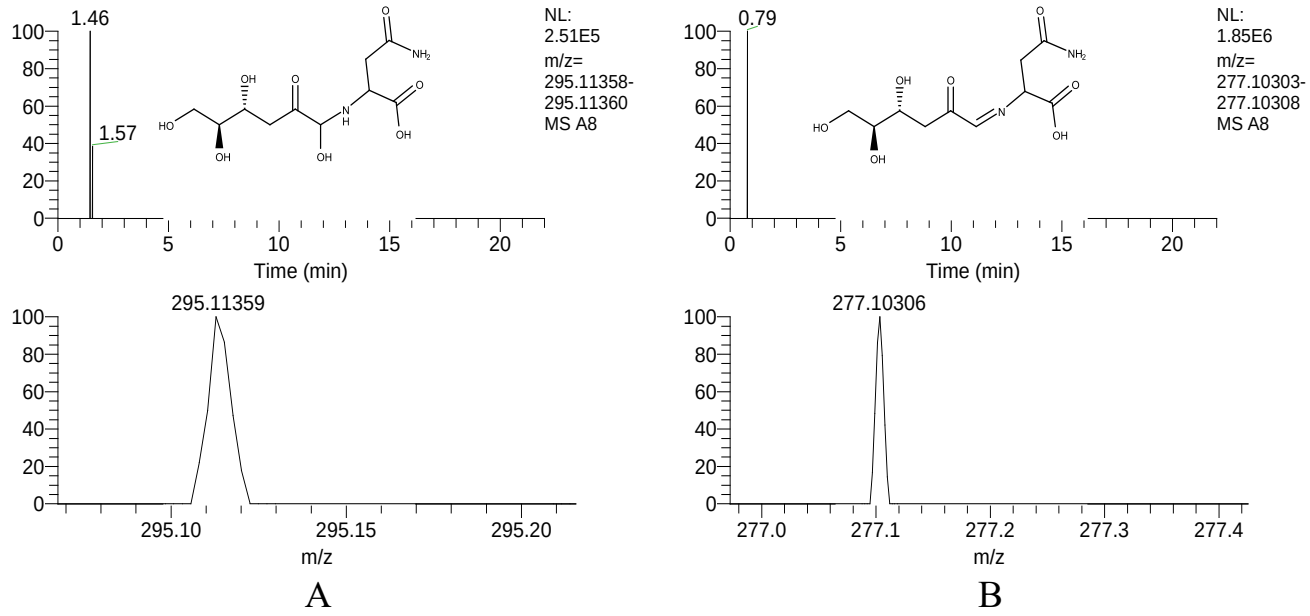

Figure 4.1. Typical extracted ion chromatogram and mass spectrum of the adducts (A) and the schiff bases (B) of 3-DG-ASN possibly formed in apple juice concentrates.

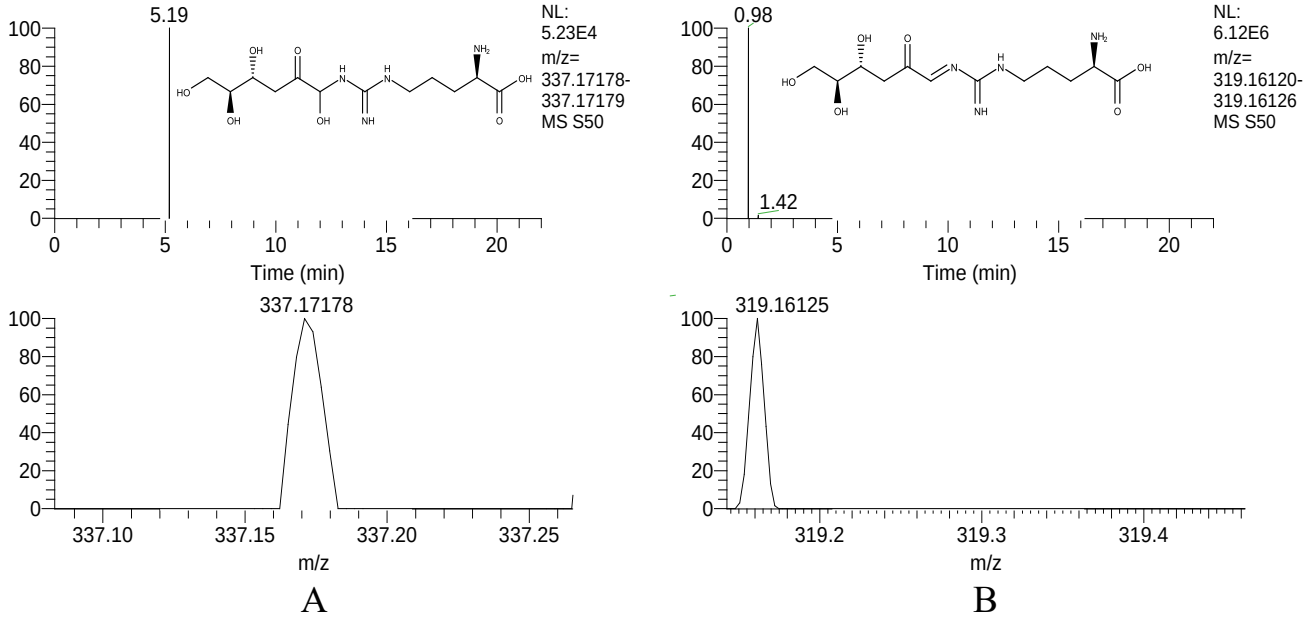

Figure 4.2. Typical extracted ion chromatogram and mass spectrum of the adducts (A) and the schiff bases (B) of 3-DG-ARG possibly formed in raisins.

4.4. CONCLUSION

The effect of the different initial concentrations of fruit juice concentrates and different pH levels of dried fruits on the formation of α-dicarbonyl compounds and HMF during the storage was reported first in this study. Additionally, the confirmation of free amino acid adducts of α-dicarbonyl compounds and HMF was also presented first to provide a better understanding of the role of Maillard reaction during the storage of fruit products. The results revealed that the concentrations of α-dicarbonyl compounds and HMF were significantly increased ($p<0.05$) with the increase in Brix levels. Besides, the changes in the initial levels of fruit juice concentrates caused the change in the main α-dicarbonyl compound profile from glucosone to 3-DG when the Brix level changed from 30 °Bx to 70 °Bx. Conversely, sugar compositions showed no correlation ($p<0.05$) with the increase in the brix levels of fruit juice concentrates whereas the increase in the loss of free amino acids was observed. For dried fruits, the concentrations of α-dicarbonyl compounds increased when the pH level changed from high-acidic (2.64) to nötr (6.61) during the storage. The major α-dicarbonyl compound in fruit products was found as 3-DG and the concentration of 3-DG in dried date at the end of the storage was found as 7251±896.6 mg/kg which has been the highest level of α-dicarbonyl compounds reported in the literature until now. Besides, the sugar concentration in dried fruits showed no significant change ($p<0.05$) during the storage while the loss of free amino acids

increased independently from the pH changes. Evaluating the decrease in the concentrations of free amino acids in both fruit juice concentrates and dried fruits, it is now possible to state that the Maillard reaction contributed to the non-enzymatic reactions through Michael type addition and Schiff base formation during the storage of fruit products. Despite the complicated nature of the Maillard reaction, this study suggests significant insights for future researches into the investigation on the kinetics of amino acid addition to α-dicarbonyl compounds or HMF under acidic conditions at low temperatures.

CHAPTER 5

CHANGES IN α-DICARBONYL COMPOUNDS AND 5-HYDROXYMETHYLFURFURAL DURING PROCESSING OF FRUIT JUICE PRODUCTS

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5.1. INTRODUCTION

Storage and process of foods have a significant impact on the formation and accumulation of α-dicarbonyl compounds and 5-Hydroxymethylfurfural (HMF). Until now in this thesis, the formation of α-dicarbonyl compounds and HMF in various fruit products has been investigated in depth during different storage conditions. On the other side, it has been well known that processing of food products also lead to the formation of α-dicarbonyl compounds and HMF. In the production of fruit juices, concentrates and purees, thermal operations such as pasteurization and concentration are performed for microbial stability and long shelf life. Among several methods for pasteurization, the most common conditions are 85 °C for 15-30 s or 95-100 °C for a few seconds [44]. Concentration is usually performed at 50-80 °C until the desired Brix value is obtained. There have been lots of study reported the effect of heating especially at elevated temperatures accelerating the formation of α-dicarbonyl compounds in food-like model systems or real foods [6, 225]. In addition to these thermal treatments, the enzyme treatment is also carried out at 40-50 °C for 1 or 2 h for the production of clear juices [44]. The enzyme treatment can cause the hydrolysis of proteins that resulted in the increase in free amino acid concentration which may trigger Maillard reaction in fruit juices. Although the effect of thermal operations in various foods on the formation of α-dicarbonyl compounds has been studied a lot, it still unclear how other critical processing stages such as enzymatic treatment, deaeration or the duration of process affect the formation of α-dicarbonyl compounds and HMF.

Therefore, this study aims to investigate the processing effect on the formation α-dicarbonyl compounds and HMF in different fruit products obtained from the critical phases of the industrial-scale processes. In this respect, apple juice, orange juice and peach puree samples were selected as typical examples of clear juice, cloudy juice and puree products, respectively, which have different processing procedures.

5.2. EXPERIMENTAL

5.2.1. Chemicals and consumables

Formic acid (98%) was purchased from JT Baker (Deventer, The Netherlands). HMF (98%) was purchased from Acros (Geel, Belgium). 3-DG (75%), glucosone (≥98%), quinoxaline (99%), 2-methylquinoxaline (97%), 2,3-dimethylquinoxaline (97%), o-phenylenediamine (98%), 5-Methylquinoxaline (98%), L-Theanine (≥98%), diethylenetriaminepentaacetic acid (98%), methanol, and acetonitrile were purchased from Sigma-Aldrich (Steinheim, Germany). Disodium hydrogen phosphate anhydrous and sodium dihydrogen phosphate dihydrate were purchased from Merck (Darmstadt, Germany). The Carrez I and Carrez II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 ml of water, respectively. Ultra-pure water was used throughout the experiments (Milli Q-System, Millipore, Milford, MA). Syringe filters (nylon, 0.45 lm) and Oasis HLB cartridges (30 mg, 1mL) were supplied by Waters (Milford, MA).

5.2.2. Sample preparation

The apple juice concentrate, orange juice, and peach puree concentrate samples from different phases were obtained from a universal fruit juice company in Turkey. Accordingly, the sampling points of the production of apple juice concentrate, orange juice, and peach puree concentrate were shown in **Figure 5.1.A, B, and C,** respectively. All samples were kept frozen at -18^{o}C prior to analysis.

Fruit juice samples from different stages of processing were only diluted with water prior to analysis and then centrifuged at 12,000 xg for 3 min. The clear supernatant-A was used for the determination of α-dicarbonyl compounds and free amino acids in the samples.

The samples were cleaned up for HMF and sugar analysis. For Carrez clarification, 1 mL of the sample was mixed with 50 µl of Carrez I and 50 µl of Carrez II solutions. The mixture was centrifuged at 10,000 xg for 5 min. The clear supernatant-B was used for the determination of HMF and sugar in the samples.

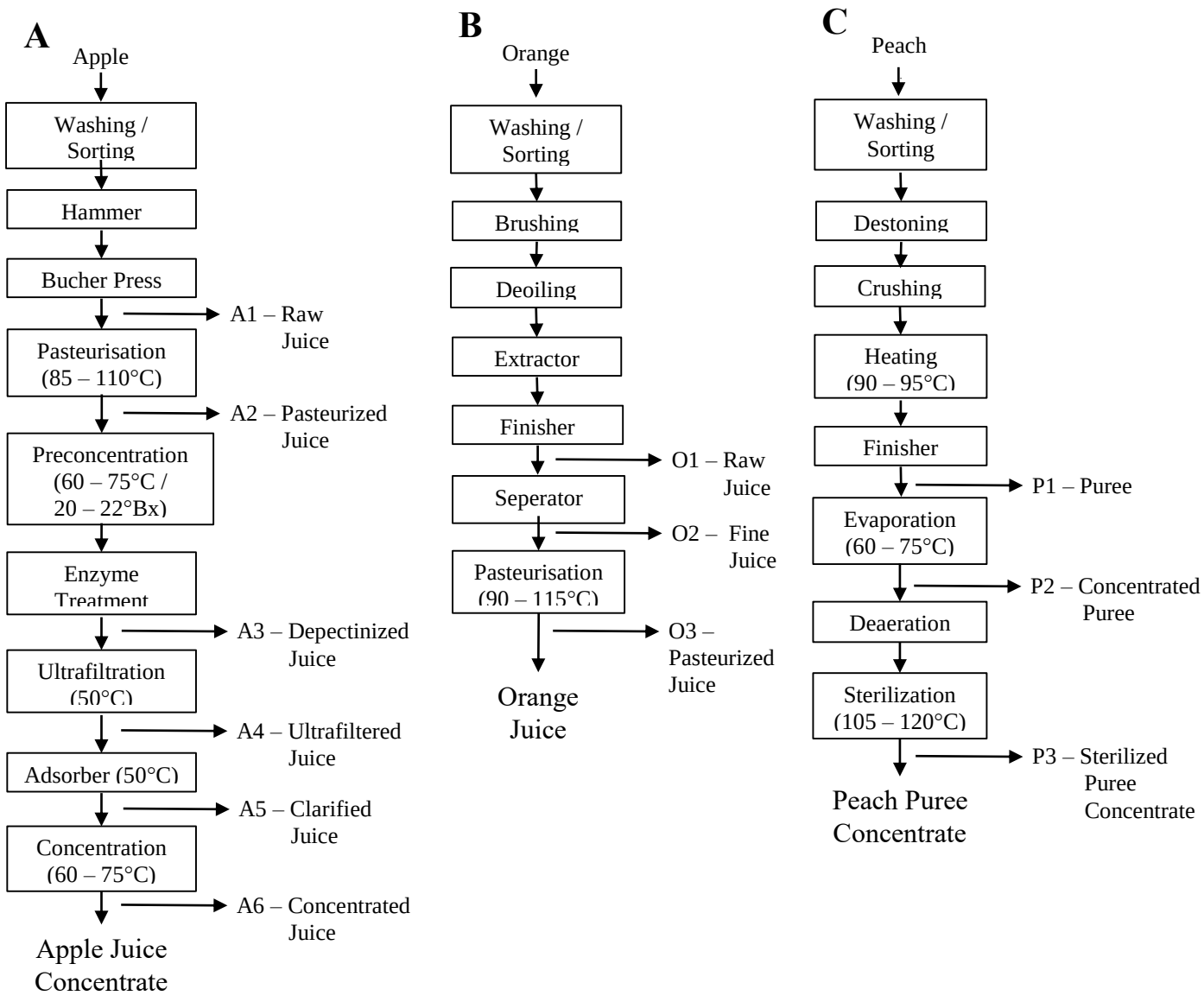


Figure 5.1. Flowchart of sampling points during the production of apple juice concentration (A), orange juice (B), peach puree concentrate (C).

5.2.3. Analysis of sugars

Sugars were determined using an analytical method described elsewhere with minor modifications [6]. One mL of the clear supernatant-B was passed through a preconditioned (by passing 1 mL methanol and 1 mL water) OASIS HLB cartridge. The first 8 drops of the eluent were discarded and the rest was collected into a vial for analysis. The analysis was performed on an Agilent 1200 HPLC system (Waldbronn, Germany) equipped with a quaternary pump, and autosampler coupled with an Agilent 1100 refractive index detector and temperature-controlled column oven. The chromatographic separations were performed on a Shodex Sugar SH-1011 column (300 mm x 8 mm, 6 µm) conditioned at 50 °C. The mobile phase was 5 mM H_2SO_4 in water (v/v) at a flow rate of 1 mL/min. The injection volume was 10 µL. The concentrations of sucrose, glucose, and fructose were calculated from the calibration curves built for each compound in the range between 0.25 and 2.5 g/L (0.25, 0.5, 0.75, 1, and 2.5 g/L).

5.2.4. Analysis of free amino acids

Free amino acids were determined using an analytical method described elsewhere with some modifications [6]. One mL of clear supernatant-A was mixed with one mL of ACN with 0.1 % formic acid and centrifuged at 7000g for 3 min. The supernatant was immediately filtered through a 0.45 µm syringe filter and put into an autosampler vial. The samples were analyzed by an Agilent 1260 Infinity II system coupled to a triple quadrupole detector operated in positive electrospray ionization mode. Chromatographic separations were performed on a Merck ZIC®-HILIC column (150 x 4.6 mm, 3.5 µm, 200Å) by using a gradient mixture of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 1 mL/min. The eluent composition starting with 20% A held for 3 min and then linearly increased to 60% in 2 min and held for 3 min. Then, it was decreased to the initial conditions (20% A) in 1 min and held for 3 min. The column was at 30°C and the autosampler was at 10 °C during the analysis. The electrospray source had the following settings: gas temperature 300°C; the gas flow of 10 L/min; nebulizer 40 psi; capillary voltage of 1.5 kV; sheat gas temperature 375°C; sheat gas flow 12 L/min; nozzle voltage 500 V. L-theanine was used as an internal standard (0.5 mg/L). Quantifications were performed using the calibration curves built for all amino acids in a range between 0.1 and 5.0 mg/L (0.1, 0.25, 0.5, 1, 2.5 and 5.0 mg/L).

5.2.5. Analysis of α-dicarbonyl compounds

α-Dicarbonyl compounds were determined using an analytical method based on derivatization with o-phenylenediamine described elsewhere with some modifications [8]. Five hundred µL

of clear supernatant-A was mixed with 150 μL of 0.2% o-phenylenediamine solution containing 11 mM diethylenetriaminepenta acetic acid and 150 μL of 0.5 M sodium phosphate buffer (pH 7). The mixture was immediately filtered through a 0.45 μm syringe filter into an autosampler vial. It was kept at room temperature, at dark for 2 h prior to measurement.

UPLC-ESI-MS Measurement. α-Dicarbonyl compounds were determined by using a Waters TQD LC-MS/MS system according to the published procedure [160]. 5-Methylquinoxaline with a concentration of 0.5 mg/L was used as an internal standard. Working solutions of glucosone and 3-DG were derivatized and then the concentrations of glucosone, 3-DG, quinoxaline, and 2-methylquinoxaline were calculated using calibration curves built in the range between 0.1 and 5 mg/L (0.1, 0.5, 1, 2, 5 mg/L). Also, the calibration curve of glucosone was used for semi-quantitation of threosone derivatives and 3-DG calibration curve was used for semi-quantitation of 3-DP since both have the same proton-accepting groups. All working solutions were prepared in water.

5.2.6. Analysis of HMF

HMF was determined using an analytical method described elsewhere with some modifications [160]. One mL of clear supernatant-B was filtered through a 0.45 μm syringe filter and put into an autosampler vial. The filtered sample was injected onto an Agilent 1200 series HPLC system consisting of a quaternary pump, an autosampler, a diode array detector, and a temperature-controlled column oven. The chromatographic separations were performed on an Atlantis dC18 column (250 × 4.6 mm, 5 μm) using a gradient mixture of (A) 10 mM formic acid in water and (B) acetonitrile as the mobile phase at a flow rate of 1 mL/min at 30 °C. The gradient mixture was started from 10% B and increased to 30% B in 10 min, 30% B remained for 2 min, then it was decreased to 10% B in 2 min and then 10% B remained for 6 min. The chromatographic run was completed in 20 min. The injection volume was 10 μL. Data acquisition was performed by recording chromatograms at 285 nm. The concentration of HMF was calculated using a calibration curve built in the range between 0.1 and 20 mg/L (0.1, 1, 2, 5, 10, 20 mg/L).

5.2.7. Analysis of pH and Brix

The pH of the juice samples was measured using a PHM210 model pH meter (MeterLab, France) and the brix of the juice samples was measured using a Pocket Pal-3 model refractometer (Atago, Japan).

5.2.8. Statistical analysis

All analyses were run in duplicate with two samples from the same equipment and all data of the process stages of apple juice concentrate, orange juice, and peach puree concentrate production were adjusted to 11.2°Bx, 11.2°Bx and 10.0°Bx, respectively, according to the reference levels of directives of the European Parliament and the Council [259]. The data were subjected to analysis of variance (one-way ANOVA). The Statistical Package for the Social Sciences (SPSS 17.0) statistical package was used for the evaluation of statistical significance of the differences between mean values by the Duncan test. $P<0.05$ was considered to be statistically significant for the results.

5.3. RESULTS AND DISCUSSION

5.3.1. Changes in pH value and soluble solids content

The pH showed no significant changes during all processing steps, ranging from 3.6 to 3.7 for apple juice samples, 3.6 to 3.8 for orange juice samples, and 3.9 to 4.0 for peach puree samples. These results suggested that processing phases had no significant effect on pH ($p>0.05$). For apple juice concentrate production (**Figure 5.1.A**), after pressing (A1), the soluble solids content was 17.4 ± 0.1°Bx, and after pasteurization (A2), it reduced to 15.6 ± 0.0°Bx due to the balance of juice with water before heat treatment. After enzyme treatment (A3), it increased to 20.8 ± 0.1°Bx through the preconcentration of juice. Then, it decreased to 6.8 ± 0.8°Bx with ultrafiltration (A4) due to the water addition to enhance the filtration efficiency and it reached to 9.8 ± 0.5°Bx after adsorption (A5), during which, evaporation proceeded since the operation temperature was 50°C. Finally, it increased to 75 ± 0.4°Bx after the concentration step (A6). For orange juice production (**Figure 5.1.B**), the content of soluble solids showed no significant change ($p>0.05$) as 11.5 ± 0.2°Bx, 10.9 ± 0.4°Bx and 11.1 ± 0.2°Bx, after finisher (O1), separator (O2) and pasteurization (O3), respectively. Finally, for peach puree production (**Figure 5.1.C**), the initial Brix value was measured as 10.7 ± 0.2°Bx and after evaporation the puree (P2), it increased to 28.1 ± 0.1°Bx and remained 28.3 ± 0.1°Bx after sterilization (P3). Hereafter, to compare the process stages each other, all evaluations were made considering the reference Brix levels described in 5.2.8.

5.3.2. Changes in reactants

Sugars play a crucial role in the formation of α-dicarbonyl compounds and HMF due to dehydration and degradation reactions. The concentrations of reducing sugars (glucose and

fructose) and sucrose were monitored during the production. Fructose which can easily participate in caramelization and Maillard reaction was in the highest concentration in apple and orange juice during processing [5]. In peach puree samples, the dominant sugar was sucrose that can rapidly hydrolyze to fructose and glucose in acidic conditions [256]. The concentrations of sucrose and reducing sugars showed no significant changes ($p>0.05$) during the production of all samples **(Table 5.1).** This result was in accordance with the previous report in which the concentrations of the sugars in apple juice showed no significant difference during the process [276].

Amino acids are key participants in the Maillard reaction to form α-dicarbonyl compounds [6]. Twenty free amino acids were detected during the processing of apple juice concentrate, orange juice, and peach puree concentrate **(Table A6 in Annex 2 in supplementary material)**. The major amino acid found in apple juice and peach puree was asparagine while proline was the dominant one in orange juice. These results match those observed earlier studies [276-278]. The concentrations of total free amino acids remained stable in orange juice and peach puree concentrate during processing **(Table 5.1)**. However, the concentrations of total free amino acids increased with the enzyme treatment (A3) in apple juice. It is obviously clear that the commercial enzymes used for the depectinization of juice cause the hydrolysis of proteins. Following the enzymatic treatment, ultrafiltration, resin decolorization and concentration performed and the content of total amino acid in clarified juice (A5) as well as in concentrated juice (A6) was found lower than that of in depectinized juice (A3). A possible explanation for this might be the interaction between amino acids and resin depending on the type of resin. In other respects, enzymatic treatment in apple juice may provide more reactants for the Maillard reaction. However, it is well known that the Maillard reaction occurs more quickly in alkaline conditions compare to acid conditions [279]. With all in this mind, it seems possible that resin adsorption might cause the decrease of amino acids during the production of apple juice concentrate.

Table 5.1. Concentrations of sugars, total reducing sugars and total free amino acids in apple, orange juice samples (g/L) and peach puree samples (g/kg) from the process stages of juice processing.

Apple		**Sucrose**	**Glucose**	**Fructose**	**Total Reducing Sugars**	**Total Free Amino Acids**
Raw Juice	A1	21 ± 0.84^{a}	19.5 ± 0.4^{a}	60 ± 0.56^{a}	79.5 ± 1^{a}	4.6 ± 0.29^{bc}
Pasteurized Juice	A2	20.3 ± 0.32^{a}	19 ± 0.49^{a}	59.1 ± 0.39^{a}	78.1 ± 0.86^{a}	4.2 ± 0.08^{ab}
Depectinized Juice	A3	19.8 ± 0.04^{a}	19.6 ± 0.21^{a}	59.4 ± 0.84^{a}	79 ± 1.03^{a}	5.3 ± 0.167^{d}
Ultrafiltrated Juice	A4	19.8 ± 1.08^{a}	19.1 ± 0.29^{a}	59 ± 2.11^{a}	78.1 ± 2.34^{a}	4.8 ± 0.26^{cd}
Clarified Juice	A5	19.8 ± 0.53^{a}	19.3 ± 0.23^{a}	58.7 ± 0.74^{a}	78.1 ± 0.96^{a}	4.3 ± 0.30^{abc}
Concentrated Juice	A6	20.4 ± 0.92^{a}	19.1 ± 0.25^{a}	59.3 ± 3.02^{a}	78.4 ± 3.28^{a}	4.1 ± 0.12^{a}
Orange						
Raw Juice	O1	26.5 ± 0.15^{a}	27.1 ± 0.84^{a}	29.7 ± 0.25^{a}	56.8 ± 1.09^{a}	4.9 ± 0.25^{a}
Fine Juice	O2	26.9 ± 0.06^{a}	27.1 ± 0.08^{a}	29.1 ± 0.2^{a}	56.2 ± 0.28^{a}	4.8 ± 0.07^{a}
Pasteurized Juice	O3	26.2 ± 0.42^{a}	26.6 ± 0.66^{a}	28.8 ± 0.17^{a}	55.4 ± 0.83^{a}	4.7 ± 0.11^{a}
Peach						
Puree	P1	30.9 ± 3.31^{a}	16.7 ± 1.91^{a}	23.9 ± 2.09^{a}	40.7 ± 4.00^{a}	3.7 ± 0.07^{a}
Concentrated Puree	P2	30.1 ± 3.33^{a}	16.7 ± 2.14^{a}	24.8 ± 1.52^{a}	41.5 ± 3.66^{a}	3.6 ± 0.07^{a}
Sterilized Puree Concentrate	P3	29 ± 1.15^{a}	18.5 ± 1.89^{a}	24 ± 0.36^{a}	42.5 ± 2.25^{a}	3.5 ± 0.15^{a}

Mean values in the same column with different letters are significantly different at the 5 % confidence level.
nd = not detectable.

5.3.3. Changes in reaction products

Dehydration and oxidation reactions of 1,2-enediol intermediate, which is formed from sugars, results in the formation of α-dicarbonyl compounds [4]. Changes in each α-dicarbonyl compounds including 3-DG, glucosone, GO, MGO, DA, threosone, and 3-DP during the processing of apple juice concentrate, orange juice, and peach puree concentrate were given in **Table 5.2.** Glucosone and 3-DG were the main α-dicarbonyl compounds in the concentration range between 1.02 – 17.12 mg/L, 0.91 – 9.9 mg/L in apple juice concentrate during processing, respectively. Despite the lower levels compared to glucosone and 3-DG, GO, MGO, DA, and threosone were detected in apple juice samples. Glucosone formation during the processing of

apple juice concentrate was quite higher than the other α-dicarbonyl compounds. Thus, in the final sample (A6), glucosone was found as 61.1 % as shown in **Figure 5.2.A.** Contrarily, some previous studies reported that 3-DG was the major α-dicarbonyl compound in different fruit juices, like apple juices, orange juices and peach nectars [9, 29, 65]. On the other hand, the dominant α-dicarbonyl compound formed in model sugar solutions heated at temperatures below 100°C was glucosone [163]. So indeed, Gürsul Aktağ and Gökmen [260] reported that glucosone was the major dicarbonyl compound in apple, orange juice, and peach nectar which were analyzed immediately after produced. In the presence of molecular oxygen, sugars such as glucose, fructose, mannose tend to oxidatively decompose into α-dicarbonyl compounds, such as glucosone and its breakdown products [280]. In apple juice concentrate production, the most effective processing step on the formation of each α-dicarbonyl compounds was pasteurization (A2). A possible explanation for this might be that the temperature of pasteurization (85 - 110°C) was the highest among the other processing temperatures. It is well known that heating at elevated temperatures accelerates the formation of α-dicarbonyl compounds [6]. After enzyme treatment (A3) in **Figure 5.1A**, the increase of α-dicarbonyl compounds formation slowed down. This might be explained by the fact that the temperature of the enzyme treatment (50°C) was lower than that of pasteurization. Another possible explanation is that the active side chains of the enzymes or free amino acids produced through protein enzymolysis might trap the α-dicarbonyl compounds [281] while the Maillard reaction and/or caramelization might be taking place at the same time. This simultaneous production and consumption at the same stage might cause the deceleration of the formation of α-dicarbonyl compounds. After the steps of ultrafiltration (A4) and adsorption (A5), the concentration of α-dicarbonyl compounds was continued to increase. The reason might be that the processing stages were performed still at high temperatures (50°C) and the α-dicarbonyl compounds content was getting concentrate with the separation of other compounds found in the juice through ultrafiltration and adsorption. In the final stage of the process (A6), the level of α-dicarbonyl compounds in the apple juice concentrate reached the maximum concentration as expected, due to the thermal load.

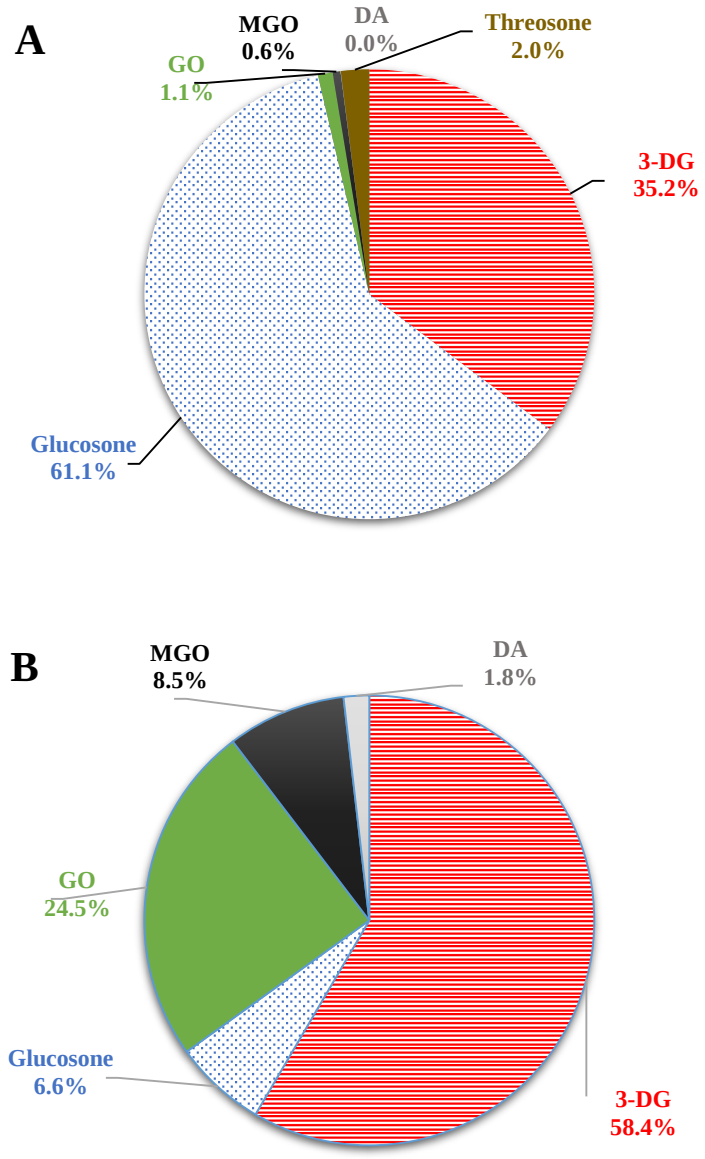
Table 5.2. Concentrations of α-dicarbonyl compounds and 5-hydroxymethylfurfural in apple, orange juice samples (mg/L) and peach puree samples (mg/kg) from the process stages of juice processing.

Process stage		**α-Dicarbonyl Compounds (α-DCs)**							**HMF**
Apple		**3-DG**	**Glucosone**	**GO**	**MGO**	**DA**	**Threosone**	**Total α-DCs**	
Raw Juice	A1	0.91 ± 0.01^{a}	1.02 ± 0.02^{a}	0.0 ± 0.00^{a}	0.02 ± 0^{a}	0.04 ± 0^{a}	0.05 ± 0^{a}	2.04 ± 0.03^{a}	nd^{a}
Pasteurized Juice	A2	3.81 ± 0.04^{b}	8.13 ± 0.07^{b}	0.1 ± 0.01^{b}	0.22 ± 0^{b}	0.09 ± 0.01^{ab}	0.19 ± 0.01^{b}	12.55 ± 0.15^{b}	0.14 ± 0.03^{b}
Depectinized Juice	A3	4.36 ± 0.06^{c}	8.47 ± 0.11^{c}	0.14 ± 0.00^{bc}	0.13 ± 0.02^{ab}	0.19 ± 0.04^{bc}	0.30 ± 0.01^{c}	13.59 ± 0.23^{c}	0.32 ± 0^{c}
Ultrafiltrated Juice	A4	5.02 ± 0.04^{d}	11.03 ± 0.03^{d}	0.17 ± 0.01^{cd}	0.56 ± 0.07^{d}	0.37 ± 0.06^{d}	0.43 ± 0.03^{d}	17.59 ± 0.23^{d}	0.20 ± 0.01^{b}
Clarified Juice	A5	6.5 ± 0.04^{e}	17.47 ± 0.16^{e}	0.21 ± 0.01^{d}	0.37 ± 0.05^{c}	0.26 ± 0.04^{c}	0.52 ± 0.00^{e}	25.33 ± 0.28^{e}	nd^{a}
Concentrated Juice	A6	9.9 ± 0.26^{f}	17.17 ± 0.09^{e}	0.30 ± 0.04^{e}	0.16 ± 0.03^{b}	0.01 ± 0.00^{a}	0.57 ± 0.02^{e}	28.09 ± 0.43^{f}	0.83 ± 0.05^{d}
Orange		**3-DG**	**Glucosone**	**GO**	**MGO**	**DA**		**Total α-DCs**	
Raw Juice	O1	4.74 ± 0.62^{a}	0.82 ± 0.16^{a}	1.73 ± 0.08^{a}	1.29 ± 0.02^{a}	0.57 ± 0.01^{a}		9.16 ± 0.88^{a}	nd^{a}
Fine Juice	O2	13.48 ± 1.66^{b}	1.85 ± 0.23^{a}	4.65 ± 0.70^{b}	1.55 ± 0.01^{a}	0.65 ± 0.01^{b}		22.19 ± 2.61^{b}	1.12 ± 0.05^{b}
Pasteurized Juice	O3	18.24 ± 0.86^{c}	2.07 ± 1.16^{a}	7.66 ± 1.03^{b}	2.67 ± 0.77^{a}	0.57 ± 0.00^{a}		31.2 ± 3.82^{c}	1.08 ± 0.06^{b}
Peach		**3-DG**	**Glucosone**	**GO**	**MGO**	**Threosone**	**3-DP**	**Total α-DCs**	
Puree	P1	1.44 ± 0.12^{a}	1.57 ± 0.05^{a}	3.82 ± 0.24^{a}	1.46 ± 0.1^{b}	0.24 ± 0.03^{b}	0.37 ± 0.01^{a}	8.90 ± 0.56^{a}	nd
Concentrated Puree	P2	10.44 ± 1.07^{b}	13.28 ± 0.44^{b}	3.46 ± 0.41^{a}	1.24 ± 0.12^{ab}	0.33 ± 0.07^{b}	0.84 ± 0.05^{b}	29.59 ± 2.15^{b}	nd
Sterilized Puree Concentrate	P3	29.71 ± 1.56^{c}	12.54 ± 1.73^{b}	2.87 ± 0.47^{a}	1.06 ± 0.14^{a}	0.11 ± 0.03^{a}	2.02 ± 0.12^{c}	48.31 ± 4.05^{c}	nd

Mean values in the same column with different letters are significantly different at the 5 % confidence level. nd: not detectable.

In the orange juice processing, 3-DG, and then GO were the predominant α-dicarbonyl compounds in the concentration ranging in 4.74 – 18.24 mg/L and 1.73 – 7.66 mg/L, respectively **(Table 5.2).** Following 3-DG and GO, MGO, glucosone, and DA were detected in all sampling points of orange juice production. The percentage distribution of each α-dicarbonyl compounds calculated for the orange juice (O3) from the final step of the process was shown in **Figure 5.2.B**. The present findings seem to be consistent with other researches that found 3-DG as the major α-dicarbonyl compound in fruit juices [9]. The reason why the main α-dicarbonyl compound was different in apple and orange samples was most likely due to the differences in processing steps. For instance, deaeration is the essential process of reducing air from orange juice to prevent undesirable quality changes such as ascorbic acid degradation, foam formation, off-flavor, and browning [64]. Hence, the vacuum deoiling step can simultaneously deaerate the juice, and so oxidation reactions resulting in the formation of glucosone and threosone can be reduced. Besides, there is no enzyme treatment step for 1-2 hours or clarification steps such as ultrafiltration and adsorption during orange juice production contrary to the production of apple juice concentrate. Therefore, the lack of these extra steps might also lead to a reduction in the oxidation reactions of α-dicarbonyl compounds. On the other hand, another possible explanation for lower glucosone concentrations in orange juice could be due to its decomposition to GO. Indeed, the finding of the high concentration of GO following 3-DG supports this approach. During orange juice production, the total dicarbonyl concentration increased dramatically from 9.16 to 22.19 mg/L after the separation step (O2), and it reached 31.2 mg/L after pasteurization (O3). The deceleration in the increase of α-dicarbonyl compounds during pasteurization at high temperatures such as 95 – 110 °C might be due to the elimination of α-dicarbonyl compounds to form AGEs or other products such as Strecker degradation products [6]. In the peach puree concentration process, the initial contents of dominant α-dicarbonyl compounds, GO and glucosone, were found as 3.82 mg/kg and 1.57 mg/kg, respectively (**Table 5.2**). However, after evaporation (P2) and finally sterilization (P3), 3-DG became the major α-dicarbonyl compound in the concentration of 29.71 mg/kg. On the other hand, the concentration of glucosone which was increased dramatically after evaporation showed no significant change after sterilization ($p>0.05$). There were also no significant changes in the concentrations of GO during processing ($p>0.05$). The deaeration process between evaporation and sterilization (**Figure 5.1.C**) might be one of the main reasons for the behavior of glucosone and GO. In the final product (P3), 3-DG had the maximum ratio with the percentage of 61.5 % and glucosone followed 3-DG with a percentage of 26.0 % as given in

Figure 5.2.C. Similarly, Kocadağlı and Gökmen [8] reported that 3-DG followed by glucosone were the major α-dicarbonyl compounds in commercial mixed fruit purees including peach purees. These were not the only α-dicarbonyl compounds found in peach puree samples during processing but also MGO, threosone, and 3-DP were detected with comparably lower levels **(Table 5.2)**. Evaluating the effect of each process on the formation of total α-dicarbonyl compounds in peach puree samples, the most effective step was found to be the evaporation stage (P2). Considering the temperature of sterilization (105 - 120°C), the main reason for this finding might be that overheating promotes the reaction to proceed to advanced stages [282].



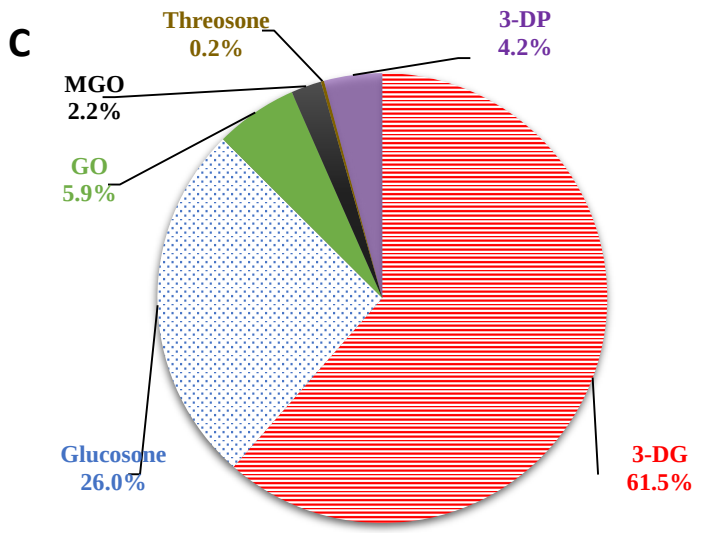


Figure 5.2. The percentage distribution of α-dicarbonyl compounds found in the final stage of the processing of apple juice concentrate (A), orange juice (B) and peach puree concentrate (C).

Changes in HMF concentration during the productions of apple juice concentrate, orange juice, and peach puree concentrate were given in **Table 5.2**. Accordingly, HMF was not detected in peach puree samples during all processing phases. For orange juice production, HMF was measured as 1.12 mg/L after separation, and remained constant after pasteurization. During apple juice processing, HMF was detected as 0.14 mg/L after pasteurization, and it increased to 0.32 mg/L with enzyme treatment. The reason of the increase during depectinization might be due to the increase in the concentration of the reactants with enzyme treatment. Then it became decreasing with ultrafiltration, and it was not detectable at the step of adsorption. Resin decolorization might cause the adsorption of HMF in this regard. Finally, HMF was found as 0.83 mg/L due to the heat treatment in the concentration step. The Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union (AIJN) has declared a maximum HMF level of 10 mg/L for fruit juices [198]. In this respect, apple juice concentrate, orange juice, and peach puree did not pose a danger to our results. In addition, the results also comply with earlier studies which found in small quantities of HMF in various juices [55, 64].

5.4. CONCLUSION

To the best of our knowledge, changes in α-dicarbonyl compounds and HMF during industrial-scale apple juice concentrate, orange juice, and peach puree concentrate production were reported first in this study. The results of α-dicarbonyl compounds showed different trends between apple juice, orange juice, and peach puree samples during processing. Glucosone was

identified as the main dicarbonyl compound formed in apple juice samples, while 3-DG was the dominant in orange juice and peach puree samples, depending on the process phase. These findings support that the processing style had a strong effect on the formation and the type of α-dicarbonyl compounds. The temperature of operation, the duration of the process, and the presence of molecular oxygen were the critical factors affecting the fate of α-Dicarbonyl compounds and HMF. On the other hand, α-dicarbonyl compounds were highly quantified, while the HMF levels were quite low or no detectable in this study. Therefore, it is clear that the measurement of HMF as a quality indicator in processed fruit products is not sufficient by oneself. It is recommended that α-dicarbonyl compounds should be monitored together with HMF in order to evaluate the quality and safety of processed fruit products. In conclusion, understanding the influence factors of the formation of process contaminants (α-dicarbonyl compounds and HMF) during processing can help to reduce their formation in fruit juice and puree samples at the right time.

CONCLUSION AND GENERAL DISCUSSION

In recent years, the investigation of the formation of α-dicarbonyl compounds in foods is increasingly of importance in terms of quality and safety issues. It has been known that Maillard reaction and caramelization are the reactions mainly responsible for the formation of α-dicarbonyl compounds during the storage and/or processing of foods. Although there have been several studies on the formation of α-dicarbonyl compounds through Maillard reaction especially in food-like model systems with neutraline and/or alkaline media, there is still no clear explanation of the fate of α-dicarbonyl compounds in acidic sugary food systems. Since Maillard reaction and caramelization occur simultaneously in foods, such complicated reactions make it difficult to clarify the reaction mechanisms of α-dicarbonyl compounds in real foods. Therefore, there are still many questions as mentioned in "Introduction" need to be answered especially in acidic sugar-rich real food systems such as fruit products.

To answer the questions of what the level of α-dicarbonyl compounds in fruit products is, and what their importance in terms of dietary exposure are, the level of α-dicarbonyl compounds and HMF in a large number of fruit products has been analyzed and the daily intake level has been calculated in **Chapter 2.** In this study, a wide range of α-dicarbonyl compounds at worrying levels have been determined, being 3-DG the highest in raisin. Although the maximum level of α-dicarbonyl compounds has been found in dried fruit products, the dietary intake calculations showed that fruit juice products also pose a risk. The results indicated that the main α-dicarbonyl compound profile changed from 3-DG to glucosone under aqueous conditions i.e. fruit juices. In addition, α-dicarbonyl compounds have been found to carry a great risk rather than HMF considering their concentrations and daily intake levels in fruit products. Contrary to what is known, the determination of only HMF as a quality marker of processed foods is not enough to make a reliable evaluation of the quality and safety of foods. In the end, this study encouraged the further investigation of α-dicarbonyl compounds together with HMF in detail in different fruit products during storage and processing.

The effect of storage on the formation of α-dicarbonyl compounds has been investigated in aqueous fruit products such as fruit juices in **Chapter 3,** and in mid-and low-moisture fruit products such as fruit juice concentrates and dried fruits in **Chapter 4**. In **Chapter 3,** prolonged storage at high temperatures has been found to cause a higher accumulation of α-dicarbonyl compounds at concerning levels even in fruit juices which contain lower levels of α-dicarbonyl compounds than other fruit products. In contrary to the literature, glucosone has

been found as the main α-dicarbonyl compound in apple and orange juices. This finding provided a new perspective in terms of disregarded α-dicarbonyl compounds such as glucosone in foods. From a kinetic point of view, it was first hypothesized in this study that the sugar decomposition pathway is mainly responsible for the formation of α-dicarbonyl compounds in fruit juices during prolonged storage. One multiresponse kinetic model was built which fitted well to the experimental data obtained from three different types of fruit juices. Thus, it could be possible to explain the formation mechanism of α-dicarbonyl compounds and HMF in acidic sugary beverages from a comprehensive perspective. The proposed multiresponse kinetic model provided a better insight into the formation mechanism of α-dicarbonyl compounds during the storage of juices by specifying the kinetically important steps. The controversial and unknown issues of the reaction network have been enlightened in this way, highlighting the importance of isomerization of glucose and fructose via 1,2-enolization, and formation of HMF from fructose rather than the 3-DG pathway. One of the major achievements of this thesis is the establishment of a comprehensive kinetic model for a real food that makes it possible to gain the ability to control undesired changes during the storage of fruit juices from a quality and safety viewpoint.

The effect of parameters during storage of mid-and low-moisture fruit products has been investigated in **Chapter 4.** From the intrinsic parameters affecting the formation of α-dicarbonyl compounds in fruit products, the effects of initial reactant concentrations and pH have been investigated in fruit juice concentrates (mid-moisture) and dried fruits (low-moisture), respectively. From the quantitative point of view, the concentration of 3-DG, major dicarbonyl in dried fruits, was found to be the highest at the end of the storage. It is important to note that the concentration of 3-DG in dried date at the end of the storage is the highest level reported in the literature to date. This clearly showed that storage has a strong effect on the excessive accumulation of α-dicarbonyl compounds. In addition, results revealed that the increase in the initial reactant concentration in fruit juice concentrates lead to the increase in the loss of free amino acid concentration whereas no significant change in sugar concentration. This result was contrary to the previous results where total free amino acids remained stable during the same storage conditions of fruit juices. This finding raised a new question, does Maillard reaction play a role in the fate of α-dicarbonyl compounds in fruit products when the conditions changes from aqueous to highly concentrated? Confirmation of the adducts of α-dicarbonyl compounds and HMF with amino acids by high-resolution mass spectrometry answered this question as Maillard reaction occurred during the storage of both fruit juice

concentrates and dried fruits. Indeed, the increase in the dicarbonyl concentration when the pH level changed from high-acidic (2.6) to neutral (6.6) in dried fruits also support the role of Maillard reaction on non-enzymatic reactions in fruit products during storage. Proving the role of Maillard reaction in low moisture fruit products has raised new questions which may lead to further investigation on the reaction kinetics of amino acids under acidic and mild conditions.

Besides the effect of storage on the formation of α-dicarbonyl compounds, the fate of α-dicarbonyl compounds at different stages of the process of fruit products has also been investigated in **Chapter 5.** In this regard, three different fruit products, apple juice, orange juice, and peach puree, were selected as representative for different fruit production process from industrial-scale production. The results revealed that the processing style specified the main type of dicarbonyl compound formed in the fruit products. For example, orange juice and peach puree concentrate which were deaerated during processing contained 3-DG as the main dicarbonyl whereas glucosone was the major one in apple juice concentrate in the presence of oxygen. Interestingly, the formation of main α-dicarbonyl compounds such as glucosone and 3-DG increased during the processing of all fruit types although some steps like ultrafiltration or clarification, at temperatures 50 °C, expected to cause a decrease in the formation of α-dicarbonyl compounds. It is understood that continuous mild temperature conditions even below 100 °C can cause the accumulation of α-dicarbonyl compounds in aqueous fruit products. The results give rise to the necessity of the development of alternative technologies to thermal treatments. Last but not least, the concentrations of α-dicarbonyl compounds were found to be much higher than HMF during all processing stages of fruit products. This finding is in accordance with the previous results in this thesis regarding the quite low or not detectable levels of HMF despite the high level of α-dicarbonyl compounds in aqueous acidic fruits. Therefore, it is strongly recommended that HMF and α-dicarbonyl compounds should be measured together as quality indicators to make a true evaluation of the quality and safety of fruit juices.

Overall, this thesis study contributes greatly to understanding the fate of α-dicarbonyl compounds together with HMF in fruit products as real foods, in depth. Therefore, the results could be effectively used in further studies on the development of mitigation and/or inhibition strategies of α-dicarbonyl compounds in foods. In aqueous fruit products, glucosone was the key intermediate should be paid special attention. This thesis pointed out the importance of α-dicarbonyl compounds which have the potential to use as a chemical marker in processed foods.

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APPENDIX

ANNEX 1. Supplementary Tables For Chapter 4

Table A1. The concentrations of free amino acids (mg/kg) in apple juice concentrates during storage. All data were adjusted to 11.2°Bx.

Brix	Week	Ala	Arg	Asn	Asp	Gaba	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
30	0	8.5 ±0.4^{a}	3 ±0.1^{e}	570.7 ±27.2^{d}	151.1 ±7.2^{a}	24.8 ±1.2^{de}	25.4 ±1.2^{d}	57 ±2.7^{f}	0.8 ±0^{c}	1.8 ±0.1^{f}	7.2 ±0.3^{f}	2 ±0.1^{e}	1.7 ±0.1^{e}	1.1 ±0.1^{d}	7.1 ±0.3^{f}	2.1 ±0.1^{b}	11.5 ±0.5^{de}	8.7 ±0.4^{g}	3 ±0.1^{d}	5.9 ±0.3^{d}	1.7 ±0.1^{d}
30	2	8.9 ±0.8^{ab}	2.6 ±0^{bc}	586.6 ±2.9^{d}	163.3 ±0.8^{bc}	36.4 ±0.2^{h}	8.6 ±0^{c}	55.9 ±0.3^{f}	0.8 ±0^{b}	1.5 ±0^{e}	6 ±0^{cde}	2.1 ±0^{f}	1.6 ±0de	0.9 ±0^{c}	7 ±0^{ef}	2.4 ±0de	11.9 ±0.1^{e}	7.9 ±0^{ef}	0.8 ±0^{c}	9.7 ±0^{f}	1.5 ±0^{bc}
30	4	8.6 ±0.7^{a}	2.8 ±0^{cd}	562 ±10^{d}	165.2 ±2.9^{bcd}	22.2 ±0.4^{b}	2.8 ±0^{b}	49.5 ±0.9^{d}	0.7 ±0^{a}	1.5 ±0^{e}	6.9 ±0.1^{f}	2 ±0^{e}	1.5 ±0^{cd}	0.7 ±0^{b}	6.8 ±0.1^{def}	1.9 ±0^{a}	11.4 ±0.2^{de}	8.3 ±0.1^{fg}	0.3 ±0^{b}	5.3 ±0.1^{bc}	1.5 ±0^{bc}
30	6	9 ±1^{ab}	2.8 ±0^{d}	525.3 ±3.9^{c}	170 ±1.3^{cd}	27 ±0.2^{f}	0.7 ±0^{a}	49.8 ±0.4^{d}	0.9 ±0^{cd}	1.4 ±0de	5.9 ±0^{bcd}	2 ±0^{e}	1.5 ±0^{cd}	0.7 ±0^{b}	6.6 ±0^{cde}	2.1 ±0^{b}	11.3 ±0.1^{de}	7.7 ±0.1^{de}	0.1 ±0^{ab}	6.4 ±0^{e}	1.6 ±0^{c}
30	8	11.7 ±0.6^{bcde}	2.5 ±0^{b}	520.3 ±4.6^{c}	174.1 ±1.6^{d}	19.9 ±0.2^{a}	0.4 ±0^{a}	51.4 ±0.5^{de}	0.7 ±0^{a}	1.3 ±0^{cd}	6.1 ±0.1^{de}	1.9 ±0de	1.4 ±0^{c}	0.6 ±0^{a}	6.8 ±0.1^{def}	2.2 ±0^{bc}	11.1 ±0.1^{cd}	8 ±0.1^{ef}	0.1 ±0^{ab}	5 ±0^{ab}	1.5 ±0^{bc}
30	10	11.4 ±1^{abcde}	2.7 ±0^{cd}	447.4 ±4.6^{b}	147.8 ±1.5^{a}	19.8 ±0.2^{a}	0.2 ±0^{a}	37.2 ±0.4^{b}	0.9 ±0^{cd}	1.4 ±0de	5.6 ±0.1^{bc}	1.4 ±0^{ab}	1.5 ±0^{c}	0.6 ±0^{a}	6.5 ±0.1^{cd}	1.8 ±0^{a}	10.6 ±0.1^{bc}	6.4 ±0.1^{b}	0.1 ±0^{ab}	4.7 ±0^{a}	1.5 ±0^{bc}
30	12	9.9 ±0.8^{abc}	2.7 ±0^{cd}	446.1 ±3.7^{b}	165.6 ±1.4^{bcd}	25.7 ±0.2^{ef}	0.2 ±0^{a}	39.8 ±0.3^{b}	0.9 ±0^{d}	1.2 ±0^{c}	6.2 ±0.1^{de}	1.5 ±0^{b}	1.4 ±0^{c}	0.6 ±0^{a}	6.3 ±0.1^{bc}	2.2 ±0^{bc}	10.4 ±0.1^{b}	6.7 ±0.1^{b}	0.1 ±0^{ab}	5.8 ±0^{d}	1.4 ±0^{b}
30	14	12 ±1.3^{cde}	2.4 ±0^{a}	417.9 ±4.4^{b}	183.6 ±2^{e}	30.7 ±0.3^{g}	0.1 ±0^{a}	54 ±0.6^{ef}	1.2 ±0^{f}	1 ±0^{b}	6.3 ±0.1^{e}	1.8 ±0^{d}	1.3 ±0^{b}	0.6 ±0^{a}	5.9 ±0.1^{ab}	2.5 ±0^{e}	10.1 ±0.1^{b}	8.1 ±0.1^{ef}	0.1 ±0^{a}	5.4 ±0.1^{c}	1.5 ±0^{bc}
30	16	13.8 ±0.7^{de}	2.5 ±0^{ab}	348.8 ±2^{a}	160.7 ±0.9^{bc}	23 ±0.1^{bc}	0.2 ±0^{a}	46.3 ±0.3^{c}	1 ±0^{e}	1 ±0^{b}	5.6 ±0^{b}	1.4 ±0^{a}	1.3 ±0^{ab}	0.6 ±0^{a}	5.6 ±0^{a}	2.3 ±0^{cd}	9.3 ±0.1^{a}	7.3 ±0^{cd}	0.1 ±0^{a}	4.8 ±0^{a}	1.3 ±0^{a}
30	18	13.9 ±1.2^{e}	2.4 ±0^{a}	338.1 ±5.3^{a}	165 ±2.6^{bcd}	24.9 ±0.4^{de}	0.1 ±0^{a}	38.4 ±0.6^{b}	1.1 ±0^{f}	1 ±0^{b}	5.1 ±0.1^{a}	1.7 ±0^{c}	1.2 ±0^{ab}	0.6 ±0^{a}	5.5 ±0.1^{a}	2.3 ±0^{c}	9.4 ±0.1^{a}	6.8 ±0.1^{bc}	0.1 ±0^{a}	5 ±0.1^{ab}	1.5 ±0^{bc}
30	20	10.8 ±0.9^{abcd}	2.4 ±0^{a}	329.3 ±6.6^{a}	156.8 ±3.1^{ab}	24 ±0.5^{cd}	0.2 ±0^{a}	31.2 ±0.6^{a}	1 ±0^{e}	0.9 ±0^{a}	4.9 ±0.1^{a}	1.7 ±0^{c}	1.2 ±0^{a}	0.6 ±0^{a}	5.5 ±0.1^{a}	1.9 ±0^{a}	8.9 ±0.2^{a}	5.4 ±0.1^{a}	0.1 ±0^{a}	4.8 ±0.1^{a}	1.6 ±0^{c}
50	0	5.8 ±0.3^{ab}	3.8 ±0.2^{f}	758 ±36.1^{f}	164.1 ±7.8^{fg}	23 ±1.1^{f}	30.7 ±1.5^{d}	60 ±2.9^{h}	0.7 ±0^{f}	2.8 ±0.1^{h}	6.1 ±0.3^{f}	2.3 ±0.1^{g}	1.7 ±0.1^{g}	0.8 ±0^{h}	9.1 ±0.4^{g}	1.3 ±0.1^{e}	10.9 ±0.5^{f}	10.7 ±0.5^{h}	2.9 ±0.1^{d}	6.7 ±0.3^{i}	1 ±0^{f}
50	2	5.9 ±0.5^{abc}	3.6 ±0^{ef}	679.9 ±3.4^{e}	168.7 ±0.8^{g}	19.9 ±0.1^{e}	9.5 ±0^{c}	49 ±0.2^{g}	0.7 ±0^{e}	2.5 ±0^{f}	5.5 ±0de	2.1 ±0^{f}	1.5 ±0^{f}	0.7 ±0^{g}	8.4 ±0^{f}	1.2 ±0^{d}	10.5 ±0.1^{ef}	9.7 ±0^{g}	1 ±0^{c}	6.2 ±0^{h}	0.9 ±0de
50	4	4.8 ±0.4^{a}	3.5 ±0^{e}	585.2 ±10.4^{d}	153.4 ±2.7^{de}	16.9 ±0.3^{d}	3 ±0.1^{b}	40.2 ±0.7^{f}	0.6 ±0de	2.2 ±0^{e}	5.5 ±0.1^{e}	2 ±0^{e}	1.4 ±0^{e}	0.6 ±0^{f}	7.4 ±0.1^{e}	1.1 ±0^{c}	10.2 ±0.2^{e}	8.3 ±0.1^{f}	0.4 ±0^{b}	5 ±0.1^{g}	0.9 ±0^{cd}
50	6	6.1 ±0.7^{abc}	3.6 ±0^{ef}	484.1 ±3.6^{c}	146.2 ±1.1^{cd}	7 ±0.1^{a}	0.8 ±0^{a}	39.5 ±0.3^{ef}	0.6 ±0de	2.7 ±0^{g}	6.4 ±0^{f}	2 ±0^{e}	1.3 ±0de	0.5 ±0de	7.1 ±0.1^{e}	0.9 ±0^{a}	9.6 ±0.1^{d}	8.3 ±0.1^{f}	0.2 ±0^{a}	2.4 ±0^{a}	0.9 ±0^{abc}

Table A1 continue.

Brix	Week	Ala	Arg	Asn	Asp	Gaba	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
50	8	6.6 ±0.3^{abcd}	2.9 ±0^{d}	458.6 ±4.1^{c}	153.7 ±1.4^{de}	13 ±0.1^{c}	0.3 ±0^{a}	36.7 ±0.3^{de}	0.6 ±0^{bc}	1.6 ±0^{d}	5.2 ±0^{cd}	1.8 ±0^{d}	1.2 ±0^{d}	0.6 ±0^{e}	6.5 ±0.1^{d}	1 ±0^{bc}	9.5 ±0.1^{d}	7.5 ±0.1^{e}	0.1 ±0^{a}	3.4 ±0^{d}	0.9 ±0^{abcd}
50	10	7.8 ±0.7^{cd}	2.8 ±0^{cd}	337.7 ±3.5^{b}	126.4 ±1.3^{a}	17.7 ±0.2^{d}	0.1 ±0^{a}	38.6 ±0.4^{def}	0.5 ±0^{b}	1.4 ±0^{d}	4.9 ±0.1^{c}	1.6 ±0^{bc}	1.1 ±0^{c}	0.5 ±0^{cd}	6.2 ±0.1^{d}	1.4 ±0^{f}	7.7 ±0.1^{bc}	6.6 ±0.1^{cd}	0.1 ±0^{a}	4.3 ±0^{f}	0.9 ±0^{bcd}
50	12	7 ±0.6^{bcd}	2.5 ±0^{b}	317.8 ±2.6^{b}	128.9 ±1.1^{ab}	10.6 ±0.1^{b}	0.1 ±0^{a}	29.6 ±0.2^{c}	0.6 ±0^{d}	1.5 ±0^{d}	4.5 ±0^{b}	1.7 ±0^{c}	1 ±0^{b}	0.5 ±0^{c}	5.5 ±0^{bc}	1.1 ±0^{c}	7.3 ±0.1^{b}	5.7 ±0^{b}	0.1 ±0^{a}	2.9 ±0^{bc}	1 ±0^{e}
50	14	8.1 ±0.9^{d}	2.6 ±0^{bc}	347.7 ±3.7^{b}	163.8 ±1.7^{fg}	12.6 ±0.1^{c}	0.1 ±0^{a}	36.1 ±0.4^{d}	0.6 ±0^{b}	1.3 ±0^{c}	4.3 ±0^{b}	1.6 ±0^{bc}	1 ±0^{b}	0.4 ±0^{ab}	5.7 ±0.1^{c}	1.1 ±0^{c}	8.3 ±0.1^{c}	6.9 ±0.1^{d}	0.1 ±0^{a}	3.2 ±0^{cd}	0.9 ±0^{abcd}
50	16	8.2 ±0.4^{d}	2.6 ±0^{bc}	313.6 ±1.8^{b}	156.1 ±0.9^{ef}	10.4 ±0.1^{b}	0.1 ±0^{a}	29.9 ±0.2^{c}	0.6 ±0^{cd}	0.6 ±0^{a}	4.4 ±0^{b}	1.5 ±0^{b}	0.9 ±0^{a}	0.5 ±0^{bc}	5.5 ±0^{bc}	1.1 ±0^{c}	7.9 ±0^{bc}	6.3 ±0^{c}	0.1 ±0^{a}	2.8 ±0^{b}	0.8 ±0^{abc}
50	18	8.4 ±0.7^{d}	2.3 ±0^{a}	254.2 ±4^{a}	137.7 ±2.1^{bc}	12.3 ±0.2^{c}	0.1 ±0^{a}	25.5 ±0.4^{b}	0.6 ±0^{bc}	1.1 ±0^{b}	3.6 ±0.1^{a}	1.4 ±0^{a}	0.9 ±0^{a}	0.4 ±0^{ab}	5.1 ±0.1^{ab}	1.1 ±0^{c}	6.6 ±0.1^{a}	5.4 ±0.1^{ab}	0.1 ±0^{a}	3.5 ±0.1^{d}	0.8 ±0^{ab}
50	20	6.8 ±0.6^{bcd}	2.3 ±0^{a}	254.3 ±5.1^{a}	141.3 ±2.8^{c}	11 ±0.2^{b}	0.1 ±0^{a}	22.4 ±0.4^{a}	0.5 ±0^{a}	1 ±0^{b}	3.6 ±0.1^{a}	1.3 ±0^{a}	0.8 ±0^{a}	0.4 ±0^{a}	4.9 ±0.1^{a}	1 ±0^{b}	6.7 ±0.1^{a}	5 ±0.1^{a}	0.1 ±0^{a}	3.9 ±0.1^{e}	0.8 ±0^{a}
70	0	9 ±0.4^{d}	1.8 ±0.1^{ef}	627.5 ±6.5^{f}	189.7 ±2^{h}	20.8 ±0.2^{f}	32.8 ±0.3^{e}	55.4 ±0.6^{i}	1 ±0^{i}	1.1 ±0^{h}	7.5 ±0.1^{j}	1.8 ±0^{i}	1.6 ±0^{i}	1.4 ±0^{e}	7.1 ±0.1^{i}	2.5 ±0de	14.7 ±0.2^{i}	9.4 ±0.1^{i}	3.1 ±0^{g}	5.4 ±0.1^{g}	1.8 ±0^{g}
70	2	8.9 ±0.8^{d}	1.9 ±0^{f}	314.8 ±12.1^{e}	133 ±0.7^{f}	21.7 ±0.1^{g}	6.5 ±0^{d}	48.3 ±0.2^{h}	0.8 ±0^{h}	1 ±0^{g}	5.4 ±0^{i}	1.6 ±0^{h}	1.2 ±0^{h}	1.2 ±0^{d}	5.5 ±0^{h}	2.9 ±0^{h}	9.9 ±0^{g}	6.8 ±0^{g}	1.1 ±0^{f}	5.5 ±0^{g}	1.7 ±0^{f}
70	4	8.3 ±0.7^{bcd}	1.9 ±0^{ef}	281.2 ±40.2^{de}	145.9 ±2.6^{g}	21.2 ±0.4^{f}	2.9 ±0.1^{c}	48.5 ±0.9^{h}	0.8 ±0^{h}	0.8 ±0^{f}	4.9 ±0.1^{h}	1.5 ±0^{g}	1.1 ±0^{g}	1.2 ±0^{d}	5.2 ±0.1^{g}	2.7 ±0^{f}	10.3 ±0.2^{h}	7.1 ±0.1^{h}	0.5 ±0^{e}	5.1 ±0.1^{f}	1.7 ±0^{ef}
70	6	8.8 ±1^{cd}	1.8 ±0^{e}	254.5 ±20.6^{d}	146.4 ±1.1^{g}	13.1 ±0.1^{d}	1.2 ±0^{b}	45.8 ±0.3^{g}	0.7 ±0^{g}	0.8 ±0^{f}	4.8 ±0^{h}	1.5 ±0^{g}	1 ±0^{f}	1.2 ±0^{d}	5.3 ±0^{g}	2.8 ±0^{fg}	10.1 ±0.1^{gh}	6.9 ±0.1^{g}	0.3 ±0^{d}	4.3 ±0^{d}	1.6 ±0^{cd}
70	8	8.1 ±0.4^{bcd}	1.3 ±0^{d}	192.3 ±1.7^{c}	123.8 ±1.1^{e}	14.9 ±0.1^{e}	0.4 ±0^{a}	24.5 ±0.2^{f}	0.7 ±0^{g}	0.6 ±0^{e}	4 ±0^{g}	1.2 ±0^{f}	0.8 ±0^{e}	1.1 ±0^{c}	4.2 ±0^{f}	2.9 ±0^{g}	7.7 ±0.1^{f}	4 ±0^{f}	0.2 ±0^{c}	4.8 ±0^{e}	1.7 ±0^{f}
70	10	6.9 ±0.6^{abc}	1.1 ±0^{c}	171.7 ±1.8^{bc}	104.7 ±1.1^{c}	13.1 ±0.1^{d}	0.2 ±0^{a}	16.5 ±0.2^{e}	0.5 ±0^{e}	0.6 ±0^{d}	3.3 ±0^{f}	1 ±0^{e}	0.7 ±0^{d}	1 ±0^{b}	3.6 ±0^{e}	2.5 ±0de	6.6 ±0.1^{e}	3.3 ±0^{e}	0.2 ±0^{ab}	4.8 ±0.1^{e}	1.5 ±0^{b}
70	12	6.1 ±0.5^{a}	1 ±0^{c}	169.8 ±1.4^{bc}	115.9 ±0.9^{d}	10.4 ±0.1^{c}	0.2 ±0^{a}	13.3 ±0.1^{d}	0.5 ±0^{f}	0.6 ±0^{cd}	3.1 ±0^{e}	1.1 ±0^{e}	0.7 ±0^{c}	1 ±0^{b}	3.4 ±0^{d}	2.4 ±0^{bc}	6.6 ±0.1^{e}	2.9 ±0^{d}	0.2 ±0^{bc}	3.8 ±0^{c}	1.7 ±0de
70	14	5.9 ±0.7^{a}	0.9 ±0^{b}	141.3 ±1.5^{ab}	112.1 ±1.2^{d}	10.8 ±0.1^{c}	0.1 ±0^{a}	13.5 ±0.1^{d}	0.5 ±0^{d}	0.5 ±0^{b}	2.8 ±0^{d}	0.9 ±0^{d}	0.6 ±0^{b}	0.9 ±0^{a}	3.2 ±0^{c}	2.6 ±0^{e}	5.4 ±0.1^{d}	2.9 ±0^{d}	0.1 ±0^{a}	3.9 ±0^{c}	1.5 ±0^{ab}
70	16	6.5 ±0.3^{ab}	0.9 ±0^{b}	119.7 ±0.7^{a}	100 ±0.6^{b}	8 ±0^{b}	0.1 ±0^{a}	9.2 ±0.1^{c}	0.4 ±0^{b}	0.6 ±0^{c}	2.6 ±0^{c}	0.8 ±0^{c}	0.6 ±0^{b}	1 ±0^{b}	2.9 ±0^{b}	2.4 ±0^{cd}	4.7 ±0^{c}	2.2 ±0^{b}	0.1 ±0^{a}	3 ±0^{a}	1.6 ±0^{c}
70	18	6.2 ±0.5^{ab}	0.8 ±0^{a}	116.7 ±1.8^{a}	102.5 ±1.6^{bc}	7.7 ±0.1^{b}	0.1 ±0^{a}	8.1 ±0.1^{b}	0.3 ±0^{a}	0.5 ±0^{b}	2.4 ±0^{b}	0.8 ±0^{b}	0.5 ±0^{a}	0.9 ±0^{a}	2.8 ±0^{b}	2.3 ±0^{ab}	4.1 ±0.1^{b}	2.4 ±0^{c}	0.1 ±0^{a}	3.3 ±0.1^{b}	1.4 ±0^{a}
70	20	4.9 ±0.4^{a}	0.7 ±0^{a}	100.9 ±2^{a}	92.2 ±1.8^{a}	6.7 ±0.1^{a}	0.1 ±0^{a}	6 ±0.1^{a}	0.4 ±0^{c}	0.5 ±0^{a}	2.2 ±0^{a}	0.7 ±0^{a}	0.5 ±0^{a}	0.9 ±0^{a}	2.5 ±0.1^{a}	2.3 ±0^{a}	3.7 ±0.1^{a}	2 ±0^{a}	0.1 ±0^{a}	3 ±0.1^{a}	1.5 ±0^{ab}

Table A2. The concentrations of free amino acids (mg/kg) in pomegranate juice concentrates during storage. All data were adjusted to 11.2°Bx.

Brix	Week	Ala	Arg	Asn	Asp	Gaba	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
30	0	26.4 ±6.4^{a}	124.3 ±4.7^{e}	35.9 ±0^{c}	59.7 ±0.2^{c}	132.6 ±16.4^{a}	73 ±6.9^{c}	53.4 ±1.5^{d}	5.5 ±0.2^{a}	77.5 ±2.2^{e}	5.1 ±4.4^{a}	6.8 ±5.1^{a}	8.6 ±0.2^{f}	10.6 ±0.8^{b}	4 ±0.1^{e}	1.3 ±0.1^{ab}	35.5 ±0.3^{f}	13.7 ±0.3^{d}	23.6 ±0.7^{d}	44.8 ±7.3^{abc}	6.6 ±1.3^{b}
30	2	25.6 ±3.5^{a}	91.5 ±1.6^{cd}	32.2 ±2^{bc}	51.5 ±0.9^{abc}	148 ±32.5^{a}	11.8 ±6.2^{b}	41.8 ±2.1^{bcd}	5 ±0^{a}	65 ±5.1^{de}	6.9 ±0.9^{a}	10 ±0.2^{a}	6.8 ±0.2^{de}	1.4 ±0.6^{a}	3.5 ±0^{cde}	1.3 ±0.1^{ab}	30.3 ±1.4^{ef}	10.4 ±0.1^{abc}	6.4 ±0.8^{c}	32.6 ±14.1^{ab}	6.1 ±0.1^{ab}
30	4	31.1 ±4.1^{a}	84.1 ±2.3^{bcd}	29.9 ±0.5^{b}	55.1 ±2.5^{bc}	155.4 ±18.2^{a}	0.9 ±0.3^{a}	40.4 ±3.4^{abc}	5.4 ±0.2^{a}	59.6 ±1.5^{cd}	8.1 ±0.1^{a}	8.9 ±0.1^{a}	6.4 ±0.2^{cde}	0.6 ±0^{a}	3.4 ±0^{bcd}	1.3 ±0.1^{ab}	29.3 ±0.7^{de}	11 ±0.6^{c}	1.3 ±0.3^{b}	46.3 ±10.5^{abc}	6.2 ±0.1^{ab}
30	6	25.5 ±5.1^{a}	86.5 ±5.4^{bcd}	27.9 ±2.9^{b}	56.1 ±6.8^{bc}	141.3 ±3.2^{a}	0.2 ±0^{a}	37.6 ±4.1^{abc}	5.1 ±0.5^{a}	54.8 ±3.7^{bcd}	8.3 ±0.4^{a}	9 ±0.4^{a}	6.6 ±0.2^{de}	0.6 ±0^{a}	3.4 ±0.3^{bcd}	1.2 ±0^{a}	28 ±3.1^{bcde}	11 ±1.4^{c}	0.3 ±0^{a}	40.1 ±6.7^{abc}	6.6 ±0^{b}
30	8	31.7 ±5^{a}	100.8 ±5.3^{d}	27.2 ±2.8^{b}	56.5 ±3.3^{c}	133.2 ±5.3^{a}	0.1 ±0.1^{a}	46 ±3.4^{cd}	5.4 ±0.7^{a}	50.6 ±0.2^{bc}	4.8 ±4.3^{a}	5.4 ±3.7^{a}	7.3 ±0.3^{e}	0.6 ±0^{a}	3.6 ±0.2^{de}	1.2 ±0^{a}	28.8 ±3.1^{cde}	11.3 ±0.5^{c}	0.2 ±0^{a}	34.6 ±13.2^{abc}	6.1 ±0.3^{ab}
30	10	28.1 ±1.5^{a}	69.8 ±12.8^{ab}	21.5 ±2.8^{a}	42.2 ±1.5^{a}	149.4 ±6.9^{a}	0 ±0^{a}	36 ±3.9^{abc}	4.8 ±0.6^{a}	41.6 ±11.8^{ab}	6.8 ±0.3^{a}	7.3 ±0.8^{a}	5.6 ±0.7^{abc}	0.6 ±0^{a}	2.8 ±0.3^{ab}	1.3 ±0^{ab}	22.9 ±2.7^{abc}	8 ±0.5^{a}	0.1 ±0^{a}	40.8 ±4.5^{abc}	5.1 ±0^{ab}
30	12	33.9 ±3.9^{a}	80.8 ±1^{bc}	21.2 ±1.3^{a}	51.7 ±2^{abc}	168 ±2.8^{a}	0.1 ±0^{a}	41.4 ±4^{abcd}	4.9 ±0.5^{a}	45 ±1.3^{ab}	7.5 ±0.1^{a}	7.6 ±0^{a}	6.2 ±0^{bcd}	0.6 ±0^{a}	3.2 ±0^{abcd}	1.5 ±0.1^{b}	22.7 ±1.3^{ab}	9.9 ±0.5^{abc}	0.1 ±0^{a}	64.7 ±5.8^{c}	5.8 ±0.2^{ab}
30	14	29.5 ±1.6^{a}	74.7 ±0.7^{abc}	20.9 ±0.3^{a}	43.6 ±4^{ab}	147.2 ±2.3^{a}	0.1 ±0^{a}	37.1 ±2^{abc}	5.2 ±0.2^{a}	43.1 ±1.7^{ab}	6.6 ±0.4^{a}	7.3 ±0.4^{a}	5.8 ±0.1^{abcd}	0.6 ±0^{a}	3 ±0.1^{abc}	1.4 ±0^{ab}	23.7 ±0.5^{abcd}	9.5 ±0.4^{abc}	0.1 ±0^{a}	52.3 ±7.9^{bc}	5.3 ±0^{ab}
30	16	28.2 ±1.8^{a}	69.2 ±3.6^{ab}	19.5 ±1.2^{a}	51.3 ±6.6^{abc}	160.7 ±19.1^{a}	0.1 ±0^{a}	43.4 ±5^{cd}	4.9 ±0.3^{a}	34.9 ±1.7^{a}	6.6 ±0^{a}	7.1 ±0.2^{a}	5.5 ±0.3^{abc}	0.6 ±0^{a}	2.8 ±0^{a}	1.4 ±0.1^{ab}	22 ±1.4^{ab}	10.7 ±0.4^{bc}	0.1 ±0^{a}	49 ±5.5^{abc}	5.4 ±0.1^{ab}
30	18	22.3 ±3.7^{a}	62.7 ±1.2^{a}	18.5 ±1.6^{a}	43.5 ±3.8^{ab}	118.2 ±18.9^{a}	0.1 ±0^{a}	28.9 ±0.6^{a}	4.5 ±0.1^{a}	35 ±0.7^{a}	3.3 ±2.8^{a}	7 ±0.2^{a}	5.1 ±0^{a}	0.6 ±0^{a}	2.6 ±0^{a}	1.2 ±0.1^{a}	21.4 ±1.5^{a}	8.3 ±0.6^{ab}	0.1 ±0^{a}	20.6 ±5.8^{a}	4.8 ±0.8^{a}
30	20	24.1 ±2.7^{a}	63.3 ±4.3^{a}	18.5 ±1^{a}	51.6 ±1.7^{abc}	143.9 ±16.3^{a}	0.1 ±0^{a}	30.1 ±6.5^{ab}	4.9 ±0^{a}	34.2 ±0.9^{a}	6 ±0.4^{a}	6.5 ±0.1^{a}	5.2 ±0.2^{ab}	0.6 ±0^{a}	2.7 ±0.3^{a}	1.3 ±0.1^{ab}	22.4 ±0.6^{ab}	9.6 ±1.3^{abc}	0.1 ±0^{a}	40.5 ±10.8^{abc}	5.4 ±0^{ab}
50	0	38.2 ±5.1^{c}	109.8 ±4.5^{h}	37.6 ±1.5^{h}	83.3 ±8.3^{d}	96.2 ±4.3^{e}	119 ±26.4^{a}	182.3 ±26^{e}	3.6 ±0.2^{d}	34.6 ±1^{h}	4 ±2.9^{abc}	4.1 ±0.1^{d}	3.6 ±0.2^{h}	9.9 ±0.5^{f}	3.4 ±0.3^{g}	0.8 ±0^{abc}	32.9 ±1.5^{f}	17.4 ±3^{e}	14.7 ±1.2^{c}	56.6 ±5.3^{b}	3.7 ±0.1^{g}
50	2	36.5 ±2.4^{bc}	100.4 ±0.4^{g}	34.1 ±1.5^{g}	78.5 ±3.6^{d}	78.5 ±0.6^{d}	16.6 ±2.5^{b}	145.4 ±6^{d}	3.6 ±0.1^{d}	21.2 ±0.6^{g}	7.8 ±0.3^{c}	3.9 ±0.1^{cd}	3.4 ±0^{g}	6.8 ±0.7^{e}	3.2 ±0.1^{g}	0.7 ±0^{a}	30.9 ±1.2^{f}	15.5 ±0.7^{de}	2.7 ±0.8^{b}	28.3 ±4.5^{a}	3.8 ±0.2^{g}
50	4	38.8 ±1.5^{c}	86.8 ±0.6^{f}	29.9 ±0.4^{f}	73.4 ±1.5^{cd}	47.6 ±2.8^{cd}	2 ±0.2^{b}	126 ±2.5^{cd}	3.4 ±0^{cd}	16.2 ±0.5^{f}	5.6 ±1.7^{bc}	3.8 ±0^{c}	3 ±0^{f}	3.4 ±0.1^{d}	2.7 ±0^{f}	0.7 ±0^{ab}	28.4 ±0^{e}	13.6 ±0.2^{cd}	0.6 ±0.1^{a}	24.6 ±2.1^{a}	3 ±0.1^{f}
50	6	32.8 ±0.2^{abc}	77.3 ±1.6^{e}	24.9 ±0.1^{e}	65.7 ±3.7^{bc}	45.9 ±5.5^{bcd}	1 ±0^{b}	105.4 ±8.6^{bc}	3.6 ±0.1^{d}	13.5 ±0.2^{de}	3.5 ±1.2^{abc}	3.5 ±0.1^{b}	2.8 ±0.1^{e}	1.8 ±0.1^{c}	2.5 ±0.1^{ef}	0.8 ±0.1^{abc}	25.1 ±0.3^{d}	11.8 ±0.6^{bc}	0.3 ±0^{a}	25.4 ±12.7^{a}	2.6 ±0^{e}
50	8	30.9 ±1.3^{abc}	73.5 ±1.5^{e}	19.1 ±0.5^{d}	53.7 ±0.5^{a}	43.7 ±0.2^{abcd}	0.8 ±0^{b}	78.4 ±2.7^{ab}	2.9 ±0^{ab}	14.1 ±0^{e}	4.7 ±0.1^{abc}	3.4 ±0^{b}	2.7 ±0^{e}	1.4 ±0^{bc}	2.4 ±0^{def}	0.7 ±0^{a}	20.8 ±0.3^{c}	9.6 ±0.2^{ab}	0.3 ±0^{a}	17.8 ±1.4^{a}	2.4 ±0.1^{de}
50	10	37 ±3.6^{bc}	64.4 ±0.1^{d}	19.2 ±0.6^{d}	57.5 ±2.4^{ab}	44.4 ±7.1^{abcd}	0.8 ±0^{b}	83.5 ±0.6^{ab}	3 ±0.1^{ab}	12.4 ±0.1^{cd}	5.2 ±0.1^{abc}	3.4 ±0^{b}	2.5 ±0^{d}	0.8 ±0.1^{ab}	2.3 ±0.1^{cde}	0.8 ±0^{abc}	21.1 ±0.6^{c}	10.4 ±0.8^{ab}	0.3 ±0^{a}	21.6 ±5.3^{a}	0.9 ±0^{bcd}

Table A2 continue.

Brix	Week	Ala	Arg	Asn	Asp	Gaba	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
50	12	29.4 ±0.9^{ab}	56.6 ±2.2^{c}	18.2 ±0.1^{cd}	58.2 ±1.8^{ab}	40.7 ±0.4^{abc}	0.7 ±0^{b}	77 ±2.4^{ab}	3.2 ±0.1^{bcd}	12.1 ±0.1^{bcd}	0.9 ±0.1^{a}	3.2 ±0^{a}	2.2 ±0.1^{c}	0.6 ±0^{ab}	2.2 ±0^{bcd}	0.8 ±0^{abc}	20.5 ±0.2^{bc}	10 ±0.6^{ab}	0.3 ±0^{a}	28.2 ±0.7^{a}	1 ±0^{e}
50	14	26.3 ±1.4^{a}	48.4 ±1.2^{b}	16 ±0.6^{bc}	52 ±2.1^{a}	42.4 ±3.7^{abcd}	0.6 ±0^{b}	64.1 ±3.6^{a}	3.1 ±0.2^{abc}	11.5 ±0.4^{abc}	1.8 ±1^{ab}	3.1 ±0^{a}	2 ±0^{bc}	0.5 ±0^{a}	2 ±0^{abc}	0.9 ±0^{bc}	18.4 ±0.5^{ab}	8.7 ±0.5^{ab}	0.3 ±0^{a}	25.2 ±0.4^{a}	0.9 ±0^{abcd}
50	16	25.2 ±0.6^{a}	43.3 ±1.7^{ab}	14.9 ±0.5^{ab}	49.1 ±0.5^{a}	38.6 ±1.1^{abc}	0.5 ±0^{b}	58.3 ±0.7^{a}	2.8 ±0.1^{ab}	10.9 ±0.1^{abc}	2.2 ±1.4^{ab}	3.1 ±0^{a}	1.9 ±0.1^{ab}	0.4 ±0^{a}	1.9 ±0^{ab}	0.9 ±0.1^{c}	17.7 ±0.5^{a}	8.2 ±0.1^{a}	0.2 ±0^{a}	23.8 ±0.4^{a}	0.8 ±0^{abc}
50	18	27.8 ±3.2^{a}	39 ±0.8^{a}	14.1 ±0.1^{ab}	49.2 ±1.4^{a}	35.4 ±1.1^{ab}	0.5 ±0^{b}	54.9 ±1.5^{a}	2.7 ±0^{a}	10.7 ±0^{a}	2.9 ±0.3^{ab}	3 ±0^{a}	1.8 ±0^{a}	0.4 ±0^{a}	1.8 ±0^{a}	0.8 ±0^{abc}	16.8 ±0.3^{a}	7.8 ±0.3^{a}	0.2 ±0^{a}	22 ±0.1^{a}	0.8 ±0^{ab}
50	20	26.7 ±0.5^{a}	39.7 ±0.5^{a}	13.3 ±0.2^{a}	51.4 ±0^{a}	34.7 ±0.2^{a}	0.5 ±0^{b}	54.8 ±0.2^{a}	2.8 ±0.2^{ab}	10.8 ±0.5^{ab}	2.1 ±1.3^{ab}	3 ±0^{a}	1.8 ±0^{a}	0.4 ±0^{a}	1.8 ±0^{a}	0.8 ±0^{abc}	16.5 ±0.5^{a}	8.4 ±0^{ab}	0.2 ±0^{a}	21.7 ±0.2^{a}	0.8 ±0^{a}
65	0	35.7 ±6.4^{b}	131.8 ±21.1^{e}	59.4 ±1.2^{e}	120.8 ±3.4^{g}	224.9 ±22.5^{g}	151.7 ±21.4^{b}	225.6 ±35.8^{e}	6.5 ±0^{e}	34.9 ±11.3^{b}	9.6 ±0.2^{f}	6 ±0.2^{d}	4.9 ±0.5^{e}	14.9 ±0.6^{g}	4.4 ±0.4^{e}	2 ±0.1^{d}	50 ±0.4^{g}	17.3 ±1.6^{e}	18.3 ±1.6^{c}	105.4 ±10.3^{d}	1.8 ±0^{g}
65	2	22 ±5.3^{a}	69.5 ±5.1^{d}	35.9 ±4.9^{d}	80.9 ±6.6^{f}	160.5 ±11.7^{f}	16.5 ±8^{a}	138.5 ±7.8^{d}	4.6 ±0.5^{d}	14 ±0.1^{a}	6.7 ±0.9^{e}	5.2 ±0^{c}	3.3 ±0.1^{d}	9.2 ±0.2^{f}	3 ±0.1^{d}	1.9 ±0^{cd}	34.6 ±4.2^{f}	11.1 ±0.3^{d}	2.5 ±1^{b}	59.2 ±2.1^{c}	1.7 ±0^{f}
65	4	17.7 ±0.5^{a}	40.4 ±4.2^{c}	20 ±0.3^{c}	52.3 ±1.1^{cd}	91.4 ±7.5^{de}	1.5 ±0.1^{a}	74.6 ±14.8^{c}	3 ±0^{abc}	8.5 ±1.5^{a}	4.8 ±0.7^{d}	4.4 ±0.3^{abc}	2.3 ±0.2^{c}	5.3 ±0.6^{e}	2.2 ±0.2^{bc}	1.5 ±0^{a}	20.6 ±0.1^{d}	7.2 ±0.8^{c}	0.6 ±0^{a}	24.2 ±5.3^{ab}	1.7 ±0^{ef}
65	6	22.3 ±2.4^{a}	38.2 ±3.9^{bc}	22.7 ±1.2^{c}	63.4 ±2.4^{e}	104.8 ±7.8^{e}	0.9 ±0^{a}	65.5 ±5^{bc}	3.7 ±0.5^{cd}	8.1 ±0.9^{a}	3.9 ±0.4^{cd}	5.2 ±0.2^{bc}	2.3 ±0.1^{c}	4.3 ±0.1^{d}	2.5 ±0.1^{c}	1.8 ±0.1^{bcd}	26.3 ±0.7^{e}	7.7 ±0.3^{c}	0.6 ±0^{a}	31.2 ±1.8^{b}	1.6 ±0^{cd}
65	8	20.4 ±0.1^{a}	29.4 ±0.1^{abc}	18.3 ±0.5^{c}	54.1 ±1.2^{de}	83.4 ±7.9^{cde}	0.7 ±0^{a}	49.1 ±1.3^{abc}	3.4 ±0.1^{bc}	7.3 ±0^{a}	2.8 ±0.2^{bc}	4.7 ±0.2^{abc}	1.9 ±0^{bc}	3.3 ±0.2^{c}	2.1 ±0^{bc}	1.7 ±0.1^{abc}	21 ±0.1^{d}	6.8 ±0.2^{bc}	0.6 ±0^{a}	25.7 ±1.2^{ab}	1.7 ±0^{f}
65	10	17.8 ±0.4^{a}	19.2 ±0.3^{abc}	13.1 ±0.4^{b}	43.4 ±0^{bc}	75.1 ±3.4^{bcde}	0.5 ±0^{a}	33 ±1.9^{ab}	2.5 ±0.1^{ab}	5.9 ±0.1^{a}	2.3 ±0^{ab}	4.6 ±0.1^{abc}	1.5 ±0^{ab}	2.6 ±0^{bc}	1.8 ±0^{ab}	1.6 ±0^{abc}	15.7 ±0.8^{c}	5.1 ±0.1^{ab}	0.5 ±0^{a}	20.6 ±0.2^{ab}	1.5 ±0^{b}
65	12	17.8 ±1.3^{a}	16.2 ±1.4^{ab}	11.6 ±1.3^{ab}	38.9 ±4^{ab}	62.1 ±0.8^{abcd}	0.4 ±0.1^{a}	25.6 ±2.8^{ab}	2.8 ±0.6^{abc}	5.8 ±0.4^{a}	2.3 ±0^{ab}	4.4 ±0.3^{ab}	1.3 ±0.1^{a}	2.2 ±0.1^{ab}	1.7 ±0.1^{ab}	1.6 ±0.1^{ab}	13.3 ±1.2^{bc}	4.6 ±0.6^{a}	0.5 ±0^{a}	18.1 ±1.7^{a}	1.7 ±0de
65	14	17.1 ±1.3^{a}	14.3 ±1.3^{a}	9.9 ±0.5^{ab}	35.8 ±3.2^{ab}	57.2 ±0.8^{abc}	0.4 ±0^{a}	21.7 ±2^{a}	2.4 ±0^{a}	5.7 ±0.3^{a}	2.2 ±0.2^{ab}	4.7 ±0.1^{abc}	1.2 ±0.1^{a}	2 ±0.1^{ab}	1.6 ±0.1^{ab}	1.6 ±0^{abc}	13.2 ±0.3^{bc}	4.4 ±0.4^{a}	0.5 ±0^{a}	16.8 ±0.4^{a}	1.5 ±0^{ab}
65	16	17.5 ±0.7^{a}	12.8 ±1^{a}	9.4 ±0.7^{ab}	35.6 ±2.1^{ab}	52.6 ±0.5^{ab}	0.3 ±0^{a}	20.1 ±1.4^{a}	2 ±0.2^{a}	5.8 ±0.4^{a}	2.1 ±0.1^{ab}	4.6 ±0.1^{abc}	1.1 ±0.1^{a}	1.8 ±0.1^{ab}	1.6 ±0.1^{ab}	1.6 ±0^{abc}	11.2 ±0.5^{abc}	4.2 ±0.2^{a}	0.5 ±0^{a}	15.8 ±0.8^{a}	1.6 ±0^{c}
65	18	18.1 ±1.3^{a}	13.2 ±0.9^{a}	9.3 ±0.8^{ab}	36.2 ±3.2^{ab}	49.8 ±4.5^{ab}	0.3 ±0^{a}	19 ±1.7^{a}	2.1 ±0.4^{a}	6.7 ±0.6^{a}	2 ±0.1^{ab}	4.8 ±0.4^{abc}	1.1 ±0.1^{a}	1.9 ±0.2^{ab}	1.7 ±0.1^{ab}	1.7 ±0.1^{abc}	11 ±0.9^{ab}	4.4 ±0.4^{a}	0.5 ±0^{a}	16.5 ±1.3^{a}	1.4 ±0^{a}
65	20	13.6 ±0.4^{a}	10 ±0^{a}	7 ±0^{a}	29.3 ±0.8^{a}	44.4 ±4.2^{a}	0.2 ±0^{a}	14.7 ±0.6^{a}	1.9 ±0.1^{a}	5.2 ±0.3^{a}	1.6 ±0.1^{a}	4.3 ±0.3^{a}	0.9 ±0^{a}	1.5 ±0.1^{a}	1.4 ±0^{a}	1.5 ±0.1^{a}	8 ±0.3^{a}	3.6 ±0^{a}	0.5 ±0^{a}	13.6 ±0.6^{a}	1.5 ±0^{ab}

Mean values in the same column with different letters are significantly different at the 5% confidence level.

Table A3. The concentrations of free amino acids (mg/kg) in dried dates, raisins and dried blueberries during storage.

Month	Ala	Arg	Asn	Asp	Gaba	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
DATE																				
0	236.5 ±27.6^{d}	82.4 ±12.2^{b}	379 ±21.4^{d}	200.9 ±2.5^{c}	1782.9 ±51.4^{d}	10.6 ±0.5^{b}	56.3 ±7.3^{c}	10.6 ±1^{b}	17.5 ±1.6^{b}	nd	21.3 ±0.9^{c}	10.8 ±0.7^{b}	12.3 ±0.1^{b}	26.1 ±3.1^{b}	775.7 ±60.4^{d}	25.6 ±2.8^{c}	18 ±2.3^{b}	15.1 ±0.5^{c}	91.3 ±16.9^{b}	26.2 ±0.3^{b}
1	151.9 ±7.6^{c}	44.4 ±3.6^{a}	164.2 ±4.9^{c}	155.3 ±2.1^{b}	1026.4 ±22.3^{c}	6.3 ±0.8^{a}	30 ±1.7^{b}	7.5 ±4.2^{b}	13.9 ±0.4^{a}	nd	14.4 ±0.6^{b}	6.7 ±1^{a}	9.1 ±1^{a}	15.7 ±0.1^{a}	675.2 ±0.5^{c}	13.4 ±0.3^{b}	12.5 ±0.1^{a}	9.8 ±0.8^{b}	44.4 ±2.3^{a}	19.9 ±1.1^{a}
2	105.5 ±15.9^{b}	29.6 ±1.4^{a}	69.7 ±0.3^{b}	127.1 ±19.7^{b}	324.4 ±63.5^{b}	5.3 ±0.4^{a}	24.1 ±0.3^{ab}	nd*	11.9 ±0.6^{a}	nd	12.1 ±1.3^{ab}	5.4 ±0.3^{a}	7.9 ±0.3^{a}	15.3 ±2.5^{a}	490.9 ±2.3^{a}	8.5 ±0.8^{ab}	11 ±2.6^{a}	8.4 ±0.4^{ab}	28.7 ±5.1^{a}	17.5 ±1.4^{a}
3	89.5 ±5.7^{ab}	32.1 ±0.3^{a}	33.2 ±2.3^{a}	94.3 ±3.1^{a}	186.1 ±5.4^{a}	6.4 ±0^{a}	22.1 ±0.2^{ab}	nd	10.9 ±0.5^{a}	nd	13.5 ±0.3^{ab}	6.4 ±0.4^{a}	9.6 ±0^{a}	14 ±0.4^{a}	632.9 ±12.7^{c}	8.6 ±1.5^{ab}	11.3 ±0.6^{a}	9.5 ±0^{ab}	27 ±1.9^{a}	20.6 ±0.6^{a}
4	62.2 ±1.6^{ab}	29 ±1.6^{a}	28.2 ±7.7^{a}	75.7 ±3.1^{a}	203.3 ±29.3^{a}	5.9 ±0.4^{a}	20.1 ±1.6^{ab}	nd	11 ±1.3^{a}	nd	13.2 ±1.5^{ab}	6 ±0.7^{a}	8.5 ±0.6^{a}	14.5 ±0.3^{a}	612.7 ±26^{bc}	7.6 ±0.2^{ab}	10.8 ±0.5^{a}	8.4 ±0.5^{ab}	23.8 ±0.5^{a}	19.8 ±1.9^{a}
5	60.1 ±2.2^{a}	27.8 ±2.2^{a}	36.9 ±6.9^{ab}	78.4 ±5.2^{a}	102.8 ±7.7^{a}	5.2 ±0.3^{a}	17.1 ±0.6^{a}	nd	12.5 ±0.4^{a}	nd	10.7 ±0.7^{a}	5.4 ±0.4^{a}	8 ±0.7^{a}	12.2 ±0.2^{a}	531.5 ±8.2^{ab}	8.1 ±3.5^{ab}	9 ±0.9^{a}	8 ±0.6^{a}	20.8 ±2^{a}	17 ±1.4^{a}
6	48.7 ±2.6^{a}	27.8 ±0.2^{a}	38.1 ±6.5^{ab}	85.5 ±9.5^{a}	118.8 ±16.4^{a}	6 ±0.2^{a}	19.5 ±1.5^{ab}	nd	11.2 ±1^{a}	nd	12 ±0.5^{ab}	6 ±0.3^{a}	8.2 ±0^{a}	14.4 ±0.6^{a}	484.6 ±37.2^{a}	4.4 ±0.4^{a}	9.7 ±0.5^{a}	8.1 ±0^{a}	24.4 ±0.4^{a}	19.2 ±0.7^{a}
RAISIN																				
0	357 ±21.8^{c}	4590 ±38.6^{b}	261.2 ±2^{c}	47.3 ±9.3^{a}	446.2 ±0.6^{c}	47.9 ±1.9^{c}	43.7 ±6.1^{c}	24.3 ±1.3^{b}	114.8 ±0.8^{b}	162.2 ±9.5^{c}	397.5 ±8.4^{c}	47.5 ±1.8^{c}	71.3 ±2.7^{c}	284.4 ±11.5^{c}	392.7 ±39.3^{a}	205.2 ±18.2^{c}	136.7 ±6.1^{b}	176.7 ±10^{c}	188.1 ±10.7^{c}	122.4 ±2.4^{c}
1	349.8 ±28^{bc}	1437.8 ±559.6^{a}	160.8 ±13.9^{b}	41.4 ±4.1^{a}	252.6 ±45.1^{b}	17.9 ±3.3^{b}	37.7 ±3^{b}	10.1 ±1.8^{a}	32.1 ±14.1^{a}	79.2 ±21.5^{b}	153.2 ±24.9^{b}	17.8 ±3.2^{b}	30.6 ±1.8^{b}	133 ±20.3^{b}	589. 2±62.2^{a}	102.1 ±0.1^{bc}	67.5 ±34.7^{a}	28.9 ±2.4^{b}	131.3 ±20.6^{b}	59.8 ±2^{b}
2	271.8 ±10.3^{ab}	695.1 ±3.7^{a}	69 ±2.9^{a}	38 ±5^{a}	110.3 ±3.4^{a}	9.8 ±0.1^{a}	22.4 ±0.4^{ab}	20.1 ±0.5^{a}	26.5 ±0.2^{a}	30.7 ±4.9^{a}	76.3 ±7.4^{a}	9.7 ±0.1^{a}	20.8 ±3.1^{a}	89.6 ±18^{a}	612.2 ±81.6^{a}	46.2 ±19.7^{ab}	39.5 ±7.2^{a}	20.2 ±2.2^{a}	72.6 ±8.3^{a}	37.3 ±3.9^{a}
3	275.6 ±23.1^{ab}	559.8 ±7.5^{a}	63 ±9.7^{a}	34.5 ±11^{a}	96.4 ±31.5^{a}	9 ±1^{a}	20.7 ±6.1^{ab}	16.4 ±0.8^{a}	23.1 ±1.5^{a}	43.5 ±19.9^{ab}	74.1 ±15.8^{a}	8.9 ±1^{a}	19.4 ±2.5^{a}	95.6 ±8.1^{a}	523 ±13.9^{a}	39.8 ±13.6^{ab}	36.5 ±6.3^{a}	18.6 ±0.2^{a}	80.2 ±14.1^{a}	33.5 ±4.7^{a}
4	260 ±12.9^{ab}	450.5 ±38.1^{a}	60.6 ±13^{a}	30.7 ±3.4^{a}	75.8 ±13.2^{a}	9.1 ±0.7^{a}	15.5 ±0.6^{ab}	21.3 ±5.5^{ab}	21.1 ±0.2^{a}	36.8 ±7.1^{ab}	67.4 ±14.9^{a}	9.2 ±0.6^{a}	19.3 ±0.6^{a}	78.9 ±1.9^{a}	465.1 ±87.4^{a}	29.2 ±7.5^{ab}	31 ±7^{a}	18.9 ±0.3^{a}	72.4 ±1.3^{a}	37.2 ±4.2^{a}
5	254.9 ±3.9^{ab}	395.5 ±4.4^{a}	59.4 ±2^{a}	44.3 ±1.9^{a}	77.1 ±6.9^{a}	11.1 ±0.1^{a}	16.1 ±0.9^{ab}	9.9 ±0.4^{a}	22.2 ±0.9^{a}	38.8 ±3.8^{ab}	70.4 ±5.5^{a}	10.8 ±0.3^{a}	23.3 ±0.3^{a}	82.1 ±1.7^{a}	532.3 ±0.9^{a}	27.1 ±5.9^{ab}	28.6 ±3.4^{a}	21.9 ±0.6^{a}	83.4 ±7.8^{a}	44.3 ±2.5^{a}
6	188.3 ±28.6^{a}	253.7 ±10.4^{a}	24.1 ±3.8^{a}	21.5 ±5.1^{a}	40.3 ±3.3^{a}	9.4 ±0.3^{a}	11.1 ±1.6^{a}	14.9 ±4^{a}	19.9 ±1.2^{a}	16.9 ±1.1^{a}	54.7 ±2.7^{a}	9.7 ±0.2^{a}	17.8 ±0^{a}	73 ±3.2^{a}	469.1 ±39.4^{a}	21.1 ±2.1^{a}	25.2 ±2.3^{a}	18.4 ±0.9^{a}	74.1 ±1^{a}	34.6 ±0.1^{a}

Table A3 continue.

Month	Ala	Arg	Asn	Asp	Gaba	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
BLUBERRY																				
0	121.4 ±15.4^{bc}	1203.3 ±158.3^{b}	150.4 ±44.9^{c}	183.2 ±64^{a}	335.1 ±75.3^{b}	26.3 ±1.8^{b}	285.5 ±76.1^{b}	15.8 ±0.6^{a}	36.6 ±0.5^{b}	21.6 ±2.1^{c}	100.7 ±14^{a}	25.9 ±2.4^{b}	27.3 ±5.2^{a}	82.4 ±2^{a}	22 ±0.2^{ab}	122.7 ±29.9^{a}	43.6 ±3.5^{b}	77.2 ±2.8^{b}	100.8 ±1.1^{a}	117.1 ±19.8^{b}
1	109.8 ±17.1^{abc}	546.3 ±114.8^{a}	111.1 ±18.3^{bc}	147 ±22.8^{a}	174.8 ±19.3^{a}	16.5 ±4^{ab}	125.7 ±22.8^{a}	18.2 ±0.2^{a}	14.6 ±1.7^{a}	17.1 ±1.2^{bc}	53.4 ±9.4^{ab}	16.9 ±3.9^{ab}	20.9 ±4.9^{a}	56.7 ±8.6^{a}	22.5 ±1^{ab}	91 ±19.2^{a}	27.1 ±5.8^{ab}	16.7 ±0.9^{a}	67.9 ±16.3^{a}	69.9 ±16.4^{ab}
2	143.1 ±1.8^{c}	429.1 ±38.8^{a}	64.2 ±10^{ab}	157.8 ±12.6^{a}	136.5 ±21.3^{a}	19 ±1.2^{ab}	103 ±7.6^{a}	16.3 ±2.7^{a}	14.3 ±4.4^{a}	13.1 ±1.5^{ab}	57.4 ±12.6^{ab}	19.3 ±1.2^{ab}	17.4 ±6.6^{a}	52 ±19.5^{a}	19.7 ±0.4^{ab}	96.4 ±18.9^{a}	23.2 ±10.3^{ab}	12.2 ±1^{a}	85.2 ±6.7^{a}	64.3 ±12.9^{ab}
3	93.8 ±38.1^{abc}	515.5 ±57.6^{a}	54.6 ±11.7^{ab}	100.9 ±56.2^{a}	219.1 ±64.9^{ab}	14.5 ±5.9^{ab}	60.9 ±39.8^{a}	19.4 ±3.9^{a}	16.5 ±2.5^{a}	12.6 ±1.3^{ab}	50.4 ±29.5^{ab}	14.7 ±5.9^{ab}	19.3 ±6.6^{a}	53.7 ±14.3^{a}	26.3 ±4^{b}	67.5 ±38^{a}	23.8 ±6.6^{ab}	13.6 ±1.8^{a}	68.9 ±32.4^{a}	58.9 ±20.2^{a}
4	51.6 ±9.8^{ab}	300.3 ±31.5^{a}	31.6 ±2^{a}	62.4 ±33.8^{a}	98.8 ±1.5^{a}	9.6 ±3.6^{a}	31.1 ±17.5^{a}	14.1 ±2.7^{a}	12.5 ±1.3^{a}	10.1 ±0^{a}	30.3 ±16.9^{a}	9.5 ±3.9^{a}	13.9 ±4.7^{a}	40.5 ±11.1^{a}	16.9 ±1.4^{a}	41.3 ±24.3^{a}	18.1 ±5.4^{a}	11.8 ±0.5^{a}	49.6 ±22.2^{a}	39.1 ±12.8^{a}
5	47.6 ±20.8^{a}	336.2 ±63.4^{a}	35.5 ±1.3^{a}	74.2 ±42^{a}	93.4 ±14.7^{a}	10.5 ±3.5^{a}	28.6 ±17^{a}	17.1 ±4.1^{a}	15.6 ±2.8^{a}	12.7 ±3.3^{ab}	34 ±19.8^{a}	10.8 ±3.4^{a}	15.2 ±5.5^{a}	45 ±14.2^{a}	18.9 ±2.5^{a}	40.5 ±26.7^{a}	19.3 ±7.4^{a}	12.7 ±0.1^{a}	56 ±25.6^{a}	45.1 ±16.7^{a}
6	48.3 ±17.6^{a}	293.6 ±49.6^{a}	29.4 ±3.8^{a}	70.5 ±37.8^{a}	87.5 ±13^{a}	9.2 ±1.7^{a}	21.9 ±8.7^{a}	15.6 ±0.7^{a}	17.4 ±1.2^{a}	11.4 ±1.3^{ab}	34.3 ±17^{a}	9.3 ±1.7^{a}	14.3 ±3.4^{a}	44.1 ±10.4^{a}	18.6 ±0.7^{a}	34.7 ±17.8^{a}	17.8 ±3.8^{a}	13.1 ±1.5^{a}	57.2 ±17.7^{a}	43.1 ±10.8^{a}

Mean values in the same column with different letters are significantly different at the 5 % confidence level.
*not detectable.

Table A4. High-resolution mass spectrometry (HRMS) performances of the adducts of glucosone, threosone, diacetyl, methylglyoxal and glyoxal with free amino acids possibly formed in 70°Bx of apple juice concentrates at the end of the storage.

Amino Acid	Formula	Exact Mass [M+H$^+$]	Experimental Mass [M+H$^+$]	Δ (ppm)	Amino Acid	Formula	Exact Mass [M+H$^+$]	Experimental Mass [M+H$^+$]	Δ (ppm)
Glucosone adducts					**Threosone adducts**				
Ala	$C_9H_{17}O_8N$	268.10269	268.10263	-0.21315	**Ala**	$C_7H_{13}O_6N$	208.08156	208.0816	-0.02592
Arg	$C_{12}H_{24}O_8N_4$	353.16669	353.1676	2.58344	**Arg**	$C_{10}H_{20}O_6N_4$	293.14556	293.1433	-7.7805
Asn	$C_{10}H_{18}O_9N_2$	311.10851	311.10855	0.14289	**Asn**	$C_8H_{14}O_7N_2$	251.08738	-	
Asp	$C_{10}H_{17}O_{10}N$	312.09252	312.09232	-0.66155	**Asp**	$C_8H_{13}O_8N$	252.07139	252.0715	0.37532
GABA	$C_{10}H_{19}O_8N$	282.11834	282.11838	0.12295	**GABA**	$C_8H_{15}O_6N$	222.09721	222.0972	0.00029
Gln	$C_{11}H_{20}O_9N_2$	325.12416	325.12411	-0.12807	**Gln**	$C_9H_{16}O_7N_2$	265.10303	265.1032	0.57571
Glu	$C_{11}H_{19}O_{10}N$	326.10817	-		**Glu**	$C_9H_{15}O_8N$	266.08704	266.0807	0.08933
Gly	$C_8H_{15}O_8N$	254.08704	254.08829	4.8978	**Gly**	$C_6H_{11}O_6N$	194.06591	194.0658	-0.37042
His	$C_{12}H_{19}O_8N_3$	334.12449	-		**His**	$C_{10}H_{15}O_6N_3$	274.10336	274.1046	4.45837
Leu/Ile	$C_{12}H_{23}O_8N$	310.14964	310.14951	-0.44335	**Leu/Ile**	$C_{10}H_{19}O_6N$	250.12851	250.1285	-0.01711
Lys	$C_{12}H_{24}O_8N_2$	325.16054	325.16055	0.0333	**Lys**	$C_{10}H_{20}O_6N_2$	265.13941	-	
Met	$C_{11}H_{21}O_8NS$	328.10606	328.10663	1.72096	**Met**	$C_9H_{17}O_6NS$	268.08493	-	
Phe	$C_{15}H_{21}O_8N$	344.13399	344.13382	-0.5041	**Phe**	$C_{13}H_{17}O_6N$	284.11286	284.1127	-0.46392
Pro	$C_{11}H_{19}O_8N$	294.11834	294.11835	0.01417	**Pro**	$C_9H_{15}O_6N$	234.09721	234.0972	0.00028
Ser	$C_9H_{17}O_9N$	284.09761	-		**Ser**	$C_7H_{13}O_7N$	224.07648	224.0763	-0.61809
Thr	$C_{10}H_{19}O_9N$	298.11326	298.11404	2.63855	**Thr**	$C_8H_{15}O_7N$	238.09213	238.0921	0.01801
Trp	$C_{17}H_{22}O_8N_2$	383.14489	383.14474	-0.38423	**Trp**	$C_{15}H_{18}O_6N_2$	323.12376	323.1238	0.14559
Tyr	$C_{15}H_{21}O_9N$	360.12891	360.12881	-0.25814	**Tyr**	$C_{13}H_{17}O_7N$	300.10778	300.1073	-1.59455
Val	$C_{11}H_{21}O_8N$	296.13399	296.13379	-0.68887	**Val**	$C_9H_{17}O_6N$	236.11286	236.1129	0.02339
Diacetyl adducts					**Methylglyoxal adducts**				
Ala	$C_7H_{13}O_4N$	176.09173	176.09174	0.00806	**Ala**	$C_6H_{11}O_4N$	162.07608	162.0761	-0.02491
Arg	$C_{10}H_{20}O_4N_4$	261.15573	-		**Arg**	$C_9H_{18}O_4N_4$	247.14008	-	
Asn	$C_8H_{14}O_5N_2$	219.09755	219.09813	2.6528	**Asn**	$C_7H_{12}O_5N_2$	205.08190	205.0833	6.60202
Asp	$C_8H_{13}O_6N$	220.08156	220.08156	-0.16317	**Asp**	$C_7H_{11}O_6N$	206.06591	206.0668	4.39021
GABA	$C_8H_{15}O_4N$	190.10738	190.10738	-0.04409	**GABA**	$C_7H_{13}O_4N$	176.09173	176.0917	0.00806
Gln	$C_9H_{16}O_5N_2$	233.11320	233.11363	1.86215	**Gln**	$C_8H_{14}O_5N_2$	219.09755	219.0981	2.6528
Glu	$C_9H_{15}O_6N$	234.09721	234.09723	0.06546	**Glu**	$C_8H_{13}O_6N$	220.08156	220.0815	-0.16317
Gly	$C_6H_{11}O_4N$	162.07608	162.07608	-0.02491	**Gly**	$C_5H_9O_4N$	148.06043	148.0604	-0.47636
His	$C_{10}H_{15}O_4N_3$	242.11353	242.11153	-8.2854	**His**	$C_9H_{13}O_4N_3$	228.09788	228.0963	-6.87843
Leu/Ile	$C_{10}H_{19}O_4N$	218.13868	218.13869	0.01162	**Leu/Ile**	$C_9H_{17}O_4N$	204.12303	204.1231	0.06043
Lys	$C_{10}H_{20}O_4N_2$	233.14958	233.14925	-1.4472	**Lys**	$C_9H_{18}O_4N_2$	219.13393	219.1336	-1.49504
Met	$C_9H_{17}O_4NS$	236.09511	-		**Met**	$C_8H_{15}O_4NS$	222.07946	-	
Phe	$C_{13}H_{17}O_4N$	252.12303	252.12282	-0.85889	**Phe**	$C_{12}H_{15}O_4N$	238.10738	238.1074	0.22113
Pro	$C_9H_{15}O_4N$	202.10738	202.10738	-0.04147	**Pro**	$C_8H_{13}O_4N$	188.09173	188.0917	0.00755
Ser	$C_7H_{13}O_5N$	192.08665	192.08665	0.02938	**Ser**	$C_6H_{11}O_5N$	178.07100	178.071	-0.08465
Thr	$C_8H_{15}O_5N$	206.10230	206.10229	-0.02018	**Thr**	$C_7H_{13}O_5N$	192.08665	192.0867	0.02938
Trp	$C_{15}H_{18}O_4N_2$	291.13393	291.13513	4.11584	**Trp**	$C_{14}H_{16}O_4N_2$	277.11828	277.1183	0.00946
Tyr	$C_{13}H_{17}O_5N$	268.11795	268.11771	-0.90573	**Tyr**	$C_{12}H_{15}O_5N$	254.10230	254.1023	0.04369
Val	$C_9H_{17}O_4N$	204.12303	204.12303	-0.01432	**Val**	$C_8H_{15}O_4N$	190.10738	190.1074	-0.04409

Table A4 continue.

Glyoxal adducts				
Amino Acid	**Formula**	**Exact Mass [M+H⁺]**	**Experimental Mass [M+H⁺]**	**Δ (ppm)**
Ala	$C_5H_9O_4N$	148.06043	148.06036	-0.47636
Arg	$C_8H_{16}O_4N_4$	233.12443	233.12331	-4.82678
Asn	$C_6H_{10}O_5N_2$	191.06625	191.06636	0.58903
Asp	$C_6H_9O_6N$	192.05026	-	
GABA	$C_6H_{11}O_4N$	162.07608	162.07608	-0.02491
Gln	$C_7H_{12}O_5N_2$	205.08190	205.08325	6.60202
Glu	$C_7H_{11}O_6N$	206.06591	206.06627	1.7245
Gly	$C_4H_7O_4N$	134.04478	134.04478	0.00228
His	$C_8H_{11}O_4N_3$	214.08223	-	
Leu/Ile	$C_8H_{15}O_4N$	190.10738	190.10738	-0.04409
Lys	$C_8H_{16}O_4N_2$	205.11828	-	
Met	$C_7H_{13}O_4NS$	208.06381	208.06256	-5.97938
Phe	$C_{11}H_{13}O_4N$	224.09173	224.09173	-0.06176
Pro	$C_7H_{11}O_4N$	174.07608	174.07608	-0.0232
Ser	$C_5H_9O_5N$	164.05535	164.05611	4.61834
Thr	$C_6H_{11}O_5N$	178.07100	178.071	0.00104
Trp	$C_{13}H_{14}O_4N_2$	263.10263	263.10269	0.22121
Tyr	$C_{11}H_{13}O_5N$	240.08665	240.08656	-0.35783
Val	$C_7H_{13}O_4N$	176.09173	176.09172	-0.07859

Table A5. High-resolution mass spectrometry (HRMS) performances of the adducts of glucosone, 3,4-dideoxyglucosone-3-ene, 3-deoxythreosone, diacetyl, methylglyoxal and glyoxal with free amino acids possibly formed in raisins at the end of the storage

Amino Acid	Formula	Exact Mass [M+H$^+$]	Experimental Mass [M+H$^+$]	Δ (ppm)	Amino Acid	Formula	Exact Mass [M+H$^+$]	Experimental Mass [M+H$^+$]	Δ (ppm)
Glucosone adducts					**3,4-Dideoxyglucosone-3-ene adducts**				
Ala	$C_9H_{17}O_8N$	268.10269	268.10269	-0.00481	**Ala**	$C_9H_{15}O_6N$	234.09721	234.09723	0.06546
Arg	$C_{12}H_{24}O_8N_4$	353.16669	353.1676	2.58344	**Arg**	$C_{12}H_{22}O_6N_4$	319.16121	319.16125	0.13784
Asn	$C_{10}H_{18}O_9N_2$	311.10851	-		**Asn**	$C_{10}H_{16}O_7N_2$	277.10303	277.10324	0.77104
Asp	$C_{10}H_{17}O_{10}N$	312.09252	312.09253	0.02293	**Asp**	$C_{10}H_{15}O_8N$	278.08704	278.08755	1.84133
GABA	$C_{10}H_{19}O_8N$	282.11834	282.11835	0.01477	**GABA**	$C_{10}H_{17}O_6N$	248.11286	248.11287	0.002226
Gln	$C_{11}H_{20}O_9N_2$	325.12416	325.12451	1.09216	**Gln**	$C_{11}H_{18}O_7N_2$	291.11868	291.11792	-2.60186
Glu	$C_{11}H_{19}O_{10}N$	326.10817	-		**Glu**	$C_{11}H_{17}O_8N$	292.10269	292.10297	0.93586
Gly	$C_8H_{15}O_8N$	254.08704	254.088	3.7568	**Gly**	$C_8H_{13}O_6N$	220.08156	220.08157	0.04483
His	$C_{12}H_{19}O_8N_3$	334.12449	334.12515	1.98006	**His**	$C_{12}H_{17}O_6N_3$	300.11901	300.1192	0.63282
Leu/Ile	$C_{12}H_{23}O_8N$	310.14964	310.1496	-0.14816	**Leu/Ile**	$C_{12}H_{21}O_6N$	276.14416	276.14417	0.00427
Lys	$C_{12}H_{24}O_8N_2$	325.16054	325.16055	0.0333	**Lys**	$C_{12}H_{22}O_6N_2$	291.15506	291.15533	0.93278
Met	$C_{11}H_{21}O_8NS$	328.10606	328.1062	0.4188	**Met**	$C_{11}H_{19}O_6NS$	294.10058	294.10062	0.10865
Phe	$C_{15}H_{21}O_8N$	344.13399	344.13385	-0.41543	**Phe**	$C_{15}H_{19}O_6N$	310.12851	310.12854	0.08461
Pro	$C_{11}H_{19}O_8N$	294.11834	294.11838	0.11793	**Pro**	$C_{11}H_{17}O_6N$	260.11286	260.11288	0.07989
Ser	$C_9H_{17}O_9N$	284.09761	284.09763	0.06404	**Ser**	$C_9H_{15}O_7N$	250.09213	250.09213	0.01715
Thr	$C_{10}H_{19}O_9N$	298.11326	298.11334	0.28407	**Thr**	$C_{10}H_{17}O_7N$	264.10778	264.10776	-0.07865
Trp	$C_{17}H_{22}O_8N_2$	383.14489	383.1449	0.01402	**Trp**	$C_{17}H_{20}O_6N_2$	349.13941	349.13947	0.15038
Tyr	$C_{15}H_{21}O_9N$	360.12891	360.12891	-0.00392	**Tyr**	$C_{15}H_{19}O_7N$	326.12343	326.12344	0.04662
Val	$C_{11}H_{21}O_8N$	296.13399	296.134	0.0325	**Val**	$C_{11}H_{19}O_6N$	262.12851	262.12854	0.1001
3-Deoxythreosone adducts					**Diacetyl adducts**				
Ala	$C_7H_{13}O_5N$	192.08665	192.08665	0.02938	**Ala**	$C_7H_{13}O_4N$	176.09173	176.09174	0.00806
Arg	$C_{10}H_{20}O_5N_4$	277.15065	277.15009	-2.02338	**Arg**	$C_{10}H_{20}O_4N_4$	261.15573	-	
Asn	$C_8H_{14}O_6N_2$	235.09246	235.09337	3.85328	**Asn**	$C_8H_{14}O_5N_2$	219.09755	219.09764	0.56349
Asp	$C_8H_{13}O_7N$	236.07648	236.0764	-0.32813	**Asp**	$C_8H_{13}O_6N$	220.08156	220.08157	0.04483
GABA	$C_8H_{15}O_5N$	206.10230	206.10231	0.05386	**GABA**	$C_8H_{15}O_4N$	190.10738	190.10738	-0.04409
Gln	$C_9H_{16}O_6N_2$	249.10811	249.10844	1.33076	**Gln**	$C_9H_{16}O_5N_2$	233.11320	233.11359	1.66578
Glu	$C_9H_{15}O_7N$	250.09213	250.09213	0.01715	**Glu**	$C_9H_{15}O_6N$	234.09721	234.09723	0.06546
Gly	$C_6H_{11}O_5N$	178.07100	178.07101	0.08673	**Gly**	$C_6H_{11}O_4N$	162.07608	162.07608	-0.02491
His	$C_{10}H_{15}O_5N_3$	258.10845	-		**His**	$C_{10}H_{15}O_4N_3$	242.11353	-	
Leu/Ile	$C_{10}H_{19}O_5N$	234.13360	234.13361	0.02886	**Leu/Ile**	$C_{10}H_{19}O_4N$	218.13868	218.13869	0.001162
Lys	$C_{10}H_{20}O_5N_2$	249.14450	249.14476	1.0512	**Lys**	$C_{10}H_{20}O_4N_2$	233.14958	233.14958	-0.00738
Met	$C_9H_{17}O_5NS$	252.09002	252.09002	0.391	**Met**	$C_9H_{17}O_4NS$	236.09511	-	
Phe	$C_{13}H_{17}O_5N$	268.11795	268.11795	0.00485	**Phe**	$C_{13}H_{17}O_4N$	252.12303	252.12305	0.04892
Pro	$C_9H_{15}O_5N$	218.10230	218.10233	0.12086	**Pro**	$C_9H_{15}O_4N$	202.10738	202.10738	-0.04147
Ser	$C_7H_{13}O_6N$	208.08156	208.08157	0.04742	**Ser**	$C_7H_{13}O_5N$	192.08665	192.08665	0.02938
Thr	$C_8H_{15}O_6N$	222.09721	222.09724	0.1377	**Thr**	$C_8H_{15}O_5N$	206.10230	206.10231	0.05386
Trp	$C_{15}H_{18}O_5N_2$	307.12885	307.12863	-0.70517	**Trp**	$C_{15}H_{18}O_4N_2$	291.13393	291.13181	-7.30992
Tyr	$C_{13}H_{17}O_6N$	284.11286	284.11282	-0.14168	**Tyr**	$C_{13}H_{17}O_5N$	268.11795	268.11795	0.00485
Val	$C_9H_{17}O_5N$	220.11795	220.11795	0.0059	**Val**	$C_9H_{17}O_4N$	204.12303	204.12303	-0.01432

Table A5 continue.

Methylglyoxal adducts					Glyoxal adducts				
Amino Acid	**Formula**	**Exact Mass [M+H$^+$]**	**Experimental Mass [M+H$^+$]**	**Δ (ppm)**	**Amino Acid**	**Formula**	**Exact Mass [M+H$^+$]**	**Experimental Mass [M+H$^+$]**	**Δ (ppm)**
Ala	$C_6H_{11}O_4N$	162.07608	162.07608	-0.02491	**Ala**	$C_5H_9O_4N$	148.06043	148.06042	-0.06413
Arg	$C_9H_{18}O_4N_4$	247.14008	247.14005	-0.1473	**Arg**	$C_8H_{16}O_4N_4$	233.12443	233.12376	-2.86317
Asn	$C_7H_{12}O_5N_2$	205.08190	205.0826	3.4027	**Asn**	$C_6H_{10}O_5N_2$	191.06625	191.06677	2.74528
Asp	$C_7H_{11}O_6N$	206.06591	206.0658	-0.57099	**Asp**	$C_6H_9O_6N$	192.05026	-	
GABA	$C_7H_{13}O_4N$	176.09173	176.09174	0.00806	**GABA**	$C_6H_{11}O_4N$	162.07608	162.07608	-0.02491
Gln	$C_8H_{14}O_5N_2$	219.09755	219.09767	0.56349	**Gln**	$C_7H_{12}O_5N_2$	205.08190	205.0826	3.4027
Glu	$C_8H_{13}O_6N$	220.08156	220.08157	0.04483	**Glu**	$C_7H_{11}O_6N$	206.06591	206.0658	-0.57099
Gly	$C_5H_9O_4N$	148.06043	148.06042	-0.06413	**Gly**	$C_4H_7O_4N$	134.04478	134.04477	-0.11155
His	$C_9H_{13}O_4N_3$	228.09788	-		**His**	$C_8H_{11}O_4N_3$	214.08223	214.08191	-1.50961
Leu/Ile	$C_9H_{17}O_4N$	204.12303	204.12303	-0.01432	**Leu/Ile**	$C_8H_{15}O_4N$	190.10738	190.10738	-0.04409
Lys	$C_9H_{18}O_4N_2$	219.13393	219.13379	-0.65945	**Lys**	$C_8H_{16}O_4N_2$	205.11828	-	
Met	$C_8H_{15}O_4NS$	222.07946	-		**Met**	$C_7H_{13}O_4NS$	208.06381	208.06265	-5.53935
Phe	$C_{12}H_{15}O_4N$	238.10738	238.10739	0.02888	**Phe**	$C_{11}H_{13}O_4N$	224.09173	224.09174	0.00634
Pro	$C_8H_{13}O_4N$	188.09173	188.09174	0.00755	**Pro**	$C_7H_{11}O_4N$	174.07608	174.07608	-0.0232
Ser	$C_6H_{11}O_5N$	178.07100	178.07101	0.08673	**Ser**	$C_5H_9O_5N$	164.05535	164.05504	-1.89234
Thr	$C_7H_{13}O_5N$	192.08665	192.08665	0.02938	**Thr**	$C_6H_{11}O_5N$	178.07100	178.07101	0.08673
Trp	$C_{14}H_{16}O_4N_2$	277.11828	277.11826	-0.10066	**Trp**	$C_{13}H_{14}O_4N_2$	263.10263	263.10263	-0.01077
Tyr	$C_{12}H_{15}O_5N$	254.10230	254.10231	0.04369	**Tyr**	$C_{11}H_{13}O_5N$	240.08665	240.08665	0.0235
Val	$C_8H_{15}O_4N$	190.10738	190.10738	-0.01109	**Val**	$C_7H_{13}O_4N$	176.09173	176.09174	0.00806

ANNEX 2. Supplementary Tables For Chapter 5

Table A6. Concentrations of free amino acids in apple, orange juice samples (mg/L) and peach puree samples (mg/kg) obtained from the process stages of juice processing.

Process stages		**Free Amino Acids**																		
Apple		**Ala**	**Arg**	**Asn**	**Asp**	**Cys**	**Glu**	**Gly**	**Gln**	**His**	**Ile**	**Leu**	**Lys**	**Met**	**Phe**	**Pro**	**Ser**	**Thr**	**Trp**	**Tyr**	**Val**
Raw Juice	A1	27.2	38.4	2300.8	982.7	nd	205.9	nd	253.7	29.2	153.1	56.9	20.9	20.3	152.5	44.8	80.6	67.2	89.9	90.1	20.1
		±2.4^{ab}	±2.7^{bc}	±177.4^{ab}	±44.5^{b}		±1.9^{ab}		±26.5^{d}	±3.7^{ab}	±5.0^{c}	±4^{b}	±1.5^{de}	±0.2^{e}	±5.7^{bc}	±1.1^{cd}	±2.6^{c}	±3.5^{c}	±5.7^{d}	±5.6^{c}	±0.3^{d}
Pasteurized	A2	29.9	31.8	2300.6	864.1	nd	183.4	nd	211.0	18.6	123.4	39.1	12.4	12.8	99.4	36.3	62.0	42.9	53.6	60.5	16.8
Juice		±5.2^{ab}	±1.1^{b}	±17.3^{ab}	±31.8^{ab}		±1.3^{a}		±1.3^{bc}	±0.9^{a}	±0.7^{bc}	±0.3^{a}	±0.5^{ab}	±0.2^{d}	±4.7^{a}	±1^{bcd}	±5.8^{bcd}	±1.2^{ab}	±3.3^{b}	±2.8^{ab}	±0.7^{c}
Depectinized	A3	35.1	46.1	3139.6	831.7	nd	226.4	nd	231	73.4	114.3	60.0	22.5	12.3	160.1	33.3	67.2	60.7	88.0	101.6	14.7
Juice		±3.4^{ab}	±3.6^{c}	±81.5^{c}	±24.2^{a}		±0.5^{ab}		±5.0^{cd}	±14.4^{c}	±3.6^{abc}	±4.4^{b}	±1.3^{e}	±0.1^{c}	±9.6^{c}	±0.1^{bc}	±2.7^{cd}	±2.2^{bc}	±4.6^{d}	±5.4^{c}	±0.3^{c}
Ultrafiltrated	A4	28.0	36.8	2499.4	1232.7	nd	180.1	nd	183.8	58.8	91.0	47.8	17.9	9.8	127.6	26.5	53.5	48.3	70.1	80.9	11.7
Juice		±3.9^{ab}	±4.3^{bc}	±163.4^{b}	±3.3^{c}		±7.5^{a}		±11.2^{b}	±13.8^{bc}	±6.4^{ab}	±5.4^{ab}	±1.7^{cd}	±0.5^{c}	±12.7^{b}	±1.1^{b}	±4.2^{bc}	±3.7^{abc}	±6.4^{c}	±7.5^{bc}	±0.7^{b}
Clarified Juice	A5	41.0	17.8	2628.1	833.8	nd	224.3	nd	44.2	13.3	110.9	49.3	10.6	7.4	92.2	45.2	42.7	34.3	15.0	44.1	22.7
		±7.5^{b}	±0.3^{a}	±123.4^{b}	±36.1^{a}		±39.5^{ab}		±2.5^{a}	±0.5^{a}	±30^{abc}	±5.2^{ab}	±0.8^{a}	±0.9^{b}	±9.2^{a}	±7.9^{d}	±10.9^{ab}	±12.0^{a}	±1.6^{a}	±12.1^{a}	±1.2^{e}
Concentrated	A6	22.7	56.5	1989.6	901.7	nd	246.9	nd	191.0	55.6	75.0	56.3	16.0	5.0	231.7	13.3	28.4	53.9	17.6	92.5	6.1
Juice		±0.3^{a}	±1.8^{d}	±34.2^{a}	±54.5^{ab}		±1.6^{b}		±5.5^{bc}	±9.4^{bc}	±0.4^{a}	±1.9^{b}	±0.4^{bc}	±0.2^{a}	±4.1^{d}	±0.1^{a}	±1.3^{a}	±1.8^{bc}	±0.6^{a}	±1.6^{c}	±0.1^{a}
Orange																					
Raw Juice	O1	92.3	947.7	5.1	748.0	406.6	166.7	21.4	30.5	3.7	12.9	16	45.3	10.3	28.4	1958.8	277.3	50.1	13.2	nd	26.4
		±11.4^{a}	±16^{a}	±0.2^{a}	±17.5^{a}	±67.8^{b}	±4.6^{b}	±2.4^{a}	±0.3^{a}	±0.7^{a}	±1.2^{a}	±1.7^{a}	±9.9^{a}	±0.5^{a}	±2^{a}	±94.1^{a}	±12.7^{a}	±0.4^{b}	±0.5^{a}		±3.2^{a}
Fine Juice	O2	87.9	943.2	5.1	766.1	254.4	118.7	25.0	32.6	3.6	15.0	17.3	42.0	9.7	27.8	2034	290.7	49.4	13.5	nd	23.9
		±4.3^{a}	±9.3^{a}	±0.1^{a}	±6.6^{bc}	±8.6^{ab}	±9.3^{a}	±1.5^{a}	±0.7^{a}	±0.5^{a}	±1.3^{a}	±0.3^{a}	±9^{a}	±0.6^{a}	±1.6^{a}	±13.4^{a}	±2.5^{a}	±1.6^{b}	±0.3^{a}		±1.6^{a}
Pasteurized	O3	76.4	940.8	5.8	802.3	175.6	166.1	27.8	30.8	4.2	14.8	14.9	53.8	9.3	32	2018.5	259.3	35.7	14.1	nd	20.5
Juice		±4.5^{a}	±10.9^{a}	±0.3^{a}	±0.3^{c}	±16.0^{a}	±1.7^{b}	±6.1^{a}	±0.1^{a}	±0.4^{a}	±0.6^{a}	±1.2^{a}	±2.0^{a}	±0.3^{a}	±4.4^{a}	±57.9^{a}	±1.3^{a}	±3.0^{a}	±0.6^{a}		±2.4^{a}

Table A6 continue.

Process stages		Free Amino Acids																	
Peach		**Ala**	**Arg**	**Asn**	**Asp**	**Cys**	**Glu**	**Gly**	**Gln**	**His**	**Ile**	**Leu**	**Lys**	**Met**	**Phe**	**Pro**	**Ser**	**Thr**	**Trp**	**Tyr**	**Val**
Puree	P1	nd	5	3426.1	41.3	nd	61.9	nd	17.3	43.4	nd^{a}	nd^{a}	20.2	nd	7.4	nd	66.6	18.7	nd	nd	5.4
			±0.1^{b}	±33.8^{a}	±9.0^{a}		±0.9^{b}		±7.8^{ab}	±6.9^{a}			±7.6^{ab}		±0.2^{b}		±2.3^{a}	±1^{b}			±0^{a}
Concentrated	P2	nd	5.2	3355.3	34.8	nd	39.7	nd	16.4	43.7	nd^{a}	nd^{a}	19.4	nd	7.1	nd	88.3	12.9	nd	nd	6.6
Puree			±0.5^{b}	±22.0^{a}	±16.3^{a}		±6.6^{a}		±0.8^{b}	±1.2^{a}			±0.8^{b}		±0.2^{b}		±21.5^{a}	±1.2^{a}			±0.1^{a}
Sterilized	P3	nd	3.0	3253.3	58.3	nd	57.3	nd	9.6	20.6	7.7	2.7	10.6	nd	6.2	nd	53.0	14.9	nd	nd	5.7
Puree			±0.4^{a}	±133.4^{a}	±0.3^{b}		±11.3^{ab}		±1.7^{a}	±4.0^{b}	±0.8^{b}	±0.2^{b}	±1.6^{a}		±0.0^{a}		±0.3^{a}	±0.5^{ab}			±0.5^{a}
Concentrate																					

Mean values in the same column with different letters are significantly different at the 5 % confidence level. nd: not detectable.