MITIGATION OF THERMAL PROCESS CONTAMINANTS BY ALTERNATIVE TECHNOLOGIES

ALTERNATİF TEKNOLOJİLER İLE TERMAL PROSES KONTAMİNANTLARININ AZALTILMASI

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This work named **"Mitigation of Thermal Process Contaminants by Alternative Technologies"** by **BURÇE ATAÇ MOGOL** has been approved as a thesis for the Degree of **DOCTOR OF PHILOSOPHY IN FOOD ENGINEERING** by the below mentioned Examining Committee Members.

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Prof. Dr. Fatma SEVİN DÜZ
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To Ali Can
and
To my mother

ETHICS

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BURÇE ATAÇ MOGOL

ABSTRACT

MITIGATION OF THERMAL PROCESS CONTAMINANTS BY ALTERNATIVE TECHNOLOGIES

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Thermal processing leads to desired color, flavor, and texture in foods. However, certain toxic chemical contaminants, like acrylamide, hydroxymethylfurfural (HMF), free and bound chloropropanols, and furan are also consequences of thermal processing. Due to their health concern, authorities reported that their formation needed to be minimized.

The aim of this PhD thesis was to develop knowledge-based new techniques for the mitigation of thermal processing contaminants in foods. To achieve the aim, the formation of above-mentioned processing contaminants and factors affecting their formation were investigated in model and actual food systems.

A computer vision based image analysis tool was developed for real-time monitoring of color changes in biscuits during baking. Since discovery of acrylamide in foods, elimination of its formation is of great importance. Quick, reliable, and objective tools are needed to evaluate the acrylamide content of foods online and decide whether it is within

food safety level or not. Both color and acrylamide are formed through Maillard reaction, so monitoring color could be an indicator of acrylamide content of food. In this respect, different approaches were applied to biscuits, predictive models were developed based on CIE Lab values, brown and dark brown ratio % of biscuits. The models successfully predicted the acrylamide and HMF content of biscuits. The effect of the deviations in the amount of ingredients was also investigated. Finally, the algorithm was more improved to give the ability of real time monitoring and decision-making.

A combined conventional and vacuum process as a new baking technology was developed to mitigate acrylamide and HMF in biscuits. The biscuit dough was first partially baked under conventional conditions, and then post baked under vacuum for accelerated drying at 500 mbar until the desired final moisture content was attained. Doing so, exposure of biscuits to higher thermal load was prevented. Therefore, the combined process formed no acrylamide or HMF (<LOQ) in biscuits. This approach was considered as a promising alternative to produce safer biscuits for targeted consumers like infants.

The effects of temperature, time and presence of salt on the formation of free and bound chloropropanols in biscuits were determined. The kinetic examination showed that increasing temperature led to an increase in the reaction rate constants of these contaminants. Eliminating chloride from the recipe decreased 3-MCPD, 2-MCPD rate constants in biscuits by 57.5% and 85.4%, respectively, and bound-MCPD formation was prevented. So, lowering thermal load or limiting chloride concentration should be considered a means to reduce or eliminate formation of these contaminants in biscuits.

The effect of sodium chloride on sucrose decomposition leading to HMF formation was investigated. The pathway of sucrose pyrolytic decomposition, and the fructofuranosyl cation formation was confirmed. Elimination of sodium chloride prevented HMF formation and could be considered as an effective mitigation strategy.

The effect of oxidizing and reducing agents on the formation of furan was determined through ascorbic acid degradation during heating at elevated temperatures (≥100^{o}C) under low moisture conditions. Kinetic constants, estimated by multiresponse modeling, stated that adding ferric ion, as oxidizing agent, increased furan formation rate constant 369-fold than that of control model at 100^{o}C. Rate-limiting step of furan formation was determined. Conclusively, oxidation-reduction potential should be kept low to limit furan formation in foods.

Keywords: acrylamide, HMF, MCPD, furan, vacuum baking, mitigation, image analysis, color, salt.

ÖZET

ALTERNATİF TEKNOLOJİLER İLE TERMAL PROSES KONTAMİNANTLARININ AZALTILMASI

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Isıl işlem ile gıdalarda, istenen renk, tat, yapı gibi özelliklerin oluşmasının yanında insan sağlığı üzerine toksik etkisi olan kimyasal bileşikler de oluşmaktadır. Akrilamid, hidroksimetilfurfural (HMF), kloropropanoller ve furan ısıl işlem sonucunda oluşan proses kontaminantlarıdır. Sağlık üzerine etkileri endişe yarattığından bu kontaminantların oluşumlarının azaltılması önerilmektedir. Uluslararası Kanser Araştırma Ajansı tarafından akrilamid "insan için olası kanserojen" (Grup 2A), furan da "insan için muhtemel kanserojen" (Grup 2B) olarak gruplandırılmıştır. HMF ve kloropropanollerin de çeşitli toksik etkileri tespit edilmiş, *in vivo* çalışmaları da devam etmektedir.

Bu doktora tezinin amacı ısıl işlem görmüş gıdalardaki bu proses kontaminantlarının oluşumunun yeni tekniklerle azaltılmasıdır. Bunun için proses kontaminantlarının oluşumları ve oluşumlarını etkileyen faktörler incelenmiştir.

Yüzey esmerleşmesi ve termal proses kontaminantları pişirme sırasında Maillard reaksiyonu sonucunda meydana gelmektedirler. FoodDrink

Europe tarafından yayınlanan akrilamid kılavuzunda (Acrylamide Toolbox) kızarmış patates renginin akrilamid miktarının göstergesi olabileceği ifade edilmektedir. Rengin sürekli ölçümü ile (doğru bir kalibrasyonla) son ürünün akrilamid seviyesi tahmin edilebilmektedir. Bundan yola çıkarak, alternatif bir teknik olarak bilgisayar tabanlı görüntü analizi ile bisküvi yüzeyi rengi analiz edilmiştir. Bu amaçla, ortalama CIE Lab ölçümü ve renk segmentasyonu olmak üzere iki yaklaşım kullanılmıştır. Elde edilen renk bilgileri ile bisküvinin akrilamid ve HMF miktarı arasında korelasyon kurulmuştur. Bu sayede bisküvideki akrilamid ve HMF miktarları renk bilgisinden tahmin edilebilmektedir. Bu doktora tezi kapsamında gerçek zamanlı olarak esmerleşmeyi izleyen bir algoritma geliştirilmiştir. Bu algoritma ile bisküvi proses hattında analiz edilerek akrilamid miktarı tahmin edilebilmektedir. Sonuç olarak, belli değerin üzerinde akrilamid içeren ürünler için 'ret' ya da altındakiler için 'kabul' şeklinde bir karar verme mekanizması oluşturulabilmektedir. Bu sayede akrilamid, HMF gibi termal proses kontaminantlarının oluşumları kontrol edilebilmektedir.

Tez çalışması kapsamında konvansiyonel ve vakum proseslerinin kombinasyonuna dayalı yeni bir pişirme teknolojisi geliştirilmiştir. Bu teknoloji ile bisküvilerde akrilamid ve HMF oluşumunun azaltılması amaçlanmıştır. Vakum pişirme yöntemi kullanılarak elde edilen bisküvilerde akrilamid miktarı, konvansiyonel koşullarda pişirilen bisküvilerden önemli derecede az bulunmuştur ($p<0.05$). Örneğin 200^{o}C'de 15 dakika vakum altında pişen bisküviler, aynı koşullarda konvansiyonel olarak pişirilen bisküvilerden %75 daha az akrilamid içermektedir. Bunun yanında vakum altında pişirilen bisküvilerde HMF oluşumu gözlenmemiştir. Konvansiyonel pişirme ile kıyaslandığında vakum pişirmede daha düşük bir zaman-sıcaklık profili sağlanmaktadır. Konvansiyonel ve vakum pişirmenin kombine olarak kullanıldığı durumda bisküviler önce konvansiyonel fırında ile 220^{o}C'de 2-4 dakika ön pişirilmiş sonra vakum altında (500 mbar) 180^{o}C'de 4-6 dakika pişirilmiştir. Bu teknik sayesinde bisküviler uzun süre yüksek sıcaklığa

maruz kalmamış, böylece termal proses kontaminantlarının oluşumu engellenmiştir. Kombine proses ile elde edilen bisküvilerde akrilamid ve HMF oluşumu gözlenmemiştir. Bu pişirme tekniği, özellikle bebekler gibi hassas tüketici grupları için daha güvenli bisküviler elde etmek amacıyla alternatif bir yöntem olarak önerilmektedir.

Tez çalışması kapsamında bisküvilerde 3-MCPD, 2-MCPD ve bağlı MCPD türevlerinin oluşumuna pişirme sıcaklığı ve klor kaynağı olarak sofra tuzunun etkisi incelenmiştir. Elde edilen veriler kinetik olarak incelenmiş ve reaksiyon hız sabitleri belirlenmiştir. Buna göre sıcaklığın artması ile 3-MCPD, 2-MCPD ve bağlı MCPD oluşum hız sabitleri de artmıştır. 3-MCPD ve 2-MCPD'nin aktivasyon enerjileri 29 kJ mol^{-1} olarak bulunmuştur. Tuzun bisküvi reçetesinden çıkarılması 3-MCPD oluşum hız sabitini %57.5, 2-MCPD oluşum hız sabitini %85.4 azaltırken, bağlı MCPD oluşumunu tamamen engellemiştir. Farklı rafine bitkisel yağların (kanola, mısır, fındık, yer fıstığı ve zeytinyağı) bisküvilerde 3-MCPD, 2-MCPD ve bağlı MCPD oluşumu üzerine etkileri de belirlenmiştir. Farklı rafine yağlarla hazırlanan bisküvilerde oluşan 3-MCPD miktarları arasında önemli fark görülmemiştir. Elde edilen bulgulara göre ısıl işlem yükünün ya da tuz miktarının azaltılmasıyla kloropropanol türevlerinin oluşumları önemli düzeyde sınırlandırılabilmektedir.

Tez çalışması kapsamında ayrıca sofra tuzunun sakkaroz dehidrasyonu yoluyla HMF oluşumuna etkisi de incelenmiştir. Sakkarozun pirolitik dekomposizyonu ve fruktofuranozil katyon oluşum yolu model sistemde doğrulanmıştır. Tuzun Lewis asidi gibi davranarak sakkarozun dekompozisyonunu, dolayısı ile de HMF oluşumunu hızlandırdığı tespit edilmiştir. Elde edilen bulgular tuzun bisküvi gibi fırıncılık ürünlerinde reçeteden çıkarılmasının ya da uygun bir enkapsülasyon materyali ile kaplanarak eklenmesinin HMF oluşumunu azaltmada etkili olacağını göstermektedir.

Tez çalışması kapsamında son olarak, oksidasyon redüksiyon ajanlarının düşük nem ve yüksek sıcaklık ($\geq 100^{o}C$) koşullarında askorbik asit bozulması sonucu furan oluşumu üzerine etkisi incelenmiştir. Bunun için

oksidasyon ajanı olarak demir klorür veya redüksiyon ajanı olarak sistein reaksiyon ortamına eklenmiştir. Reaksiyon mekanizması çoklu cevap kinetik modelleme yöntemi ile analiz edilmiş ve reaksiyon hız sabitleri hesaplanmıştır. Buna göre 100^{o}C'de ortamda demir bulunması halinde furan oluşum hız sabiti kontrole göre 369 kat artırmıştır. Furan oluşum reaksiyon mekanizmasındaki hız belirleyici basamağın askorbik asitin hidrasyonu sonucu oluşan bir ara ürün ile diketoglukonik asit arasındaki geri dönüşümlü aşama olduğu belirlenmiştir. Ayrıca demir askorbik asitin hidrasyonu ve furan oluşum aktivasyon enerjisini sırasıyla %28.6 ve %60.9 azaltmıştır. Elde edilen sonuçlar, bebek formülasyonları gibi spesifik tüketici gruplarını hedef alan, demir ve vitaminlerce zenginleştirilen ısıl işlem görmüş gıdalar için önem taşımaktadır. Elde edilen bulgular oksidasyon-redüksiyon potansiyelinin düşürülmesi ile askorbik asit bakımından zengin gıdalarda ısıl işlem sırasında furan oluşumunun kontrol altına alınabileceğini göstermektedir.

Anahtar Kelimeler: akrilamid, HMF, MCPD, furan, vakum pişirme, azaltma stratejisi, görüntü analizi, renk, tuz.

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ABBREVIATIONS

AA	Ascorbic Acid
AACC	American Association of Cereal Chemists
ALARA	As Low As Reasonably Achievable
ANOVA	Analysis of Variance
CB	Conventional Baking
CCD	Central Composite Design
CIAA	Confederation of the Food and Drink Industries of the EU
CIE	International Commission on Illumination
Cys	Cysteine
DAD	Diode Array Detector
1,3-DCP	1,3-Dichloro-2-propanol
DHAA	Dehydroascorbic Acid
DKG	Diketogluconic Acid
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
GC-MS	Gas Chromatography Mass Spectrometry
HFBI	1-(Heptafluorobutyryl)-imidazole
HFCS	High Fructose Corn Syrup
HILIC	Hydrophilic Interaction Chromatography
HMF	5-Hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HSS	High Strength Silica
HSV	Hue, Saturation, Value
HVP	Hydrolyzed Vegetable Proteins
IARC	International Agency for Research on Cancer
Int	Intermediate
JPEG	Joint Photographic Experts Group
LC/MS/MS	Liquid Chromatography Tandem Mass Spectrometry

LOD	Limit of Detection
LOEL	Lowest Observed Effect Level
LOQ	Limit of Quantitation
MATLAB	Matrix Laboratory
3-MCPD	3-Monochloropropane-1,2-diol
MCX	Mixed-mode Cation Exchange
MRM	Multiple Reaction Monitoring
MSD	Mass Selective Detector
ND	Not Detected
NFMP	Non Fat Milk Powder
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
NOEL	No Observable Effect Level
PTFE	Polytetrafluoroethylene
PUFA	Polyunsaturated Fatty Acids
RGB	Red, Green, Blue
ROI	Region of Interest
RSM	Response Surface Methodology
SIM	Selected Ion-monitoring Mode
SMF	5-Sulfoxymethylfurfural
SPME	Solid Phase Micro Extraction
SPSS	Statistical Package for the Social Sciences
TDI	Tolerable Daily Intake
TQ	Triple Quadrupole
UFLC	Ultra Fast Liquid Chromatography
UHPLC	Ultra High-Performance Liquid Chromatography
UPLC	Ultra Performance Liquid Chromatography
VB	Vacuum Baking

INTRODUCTION

Human ancestors discovered the fire and the controlled use of it 1 million years ago. The fire gave the human ability to get warm and cook their foods. "How lucky that Earth has fire" says Richard Wrangham in his book named "Catching Fire: How Cooking Made Us Human", as fire provides cooked foods, which has many advantages over raw ones. For example, the microbiological safety, digestibility, and edibility of the food increase by the cooking. Food constituents undergo many changes during cooking and nutrients become accessible, therefore digestibility increases. Additionally, cooking leads to form many chemical reactions in foods, which are desired in terms of color and flavor. Consequently, the food become attractive and its edibility increases. Chemical reactions leading to desired color and flavor, like Maillard reaction, is accompanied by certain chemical food safety problems. Maillard reaction is the reaction between carbonyls and amino acids, or amino group in lysine residue of protein chain and responsible for the desired changes in foods. However, certain toxic chemical compounds, like acrylamide and HMF, are formed during this reaction, which create health concerns. Beside Maillard reaction, heat treatment of foods causes to form many other chemical contaminants, like furan, 3-MCPD and its esters. The authorities published many surveys and reports on the formation and occurrence of these contaminants in foods, and they indicate the need of developing strategies for the elimination of these contaminants in foods. Many researchers studied on the formation and mitigation of these compounds, but they are still hot topics and need further findings.

The main objective of this PhD thesis was to develop viable strategies on minimizing the formation of certain thermal process contaminants, namely acrylamide, hydroxymethylfurfural, free and bound chloropropanols, and furan formed in foods. To achieve this, formation routes of these compounds and the factors affecting these routes are

examined as they are of importance to understand the mechanism and develop strategies.

Within this context this PhD thesis is divided into 6 chapters:

Chapter 1 gives background information on baking, Maillard reaction, and the contaminants formed in foods during heat-treatment. The chemical properties, formation mechanisms, occurrence in foods, as well as mitigation strategies of these contaminants were summarized.

Chapter 2 describes an alternative color measurement tool, computer vision based image analysis, and its potential to predict acrylamide and HMF levels in biscuits. This chapter provides a deep insight on the relationship between surface color characteristics of biscuits and the concentrations of acrylamide and HMF. The results indicate a potential for real-time application of this color measurement technique to baking process as process and quality control tool.

Chapter 3 discusses the effect of thermal processing conditions on the formation of acrylamide and HMF. As the thermal load is the main factor in the formations of acrylamide and HMF, a new baking technology with lower thermal load that is based on the combination of partial conventional baking and vacuum post baking was proposed to minimize the formation of these compounds in biscuits.

Chapter 4 discusses the effects of temperature, sodium chloride, and oil type on the formation of free and bound chloropropanols (3-MCPD, 2-MCPD) and MCPD esters in biscuits during baking. The results of kinetic analyses suggested the elimination of sodium chloride from recipe to prevent the formation of chloropropanols in biscuits during baking.

Chapter 5 describes mechanistically the effect of sodium chloride on the formation of HMF through sucrose decomposition. The intermediate compounds and HMF formed during heating sucrose were successfully identified by orbitrap high-resolution mass spectrometry. The presence of sodium chloride increased significantly the rates of their formations

suggesting that its elimination from recipe or limiting its reactivity by encapsulation would be considered as a mitigation strategy.

Finally **Chapter 6** describes the effects of oxidizing and reducing agents on furan formation through the degradation of ascorbic acid during heating at elevated temperatures under low moisture conditions. A multiresponse mechanistic model was developed, and the formation mechanism was further enlightened. The results suggested the elimination of oxidizing agents like ferric ions to limit furan formation from ascorbic acid under the conditions stated above.

This PhD study was part of the Prometheus project (**PRO**cess contaminants: **M**itigation and **E**limination **T**echniques for **H**igh food quality **E**valuated **U**sing **S**ensors), a European Union-funded FP7 project on the identification of the effect of processing on food contaminants.

1 GENERAL INTRODUCTION

1.1 Introduction

This chapter gives the fundamental literature on the topics covered in the thesis. Throughout the thesis, thermal processing of foods is the common subject of every chapter and as a thermal process, baking is involved particularly in **Chapter 2, Chapter 3,** and **Chapter 4**. Therefore, this chapter starts with the basics of baking, then, discusses the chemical changes occurring in biscuits during baking, including Maillard reaction. Lastly, the thermal process contaminants, namely acrylamide, HMF, chloropropanols and furan are covered together with their properties, formation mechanisms, occurrence in foods and mitigation strategies.

1.2 Baking

Baking is a complex process in which certain chemical and physical changes take place simultaneously. It is a key process to develop desired product characteristics including structure, texture, flavor, and color [1]. Baking is also important in terms of shelf life stability for certain products, like biscuits [2].

There are certain changes in dough during baking (Figure 1.1). The physical changes occurring by heat treatment are defined as crust formation, melting of shortening, conversion of water to steam, gas expansion, and escape of carbon dioxide, other gases, and steam [3]. These changes must be encouraged to take place in an order. Environmental conditions, temperature, and a time optimum for the particular dough makeup are required for the desired attributes of the cookie/biscuit to be produced [3].

After certain thermal energy applied to biscuit, chemical reactions, namely sugar caramelization and Maillard reaction, start. Caramelization occurs around 148.9^{o}C and is the consequence of sugar molecules such as maltose, fructose and glucose to produce the colored substances. The Maillard reaction is the reaction between reducing

sugars and amino compounds. Both reactions are responsible for the attractive color, flavors, and aromas in baked foods. At about 176.7°C, the brown color seems and tastes like caramel, and around 246.1 to 260°C, the melanoidins become black, bitter, and insoluble [3]. Maillard reaction will be comprehensively discussed under the next heading.

In the end of baking, dough is transformed to a product due to heat and mass transfer within dough as well as within the oven chamber [1]. All heat transfer mechanisms, i.e. conduction, convection, and radiation, are involved in the baking process. Heat is transferred primarily by convection from heating medium, i.e. air, and by radiation from oven walls to the product surface, which is followed by conduction to the geometric center [4]. Conduction is also effective heat transfer mechanism from heated tray to the bottom of the dough. Some characteristics of biscuits depend on governed heat transfer mechanism during baking process. This topic will be fully discussed in **Chapter 3**.

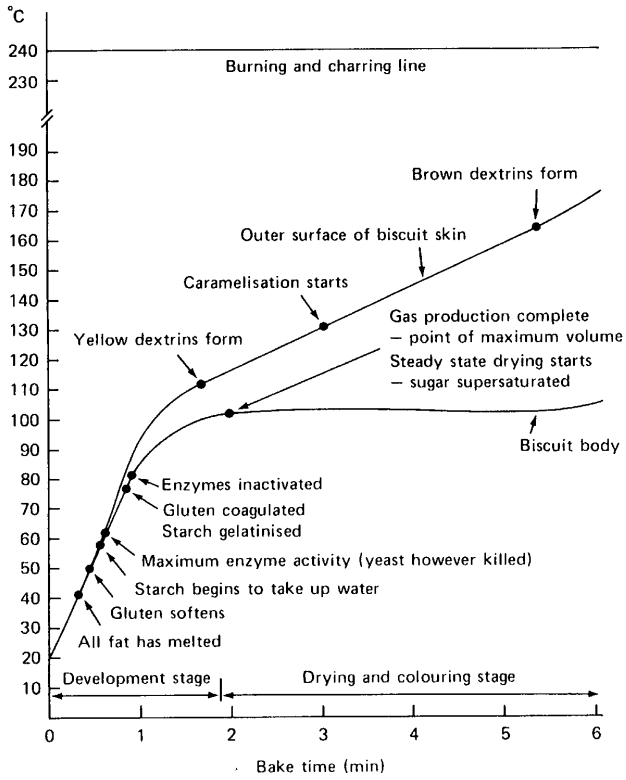


Figure 1.1 Changes during baking [2, 5]

1.3 Maillard Reaction

The thermal treatment causes not only physical changes in biscuit but also leads certain chemical reactions to occur. During the course of baking, as the moisture content of biscuit decreases and the temperature increases to a certain level chemical reactions take place. The Maillard reaction, also called non-enzymatic browning, is the main reaction occurring in biscuits during baking and responsible for the color flavor and aroma. Maillard reaction is named after the French chemist Louis-Camille Maillard, who first described it in 1912 [6]. In 1953, a coherent reaction scheme was proposed by Hodge [7] (Figure 1.2).

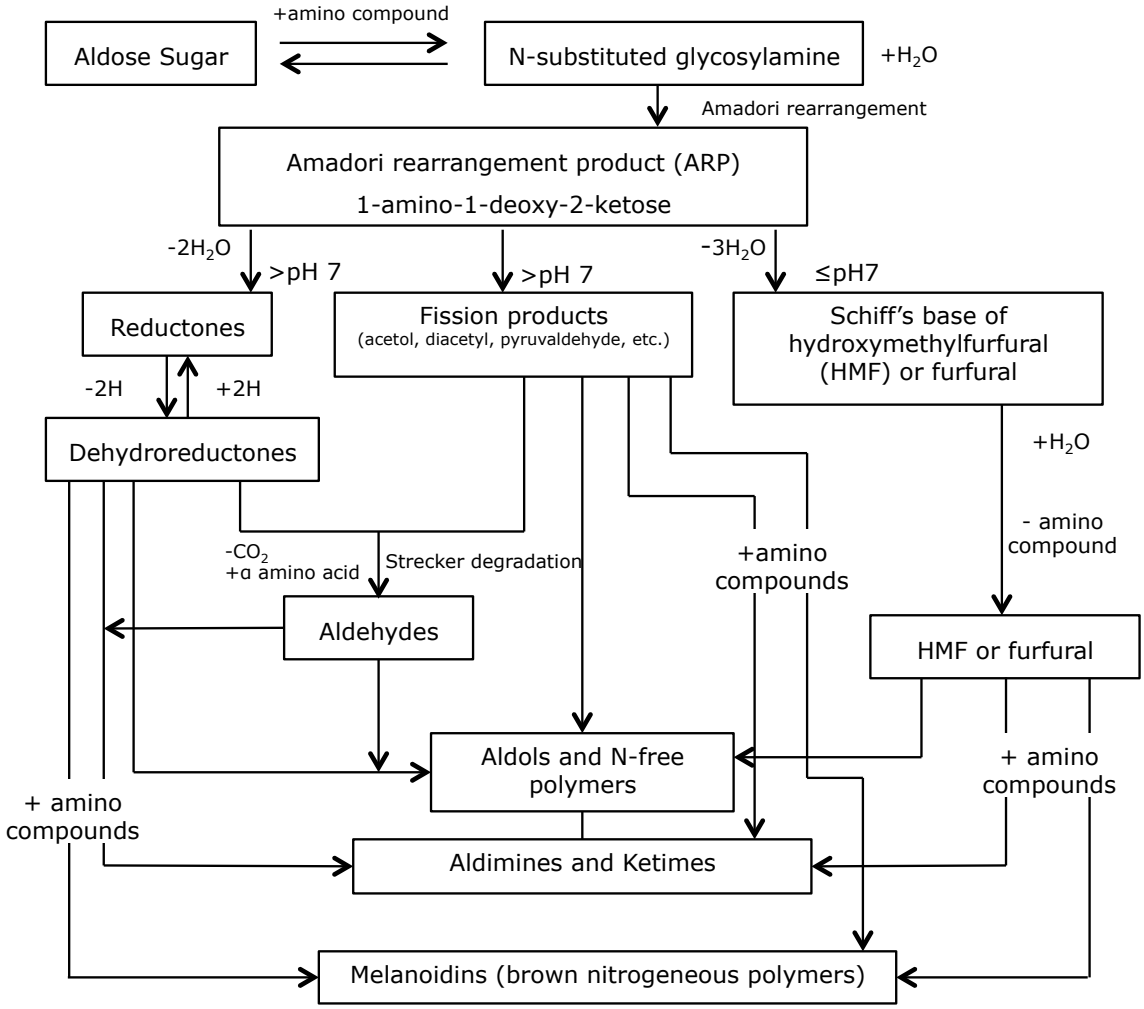


Figure 1.2 Maillard reaction scheme adapted from Hodge [7]

During early stage of the reaction, reducing sugars condense with free amino group of a compound, like amino acids, α-amino group of

proteins or ε-amino group of lysine residue present in protein structure, and gives condensation product N-substituted glycosylamine. After formation of Amadori product by Amadori rearrangement, the reaction depends on the pH of the system. Amadori product undergoes mainly 1,2-enolisation at pH 7 or below resulting furfural (from pentoses) or HMF (from hexoses) formation depending the on reducing sugar involved. When the pH>7, Amadori product undergoes mainly 2,3-enolisation producing reductones and fission products, including acetol, pyruvaldehyde and diacetyl. All these products are very reactive and they proceed to further reactions. Carbonyl groups condense with free amino groups, while dicarbonyl groups react with amino acids to form aldehydes and α-aminoketones in the so-called Strecker degradation. In the advanced stage, cyclisations, dehydrations, retroaldolisations, rearrangements, isomerisations and further condensations take place, leading, in the final stage, formation of melanoidins, known as brown nitrogeneous polymers. In the reaction between ketoses, such as fructose, and amino groups ketosylamines are formed which after the Heyns rearrangement form 2-amino-2-deoxyaldoses [8]. The Maillard reaction has been further unraveled. It was reported that 3-deoxyosuloses and 3,4-dideoxyosulos-3-enes are important intermediates for color formation (for glucose they are 3-deoxyhexosulose and 3,4-dideoxyhexosuloses-3-ene) [9]. 3-deoxy-2-hexosuloses and 1-deoxy-2,3-hexodiuloses and other dicarbonyl intermediates can undergo Strecker degradation reaction, thus catalyzing the degradation of amino acids during the reaction and, indirectly, being responsible for many of the aldehydes associated with the Maillard reaction [10]. 1-deoxy- and 3-deoxyglucosones were isolated and characterized from heated Amadori products [11]. Maillard reaction products from certain amino acid and sugars were reported to have antioxidant properties [12]. Researchers associated antioxidant capacity of Maillard reaction to the formation of brown melanoidins, which are reported to be powerful scavengers of reactive oxygen

species [13, 14]. On the other hand, Maillard reaction was associated with loss of nutritional quality, due to loss of available lysine during reaction. This issue is critical especially in cereals, where this amino acid is limiting [15].

1.4 Thermal Process Contaminants

Thermal process contaminants, also known as thermally-generated toxicants or process-induced toxicants are defined by Lineback and Stadler as "chemicals that are formed in food as a result of food processing/preparation that are considered to exert adverse toxicological effects or create a potential or real risks to humans" [16]. Among thermal process contaminants, acrylamide, HMF, chloropropanols, furan were covered within this thesis.

1.5 Acrylamide

1.5.1 Physical and Chemical Properties

Acrylamide (or acrylic amide, prop-2-enamide) is a chemical compound with a chemical formula C_3H_5NO. Its chemical structure and physical and chemical properties are given in Table 1.1 and Figure 1.3, respectively.

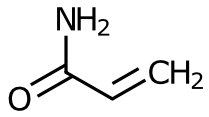


Figure 1.3 Chemical structure of acrylamide

Table 1.1 Physical and chemical properties of acrylamide

Molecular Formula	C_3H_5NO
Molar Mass	71.08 g mol^{-1}
Appearance	odorless, white crystalline solid
Density	1.322 g mL^{-1} (20°C)
Melting Point	84.5°C
Solubility	Water, ethanol, ether, chloroform

1.5.2 Formation Mechanism

Maillard reaction is desirable for color, flavor and aroma in bakery products, like breads, cookies, and biscuits. However, one of the important consequences of Maillard reaction is formation acrylamide, which is classified as a 'probable human carcinogen' by the International Agency for Research on Cancer (IARC) [17-19]. After the discovery of presence of acrylamide by Swedish researchers in foods, it was revealed that asparagine is the main precursor in the Maillard reaction to form acrylamide [18-21]. Figure 1.4 shows the formation pathway of acrylamide. At temperatures higher than 100^{o}C, asparagine condenses with reducing sugar or a carbonyl source leading to form N-glycosyl asparagine, which is present in equilibrium with its Schiff base. The moisture content of system determines the direction of the reaction. If the moisture content is high Schiff base may hydrolase to the precursors. It could also rearrange to form Amadori compound, which is not an effective precursor of acrylamide and could take role in color and flavor formation [22, 23].

Schiff base may decarboxylase to form azomethine ylide, which can lead to form the decarboxylated Amadori compound. The decarboxylation step may occur through Schiff base betain, zwitterionic form of Schiff base, or through intramolecular cyclization to form oxazolidine-5-one intermediate [23, 24]. The azomethine ylide may react to imine I or to imine II. Hydrolyses of imine I leads to the Strecker aldehyde (3-oxopropanamide), which did not release high amounts of acrylamide [22, 25]. Imine II could hydrolyze to the 3-aminopropionamide [22], which could form acrylamide by elimination of ammonia [26]. Imine II could also form acrylamide after protonation and β-elimination [27]. Decarboxylated Amadori product, formed by tautomerization of azomethine ylide, relases acrylamide and aminoketone by β-elimination [22]. Although α-hydroxycarbonyls, like reducing sugars, generate much more higher acrylamide, β-hydroxycarbonyls or any carbonyl may react as carbonyl source to form

acrylamide from asparagine [18, 22, 24, 25, 28]. In this reaction path, rate limiting step of acrylamide formation was determined as decarboxylation step of Schiff base [29].

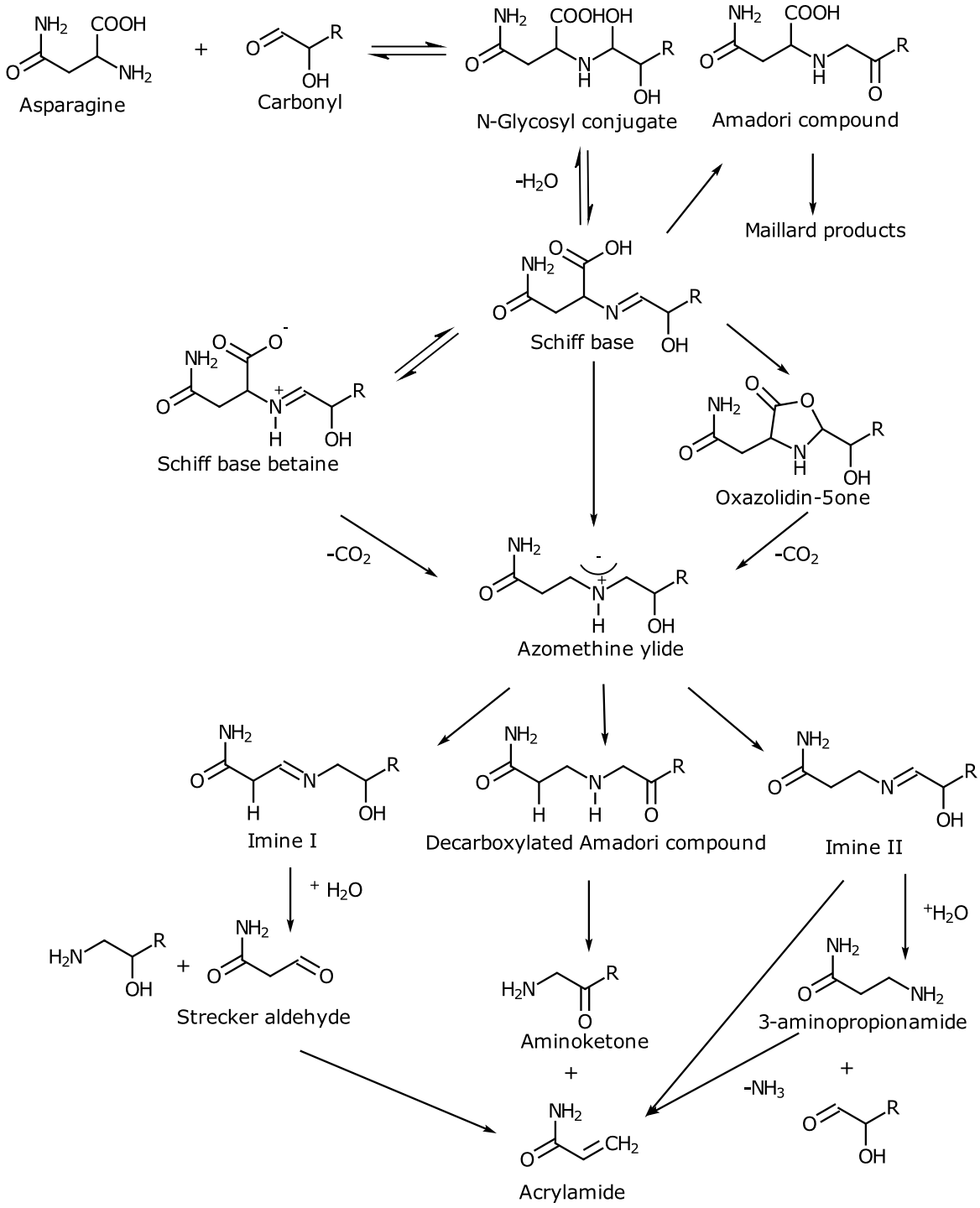


Figure 1.4 Formation pathways of acrylamide

There are also other pathways having minor role to form acrylamide. Acrolein, produced from triglycerides by strong heat treatment, could be found in some foods, such as fried foods, cooking oils and roasted coffee. Acrylic acid produced from acrolein reacts with ammonia (produced from α-amino acids via Strecker degradation in the presence of carbonyl compounds) to produce acrylamide [30]. Acrylic acid could also be formed from thermal decomposition of aspartic acid, carnosine and β-alanine [31-33]. Acrylic acid, then, could proceed to acrolein pathway, mentioned before. 3-aminopropionamide was reported as an effective precursor of acrylamide in the absence of carbonyls [34, 35].

1.5.3 Toxicity

Acrylamide is a neurotoxic compound in animals and humans. It is rapidly absorbed from the gastrointestinal tract and is widely distributed throughout the body [36, 37]. It is metabolized to glycidamide *in vivo*, which was thought to have more reactive genotoxic effects [38]. Due to their electrophilic property, both acrylamide and glycidamide could form adducts on DNA and proteins by Michael addition *in vivo* [39]. So, it can specifically react with hemoglobin, serum albumin, and enzymes [40, 41]. Conjugation of acrylamide or glycidamide with glutathione is converted to mercapturic acids [42-46]. Acrylamide was reported as a multi-organ carcinogen, may lead to form tumors at multiple sites such as lung, skin, brain, uterus, thyroid and mammary gland [47].

The No Observable Effect Level (NOEL) for neurotoxic effects in mouse and rat studies is in the range of 0.2–10 mg kg bodyweight^{-1} day^{-1} [48], which is far above dietary exposure. Tardiff and co-workers [49] estimated the tolerable daily intake (TDI) of acrylamide for neurotoxicity to be 40 μg kg^{-1}day^{-1} and for cancer to be 2.6 μg kg^{-1}day^{-1}.

1.5.4 Occurrence in Foods

Since its discovery in heat-treated foods, authorities started to investigate the levels of acrylamide in these foods. Basically, foods, rich in carbohydrate and asparagine, processed at high temperature,

and containing low moisture have the highest potential to form acrylamide. Acrylamide was reported to be present in a wide range of thermally processed foods, prepared commercially or cooked at home, including bread, crisp bread, bakery wares, breakfast cereals, potato products (crisps, French fries), and coffee [39]. European Union Member States, together with the European food industry, conducted surveys in different food groups present in market and built a database [50]. EFSA compiled data on acrylamide levels of foods in Europe for years 2007 – 2010. Table 1.2 summarizes acrylamide content of some foods present in market in 2010 adapted from EFSA report. European commission published 'recommendation on investigations into the levels of acrylamide in foods' together with indicative values in 2007 and 2010, 2011 and updated in 2013 (2013/647/EU) [51]. The indicative values are intended to indicate the need for an investigation if the acrylamide level found in a specific foodstuff exceeds the indicative value given in the recommendation. They are not safety thresholds and enforcement should be made based on risk assessments.

Table 1.2 Acrylamide levels (μg kg^{-1}) of foods in 2010, adapted from EFSA report [51, 52]

	Indicative value (μg kg^{-1})	n	Mean (μg kg^{-1})	90 percentile (μg kg^{-1})
Crackers	500	64	178	303
Infant biscuits	200	46	86	175
Crisp bread		54	249	665
Bread, soft	80	150	30	63
Breakfast cereals	400	174	138	293
Instant coffee	900	15	1123	2629
Roasted coffee	450	103	256	462
Potato crisps	1000	242	675	1538
French fries	600	256	338	725
Oven baked potato product (home cooked)		28	690	1888

1.5.5 Mitigation

Since discovery of acrylamide in foods, many efforts have been made to mitigate its formation. The organization FoodDrinkEurope, which represents the European food and drink industry, developed a 'toolbox' [53] containing tools that can be used selectively by food producers in line with their particular needs to lower acrylamide levels in their products. This toolbox discusses preventing and reducing formation of acrylamide in specific manufacturing processes and products. According to this toolbox, ALARA (As Low As Reasonably Achievable) concept is applied to acrylamide. ALARA means that the Food Business Operator should make every reasonable effort (based upon current knowledge) to reduce levels in final product and thereby reduce consumers' exposure [53]. Many researchers studied the factors affecting acrylamide formation in order to develop mitigation strategies. These factors could be categorized as agronomical/recipe factors and processing factors. Initial concentration of precursors (reducing sugar and asparagine), pH and water activity of the system, presence of other amino acids other than asparagine, presence of oxidizing fatty acids, mono, di-, and polyvalent cations, and type of leavening agent affect the formation of acrylamide during heating [54-60].

As the acrylamide backbone originates from free asparagine, decreasing asparagine content of food, expectedly leads to decrease in acrylamide formation. Within this regard, asparaginase pre-treatment have been suggested promising for acrylamide mitigation. Asparaginase converts asparagine into aspartic acid [61] and its application practiced in potato products [62, 63] and biscuit [64]. pH of the system is also important in such a way that lowering pH by means of the addition of organic acids decreased the amount of acrylamide formed in foods during heating [65], while its formation is maximum around at a pH value of 8. Another approach to mitigate acrylamide is incorporation of amino acids other than asparagine to the recipe. In such systems, other amino acids become competitive to asparagine in the chemical reactions or they

could be bound to acrylamide formed [66]. Presence of mono, di- or polyvalent cations, like NaCl, $CaCl_2$ could decrease formation of acrylamide [67]. It was stated that due to ionic and electronic association between $CaCl_2$ and asparagine which suppresses early-stage Maillard reactions [66]. Amrein et al. reported that ammonium hydrogencarbonate strongly enhanced acrylamide formation [68]. Therefore, eliminating ammonium hydrogencarbonate from formulation could result in decreasing acrylamide content of biscuits. The influence of temperature on the formation of acrylamide has been repeatedly demonstrated [18, 19, 21, 69]. Increased temperature lead to increase in concentration of acrylamide in heated food. Regarding process effect on the formation of acrylamide will be discussed in **Chapter 3**.

Mitigation of acrylamide in foods is someway possible with many methods, individually or as a combination. However, the challenge is to maintain the sensorial attribute of the food, while using these methods. Many of them include addition of new ingredients or replacing some of them in the original recipe, and/or changing processing method. Therefore, the product, in the end, might be different. This concern should be considered while deciding the right mitigation strategy.

1.6 5-Hydroxymethylfurfural

1.6.1 Physical and Chemical Properties

Hydroxymethylfurfural (5-hydroxymethyl-2-furaldehyde, HMF) is an intermediate product of the Maillard reaction [70]. It could also be formed by dehydration of hexoses under mild acidic conditions [71]. Its chemical structure and chemical and physical properties are given in Figure 1.5 and Table 1.3, respectively.

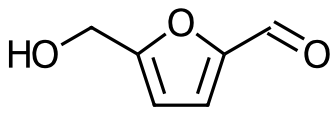


Figure 1.5 Chemical structure of HMF

Table 1.3. Physical and chemical properties of HMF [72]

Molecular Formula	$C_6H_6O_3$
Molar Mass	126.11 g mol^{-1}
Appearance	Beige colored crystalline solid
Density	1.206 g cm^{-3}
Melting Point	32–34 °C
Boiling Point	350.973-354.0878 °C / 760 mmHg
Solubility	Highly in water, methanol, ethanol, ethylacetate

1.6.2 Formation Mechanism

During thermal treatment of foods, HMF is formed both in caramelization, by dehydration of sugars, and Maillard reaction. Sugars decompose into furfural compounds by these reactions [70, 71, 73]. Formation mechanism is shown in Figure 1.6. 3-Deoxyosone, known key intermediate in HMF formation, is formed by 1,2 enolisation and dehydration of glucose or fructose and forms HMF by dehydration and cyclization reactions [74]. During Maillard reaction, positively charged amino group shifts the equilibrium to the enol form. Then, hydroxyl group is eliminated from C3 forms 2,3-enol, which is hydrolyzed at the C1 Schiff base to glycosulose-3-ene. Glycosulose-3-ene, dicarbonyl compound, forms HMF by cyclodehydration reaction [72]. Under dry and pyrolytic conditions highly reactive fructofuranosyl cation is formed from fructose and sucrose, which can be effectively and directly converted to HMF [75]. This pathway will be discussed in **Chapter 5**.

There are many factors affecting HMF formation in foods, including temperature, type of sugar, pH, water activity, and presence of divalent cations [59, 71, 76-78].

Caramelization requires higher temperatures than the Maillard reaction [72]. Similarly, different sugars have a different impact on the formation of HMF by caramelization; for example, fructose was found to be 31.2 times faster than glucose, whereas sucrose was 18.5 times faster than glucose in the rate of 5-HMF formation in three different sugar-catalyst systems [76].

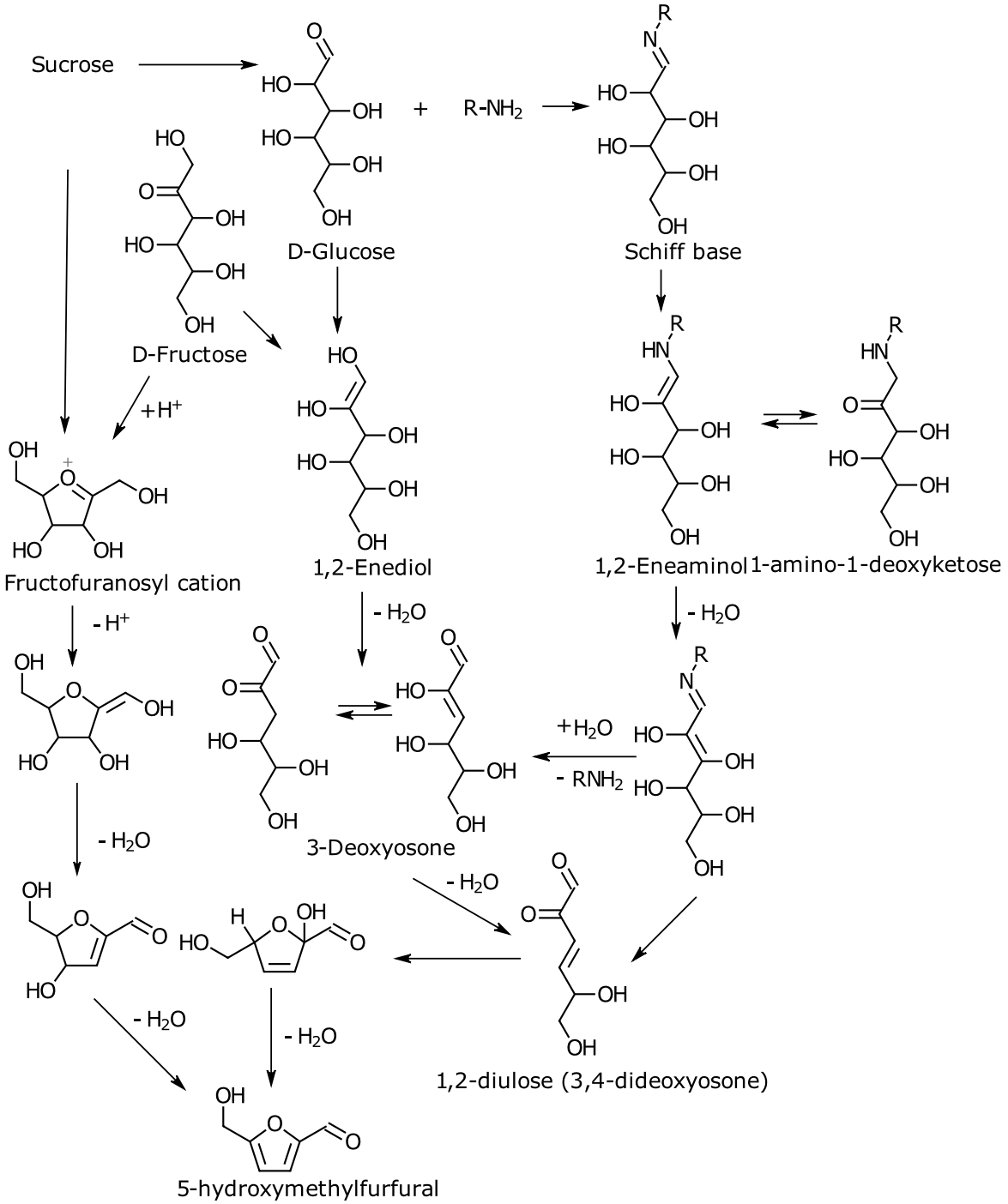


Figure 1.6 Reaction scheme for the formation of 5-hydroxymethylfurfural [74, 75].

1.6.3 Toxicity

HMF was reported as cytotoxic at very high concentrations, causes irritation to the eyes, upper respiratory tract, skin, and mucus membranes [72]. According to *in vitro* data, HMF does not pose a serious risk to human health, but there are concerns in the potential

genotoxic properties of its specific metabolites [79]. HMF is converted *in vitro* and *in vivo* by sulfotransferase into 5-sulfoxymethylfurfural (SMF), a compound reported to be mutagenic in a conventional Ames test and to initiate tumors in mice skin [80, 81]. This conversion has raised concern and EFSA concluded in its opinion on furan derivatives that there is a sufficient evidence to raise concern about genotoxic potential of SMF *in vitro*, but added that the lack of *in vivo* data in humans does not allow a final evaluation [82]. It was concluded that there are contradictory findings and limited evidence on the possible carcinogenicity of 5-HMF and the maximum dose observed with no adverse effects (NOAEL) regarding acute and subacute toxicity in animal experiments is in the range of 80–100 mg kg body weight^{-1} day^{-1} [83].

1.6.4 Occurrence in Foods

HMF is naturally present in honey, which is produced by action of the normal honey acidity on reducing sugars and sucrose usually at room temperature [72]. HMF has also been detected in a wide variety of heated foods, given in Table 1.4.

Table 1.4. HMF content of selected food products [74].

Food product	HMF content, mg kg^{-1}
Coffee	100-1900
Malt	100-6300
Cookies	0.5-74.5
Bread (white)	3.4-68.8
Breakfast cereals	6.9-240.5
Baby food (cereal-based)	0-57.2

HMF could also be formed during manufacturing of caramel colors depending on the production process. EFSA recommended that the specifications defined for caramel colors in EU legislation should be updated to include also maximum levels for HMF [84].

HMF is considered as an indicator of heat damage during thermal process [78, 85, 86]. Upper limits were set to monitor heat damage in

certain foods, namely 40 mg kg^{-1}, 20mg L^{-1} and 25 mg kg^{-1} for HMF in honey, fruit juices and concentrates, respectively [87, 88].

1.6.5 Mitigation

As both sugar caramelization and the Maillard reaction are involved in the formation of HMF, factors affecting both reactions should be considered while developing mitigation strategies. For example, limiting the content of reducing sugars by using sugar alcohol (i.e. maltitol) instead of fructose or glucose will reduce the potential formation of HMF [72]. Water content also affects HMF formation. It is more favored in low water content of cereal-based products than in liquid products such as milk [72]. Presence of some cations, like Ca^{2+}, Mg^{2+}, promotes the dehydration of glucose leading to HMF and furfural [89]. Ammonium bicarbonate increases HMF formation in cookies [77]. So, elimination of these ingredients from the formulations would decrease the formation of HMF in heated foods. The effect of pH of the dough on HMF formation in cookies has been reported [59]. Generally, increasing the pH of the dough resulted in a decreased level of HMF in bakery products. Recently, it was reported that HMF was decreased by yeast fermentation and converted to hydroxymethyl furfuryl alcohol in roasted malt, suggesting that yeast fermentation can be considered as a useful strategy for the mitigation of HMF in fermented products [90].

1.7 Chloropropanols (3-MCPD, 2-MCPD) and MCPD Esters

Chloropropanols and their fatty acid esters (chloroesters) are contaminants that are formed during the processing and manufacture of certain foods and ingredients [91]. 3-Monochloropropane-1,2-diol (3-MCPD), 1,3-dichloro-2-propanol (1,3-DCP), and their isomers 2-MCPD and 2,3-DCP are the known components of group chloropropanols. 3-MCPD and 2-MCPD found in hydrolyzed vegetable proteins (HVP) manufactured by hydrochloric acid hydrolysis [92].

1.7.1 Physical and Chemical Properties

3-MCPD is a glycerol chlorohydrin so named when one hydroxyl group of the parent molecule glycerol is replaced with a chlorine atom [93]. Physical and chemical properties of 3-MCPD are given in Table 1.5.

Table 1.5. Physical and chemical properties of 3-MCPD [94]

Molecular Formula	$C_3H_7ClO_2$
Molar Mass	110.539 g mol^{-1}
Appearance	viscous, colorless liquid
Density	1.322 g mL^{-1} (20°C)
Melting Point	-40°C
Boiling Point	213°C
Solubility	Water and ethanol

Chemical structures of MCPD isomers (positional isomer 2-MCPD and optical isomers) were shown in Figure 1.7. Optical isomers (enantiomers) of 3-MCPD are formed when -OH is replaced by -Cl at the *sn*-1 or *sn*-3 positions on the glycerol backbone.

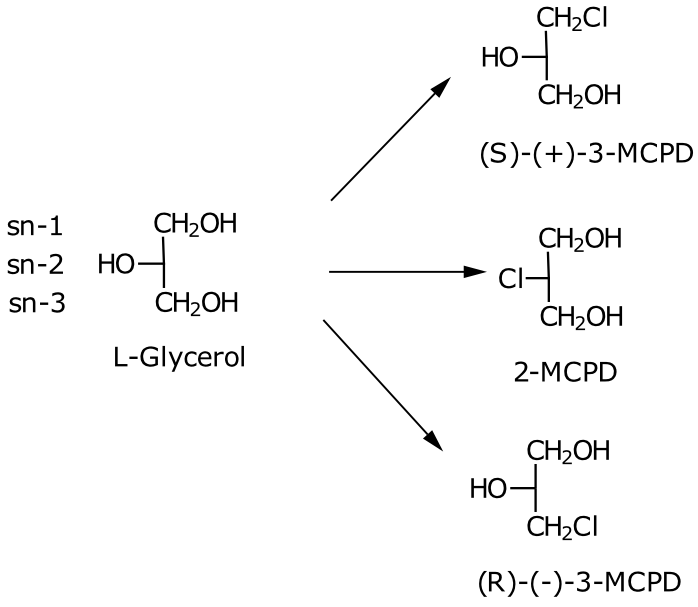


Figure 1.7 Chemical structure of glycerol and monochloropropanediol isomers

Other food-borne contaminant is ester forms of 3-MCPD formed during high-temperature processing of fat-containing matrices. They were

considered as food-borne contaminant as it was reported that 3-MCPD esters are readily hydrolysed *in vivo*, to release the free form [95, 96]. MCPD esters are likely to have similar physical and chemical properties to the naturally occurring acyl-glycerols with which they are probably associated in foods [97]. Molecular structures of some MCPD esters are shown in Figure 1.8.

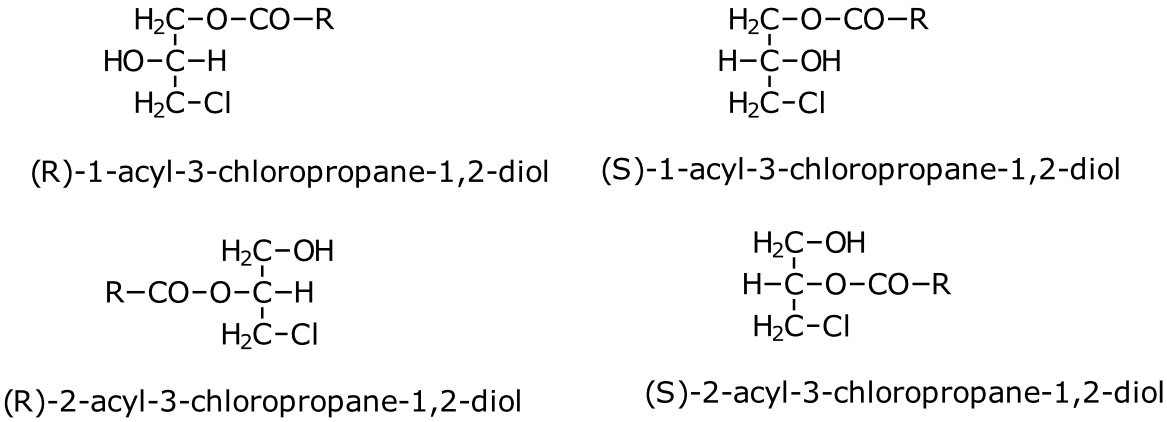


Figure 1.8 Chemical structures of some MCPD esters (R group in the structure indicates fatty acid)

1.7.2 Formation Mechanism

After discovery of 3-MCPD in acid-HVPs and soy sauces, it was reported that its precursors were hydrochloric acid and residual lipids (acylglycerols or glycerol) from the raw materials used [98, 99]. Formation mechanism [100] and degradation in model systems have also been reported [101]. There are different 3-MCPD formation paths depending on the reactants. It could be formed from hydrochloric acid and glycerol/acylglycerols or hypochlorous acid and allyl alcohol but the most probable formation in foods is from sodium chloride (chlorine source) and glycerol/acylglycerol [91]. Model experiments were carried out by Dolezal et al. [102] and Calta et al. [103] with sodium chloride and glycerol in order to simulate the formation of 3-MCPD. Dolezal et al. reported that 3-MCPD formation depends on temperature and reaches the maximum value when the model system was heated at 230^{o}C for

20h. This indicated that levels of 3-MCPD in foods could be lower but increased thermal process still have increased effect on 3-MCPD formation. Process parameters, i.e. time and temperature, play critical role in 3-MCPD formation. It was reported that toasting led to form 3-MCPD in bread depending on toasting time [104]. On the other hand, increased temperature increased the final concentration of 3-MCPD in model systems above 160°C [103, 105, 106]. Calta et al. stated that the formation of 3-MCPD strongly depended on the concentration of NaCl and reached to a maximum level at 4–7% NaCl [103]. Hamlet et al. investigated generation of MCPDs in model leavened dough system and proposed a mechanism of formation of MCPDs from glycerol via the intermediate epoxide, glycidol (I) shown in Figure 1.9 [107]. He also reported that free glycerol is a key precursor of MCPDs in leavened dough and formation of MCPDs increased with decreasing dough moisture to a point where the formation reaction was limited by chloride solubility.

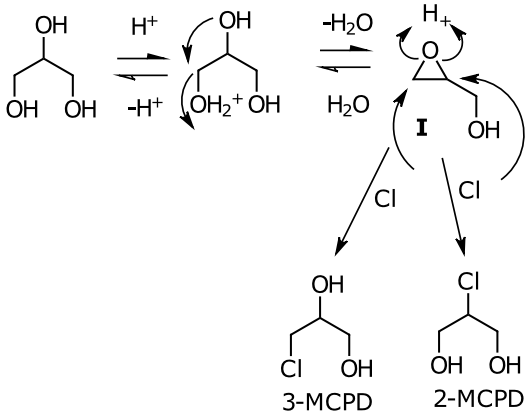


Figure 1.9. MCPD formation from glycerol proposed by Hamlet et al.[107]

MCPD esters are the esterified form of the parent chloropropanediols such as 3-MCPD and they are mainly formed during high-temperature processing of fat-containing foods [97]. Not only chloride ions and

glycerol, but also presence of tri-, di- or monoacylglycerides affect formation of 3-MCPD esters together with temperature and time [108]. Figure 1.10 shows possible formations of MCPD esters from acylglycerols.

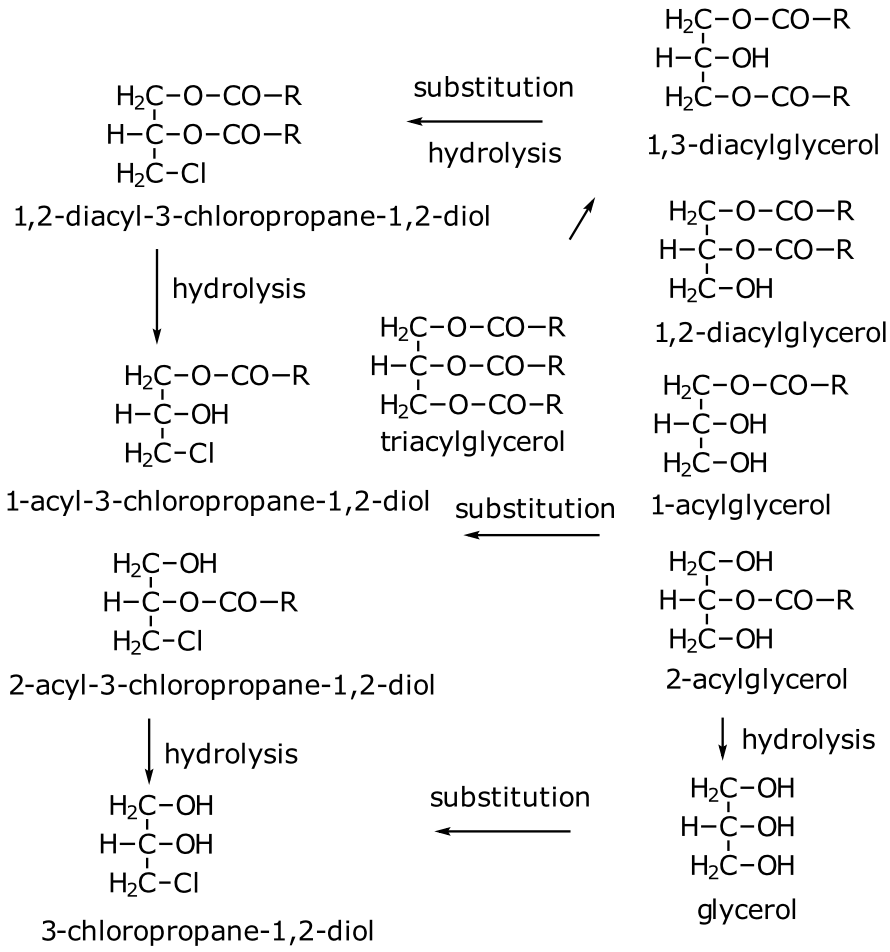


Figure 1.10 Formation of 3-MCPD and its esters from acylglycerols [109] (R indicates amino acid).

1.7.3 Toxicity

Toxicological studies have shown that 3-MCPD is carcinogenic in rat [110] and has genotoxic activity *in vitro*. However, the consensus of the expert committees reported that the genotoxic activity seen *in vitro* was not expressed *in vivo* [111-113]. Enantiomers of 3-MCPD have shown to exhibit different biological activity. (*R*)-isomer of 3-MCPD induced a period of diuresis and glucosuria [114], whereas the (*S*)-isomer possesses the antifertility activity in rats [115].

Toxicological concern of 3-MCPD esters is related with the possible release of 3-MCPD from the parent esters by lipase-catalyzed hydrolysis in the gastrointestinal tract. *In vitro* studies showed that MCPD esters are accepted as substrates by gut lipases and thus potentially could be hydrolyzed in the mammalian gut [116]. Robert et al. reported formation of 3-MCPD from vegetable oils and fats by lipase hydrolysis obtained from different sources, namely from mammalian, vegetable and fungal [117]. A recent study performed *in vivo* investigation and reported that oral bioavailability of 3-MCPD from 3-MCPD fatty acid esters in rats [95]. Results showed that 3-MCPD was released by enzymatic hydrolysis from the 3-MCPD diester in the gastrointestinal tract and distributed to blood, organs and tissues. Due to its toxicity, a tolerable daily intake of 2 µg kg body weight^{-1} on the basis of the lowest observed effect level (LOEL) and a safety factor of 500 has been set by European Commission [118]. Commission regulation has also set a maximum limit of 20 µg kg^{-1} for foodstuffs, in acid-HVP and soy sauce having 40% dry matter [113].

1.7.4 Occurrence in Foods

Researchers conducted surveys in local markets and found that wide range of foods contain 3-MCPD other than acid-HVP and soy sauce. It has also been shown to be present in foods that have not been subjected to treatment with hydrochloric acid [119]. These foods include noodles, meat, cakes as well as cereal products, such as breads and biscuits, which are common foods and eaten in large quantities (Table 1.6) [120].

Table 1.6. 3-MCPD (mg kg^{-1}) levels in retail food products from different groups: UK survey, adapted from Crews et al. [120].

Food type	**3-MCPD mg kg^{-1}**
Breadcrumbs	0.03
Meat and meat products	<0.010-0.081
Cheese	0.043
Savoury crackers	0.010-0.134
Biscuits (different types)	<0.010 - 0.032
Breads (different types)	<0.010 - 0.049
Toasted breads	<0.010 - 0.088
Breakfast cereals	<0.010

The mechanism in these foods expressed as releasing free glycerol by the high-temperature hydrolysis of triglycerides, which can react with the naturally present or added sodium chloride during manufacturing and thermal process, such as baking [111, 121-123]. 3-MCPD was found in the crust part of breads at high levels (up to 0.40 mg kg^{-1}), whereas no contaminant was detected in the breadcrumbs [124, 125]. In another research, 3-MCPD was determined in leavened dough consistently greater than unleavened dough due to the formation of glycerol during the fermentation [106, 107].

Vegetable oils contain high levels of chloroesters probably due to the high-temperature applied during deodorisation step of refining. There are no 3-MCPD esters present in virgin seed and olive oil, while esters in refined seed and olive oil exceed levels of 3-MCPD by hundreds or even thousands of times [126].

1.7.5 Mitigation

After the discovery of 3-MCPD in HVP, manufacturers implemented the necessary procedures to minimize its formation. For example, careful control of the acid hydrolysis step and subsequent neutralization or alternatively decomposition of 3-MCPD by a subsequent alkali treatment stage are known approaches, as both 2- and 3-MCPD are decomposed to glycerol in alkaline media [127].

1.8 Furan

1.8.1 Physical and Chemical Properties

Furan is a heterocyclic organic compound, consisting of a five-membered aromatic ring with four carbon atoms and one oxygen (Figure 1.11). It is an intermediate in the production process of tetrahydrofuran, pyrrole, and thiophene, in the manufacturing of lacquers and resins [128], and for the production of pharmaceuticals, agricultural chemicals (insecticides), and stabilizers [129]. Furan is also formed in a number of heated foods through thermal degradation of natural food constituents. Its physical and chemical properties are given in Table 1.7.

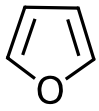


Figure 1.11 Chemical structure of furan

Table 1.7. Physical and chemical properties of furan.

Molecular Formula	C_4H_4O
Molar Mass	68.07 g mol^{-1}
Appearance	Colorless, volatile liquid
Density	0.936 g mL^{-1}
Melting Point	−85.6 °C
Boiling Point	31.3 °C
Solubility	Highly in alcohol, ether, acetone; slightly in water

1.8.2 Formation Mechanism

Maga reported that the primary source of furans in food is thermal degradation of carbohydrates such as glucose, lactose, and fructose [130]. Moreover, US FDA report indicated that variety of carbohydrate/amino acid mixtures or protein model systems (e.g., alanine, cysteine, casein) and vitamins (ascorbic acid, dehydroascorbic acid, thiamin) have been used to generate furans in food [131]. Furan could also be formed through oxidation of polyunsaturated fatty acids

(PUFA) and carotenoids at elevated temperatures [132]. Detailed formation mechanism of furan from ascorbic acid will be discussed in **Chapter 6**.

Potential routes of furan formation from different components present in food were summarized in Figure 1.12. Furan could be formed by cyclization of 4-Hydroxy-2-butenal, which is one of the lipid peroxidation products, formed due to oxidative degradation of PUFAs [132]. Furan could also be formed from thermal degradation of amino acids resulting in the formation of two key aldehyde intermediates, acetaldehyde, glycolaldehyde. They could undergo aldol addition forming 2-deoxyaldotetrose, which further react to form furan [132]. Furan could also be formed from sugars. Thermal degradation of sugars leads to form 1-deoxyosone, 3-deoxyosone, which further react to form 2-deoxyaldotetrose, and 2-deoxy-3-ketoaldotetrose, involving furan formation.

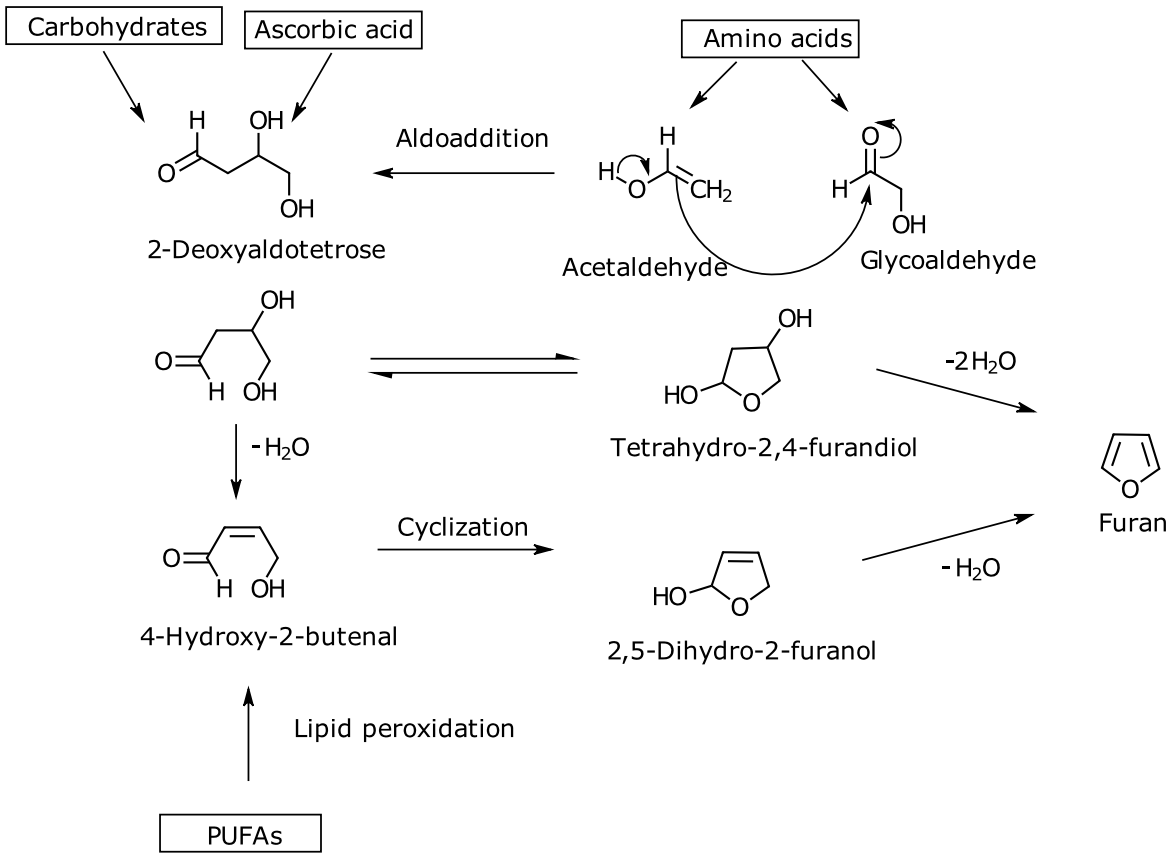


Figure 1.12 Summary of possible formation routes for furan formation [129, 132].

1.8.3 Toxicity

Furan has received considerable attention as it is an animal carcinogen and classified as 'possibly carcinogen to humans' (Group 2B) by the International Agency for Research on Cancer (IARC) [128]. Due to concern on the exposure of furan, the European Food Safety Authority (EFSA) published a risk assessment of the toxicity of furan [133]. Furan is reported to be rapidly absorbed from the gastrointestinal tract, extensively metabolized, and eliminated via expired air, urine, and feces in rats [134]. NOAELs based on a 2-year bioassay have been identified for cytotoxicity and hepatocarcinogenicity of 0.5 and 2 mg kg body weight^{-1}, respectively [135]. It was reported that the margin of exposure for furan indicated a human health concern for a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite [136]. Based on the presently available data, it appears that both

genotoxicity and chronic cytotoxicity may contribute to furan-induced tumor formation [129].

1.8.4 Occurrence in Foods

Due to its low boiling point, furan, formed during thermal processing, easily vaporizes. However, this gives rise to concern in canned or jarred foods as furan accumulates in the headspace. Monitoring furan levels in foods has outlined its occurrence in a broad range of products (roasted coffee, bakery products, baby foods, etc.) from none detectable levels to 7000 μg kg^{-1} [137-146] (Table 1.8).

Table 1.8. Furan content of certain food groups adapted from EFSA report [140].

Food product	n	Mean (μg kg^{-1})	90 percentile (μg kg^{-1})
Coffee, instant	109	394	1457
Coffee, roasted bean	30	3660	6015
Baby food	1617	31-32	67
Infant formula	11	0.2-3.2	0-2.5
Cereal product	190	15-18	49
Meat product	174	13-17	46
Soy sauce	94	27	51

1.8.5 Mitigation

There is very limited information on the mitigation strategies of furan in foods. But due to its carcinogenicity, ALARA "as low as reasonably achievable" concept should be applied to furan levels in food. As furan is a consequence of thermal process, one might think to decrease thermal load applied to food. But this could be not practical especially for the sealed containers undergo to pasteurization and sterilization for microbiological safety. Other mitigation strategy could be reducing the content of precursor. Due to its volatility, EFSA concluded in the report on furan that furan levels can be reduced in some foods through

volatilisation (e.g. by heating and stirring canned/jarred foods in an open saucepan when consumed) [133]. However, this technique would technically difficult to purge coffee of furan whilst retaining all the flavor and aroma substances that the consumer demands [147].

2 COMPUTER VISION BASED ANALYSIS OF FOODS - A NON-DESTRUCTIVE COLOR MEASUREMENT TOOL TO MONITOR QUALITY AND SAFETY

2.1 Summary

Color is an important feature of food products takes critical part in buying decision as it communicates to the consumers. It is indicator of food quality/defects and grade decisive in process. Development of surface browning and formation of contaminants, such as acrylamide and HMF are usual consequences of thermal process, particularly Maillard reaction. There are attempts to mitigate formation of acrylamide in some product groups, especially potato chips, French fries and biscuits. It was stated in the Acrylamide Toolbox of FoodDrinkEurope that color was considered as indicator for acrylamide content of French fries and continuous measurement of color could (if properly calibrated) be a reliable predictor of finished product acrylamide-levels [53]. Therefore, lighter color was recommended in these products. With this regard, a real-time color measurement tool was developed, and it was applied as an alternative tool to monitor and limit acrylamide formation in biscuits. This will allow to decide a biscuit to accept or reject in terms of chemical safety point of view.

2.2 Introduction

2.2.1 Color and Computer Vision

Food quality inspection is a key issue for the food industry. Trained inspectors usually perform this inspection visually, which is subjective, unreliable, tedious, laborious, and costly [148]. For satisfactory and steady results, automated systems should be implemented to the quality inspection process together with mechanical and instrumental devices. Computer vision technology has been used for many years in food industry, which ranks among the top ten industries using image processing techniques [149]. Computer vision based image analysis has many advantages. It offers rapid, accurate, non-contact, and non-destructive analysis of foods. Additionally, it provides a high level of

flexibility and repeatability at relatively low cost and high throughput. Besides, it can be implemented online as an integral part of processing plants for real time monitoring of the quality and it provides precise inspection and increase throughput in the production and packaging process [150]. It can also be used offline to measure certain quality features of final product.

A digital image can be considered as a discrete representation of data possessing both spatial (layout) and intensity (color) information [151]. It is viewed as a 2D matrix whose row and column indices identify a small square area of the image called a pixel [152]. A color space is a 3D model and can be represented typically as three numbers, i.e. RGB. In digital images, each pixel $x[n,m]$ has red, green and blue color values;

$$x[n,m]=\begin{bmatrix} x_r(n,m) \\ x_g(n,m) \\ x_b(n,m) \end{bmatrix} \quad \text{Eq.1}$$

where $x_r(n,m)$, $x_g(n,m)$, and $x_b(n,m)$ are values of the red (R), green (G), and blue (B) components of the $(n,m)_{th}$ pixel of $x[n,m]$, respectively. In digital images, x_r, x_g, and x_b color components are represented in 8 bits, i.e., they are allowed to take integer values between 0 and 255 ($=2^8-1$) [153].

Digital image is taken from an image acquisition system consisting of a color digital camera as illustrated in Figure 2.1. The angle between the axes of the lens and the sources of illumination is adjusted to approximately 45^o. Illumination is achieved with daylight fluorescent lamps with color temperature of 6500 K. For a reliable and reproducible analysis, it is important to create fixed conditions during image acquisition.

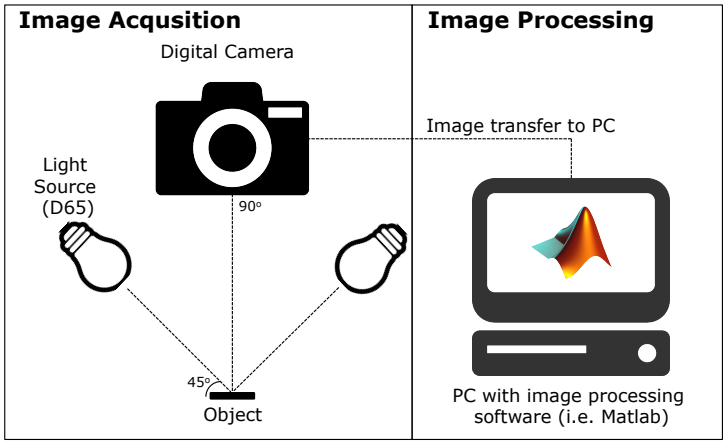


Figure 2.1 Essential components of a computer vision based image analysis system

2.2.2 Color Spaces and Color Measuring Devices

There are different color models other than RGB such as XYZ, HSV, and CIE L*a*b* that are convertible to each other. Since it is more perceptible by human, color image data is usually transformed to CIE L*a*b* in most computer vision based image analysis applications [154]. The CIE L*a*b* color space has been implemented by the Commission Internationale d'Eclairage (CIE) in 1976 as an international standard for color measurements. In CIE L*a*b* space, L* represents luminance or lightness that ranges from 0 to 100. Chromatic components, a* and b*, range from -120 to 120 and represent colors from green to red, and from blue to yellow, respectively [155-157]. Euclidean distance (ΔE), given in Eq. 2, between two different colors in the CIE L*a*b* space corresponds approximately to the difference perceived by the human eye [158].

$$\Delta E = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2} \quad (2)$$

Instruments detecting the color generally fall into one of the four categories: colorimeters, densitometers, spectral cameras, and spectrophotometers [158]. Many colorimeters measure color using three filters that match human color receptors, but with only one light

source [159]. The CIE L*a*b* color space is successfully implemented to colorimeters to measure the surface color of foods. However, many color measuring devices measure only a very small area that is not representative for whole food unless color is homogenous over the surface [155, 156].

Computer vision based image analysis could be used to obtain representative and reliable data for the foods having non-homogenous color on surface. Within this respect, there are different approaches for processing digital images to obtain specific information, which is meaningful for quality evaluation of foods. Throughout this chapter, two computer vision based image analysis approaches, namely "mean color information" and "featured color information" (segmentation) will be examined on biscuit example.

2.2.3 Mean Color Information

A processed food like fried potato chip or a baked cookie has non-homogenous brown surface that limits accuracy of color measurement. Baking and frying processes at elevated temperatures (150-250^{o}C) induces Maillard reaction and caramelization. These reactions highly depend on water activity; low water activity causes reactants to concentrate and so, promotes these reactions. Surface browning begins when sufficient amount of drying has occurred in cookies. It simply develops as a circle on the edge regions and grows to the center as the baking proceeds; finally color gradient is formed on the surface. Single measurement taken from a small area does not provide accurate mean color information for such foods. Increasing the number of measurements from different regions of the surface may increase accuracy, but to a certain extent. With this respect, computer vision based image analysis offers great advantage as it allows measuring color of food on a region of interest (ROI), which could be either entire surface or a specific region on the surface. As shown in Figure 2.2, mean CIE L*a*b* values of potato chip and cookie greatly differ when

ROI is defined as entire surface instead a small rectangular area on the surface.

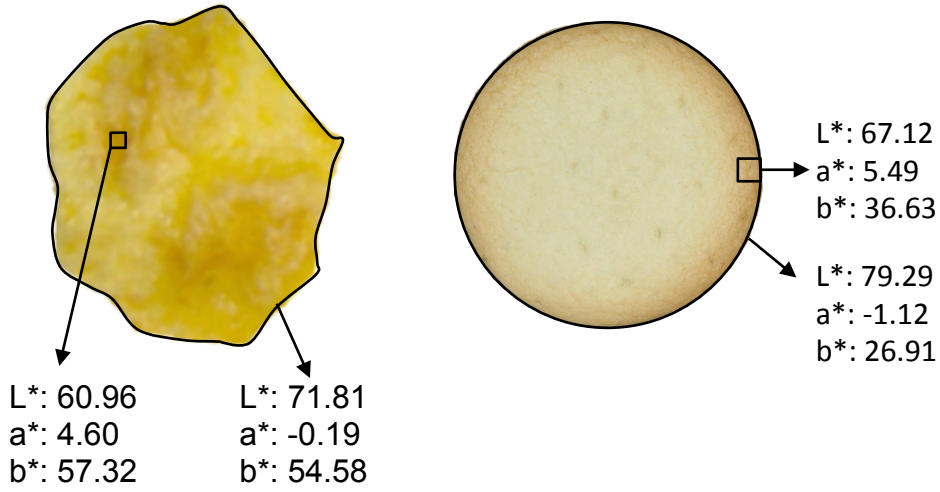


Figure 2.2 Measurement of mean color of potato chip and cookie on different ROIs by means of computer vision based image analysis.

Flexibility on defining the ROI on a digital image provides more accurate mean color information for non-homogenous foods, moreover meaningful color information for specific regions. Which is important while using this approach is the selection of relevant area to fit for purpose.

These kinds of information may be of particular importance as color indicates certain chemical changes or physical properties in foods. Mean color, such as CIE a* value is considered informative, and gives fairly well correlations with acrylamide concentration of thermally processed foods. For example, it was reported that mean CIE a∗ value of potato chips determined by computer vision based image analysis showed a good linear correlation (R^2>0.88) with acrylamide concentration [160, 161]. Another study revealed that mean CIE a* value of cookies was only roughly correlated (R^2=0.67) with acrylamide concentration [162]. 5-HMF, formed during browning reactions and considered as an indicator of heat damage during thermal process, could also be related with color of the product.

2.2.4 Featured Color Information

Owing to their non-homogenous surface color, processed foods can be better dealt with pattern recognition techniques. For example, browning ratio can be defined as a new feature for these foods. Such featured color information can be extracted from digital images by means of segmentation algorithms. Image segmentation is the process of partitioning a digital image into multiple sets of pixels based on predefined reference color values as schematically shown in Figure 2.3.

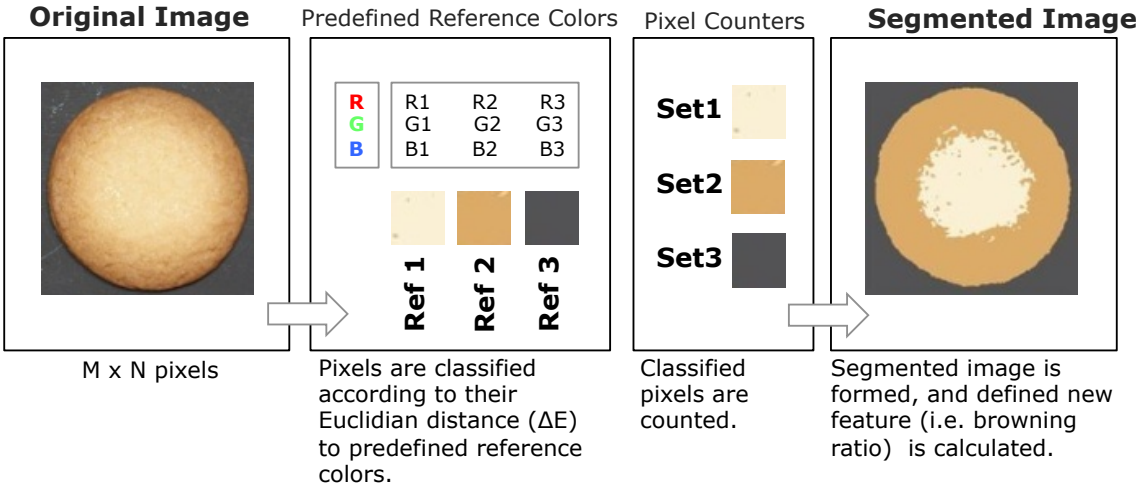


Figure 2.3 Schematic illustration of the principle of image segmentation used to calculate featured color information (i.e. browning ratio)

Using a custom-designed MATLAB® code, pixels of an image are classified into sub sets based on their Euclidean distance (ΔE) (Eq.2) to predefined reference color values. Definition of these reference values is specific to the food product, and should fit for the purpose. One additional reference must be defined for background. Sub set of background pixels are not taken into consideration for the calculation of featured color information. A defined feature can be calculated as the normalized area of a sub set pixels (Set 1, Set 2) after counting the number of pixels for all sub sets. For instance, browning ratio is simply the normalized area of Set 2 pixels for cookie sample shown in Figure 2.3.

The number of reference colors and their values can be modified in line according to need, which gives the user opportunity to segment the image from one to many color regions. So, computer vision based image analysis gives to user flexibility to calculate the percentage of any color region selected. It is also possible to calculate browning ratio of cookie and potato chip using the same algorithm. Models based on featured color information have an advantage over the mean color models in that information from every class is taken into account rather than using only a global description of the image.

Mean or featured color information extracted from an image by means of computer vision based image analysis is relevant not only for visual quality of food products, but also indicative for certain chemical and physical characteristics. It can be used as a tool to predict the levels of neo-formed compounds, namely acrylamide and HMF in thermally processed foods. In this regard, applications of these techniques on biscuits will be discussed throughout this chapter.

2.3 Experimental

2.3.1 Chemicals and Consumables

Raw material and ingredients for biscuit were kindly supplied by Kraft (Germany) and Eti (Turkey). HMF (98%) was purchased from Acros (Geel, Belgium). Formic acid (98%), acetonitrile and methanol (HPLC grade) were purchased from J.T.Baker (Deventer, Holland). Potassium hexacyanoferrate (II) trihydrate and zinc sulphate heptahydrate were purchased from Merck (Darmstadt, Germany). Carrez I and Carred II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate in 100 mL of water, and 30 g of zinc sulfate in 100 mL of water, respectively. Ultra-pure water was prepared by the system of TKA GenPure (Niederelbert, Germany). Nylon membrane syringe filters (0.45 µm) and glass vials with septum screw caps were supplied by Agilent (Waldbronn, Germany). Oasis MCX solid-phase extraction cartridges (1 mL, 30 mg), Atlantis dC18 column (4.6 mm 4.6 mm 5 µm)

and Acquity UPLC HSS T3 C18 column (100 x 2.1 mm i.d., 1.8 µm) were supplied by Waters (Milford, MA, USA).

2.3.2 Preparation of Biscuits

The biscuits were prepared according to the American Association of Cereal Chemists Method 10–54 with some modifications [163]. The recipe contains 80.0 g standard wheat flour, 35.0 g grained sucrose, 20.0 g vegetable shortening, 1.0 g NaCl, 0.4 g NH_4HCO_3, 0.8 g of $NaHCO_3$ and 17.6 ml water. All ingredients were thoroughly mixed in accordance with the AACC Method 10–54 procedure using a dough mixer Artisan Kitchen Aid 5KSM150 (MI, USA). Dough was rolled in 3 mm thickness and cut in three discs having 5 cm diameter and baked in a conventional oven (Memmert, UNE 400, Germany). Biscuits, then, baked at 180^{o}C for 11, 13, 15, 17, 19 min, at 190^{o}C for 10, 12, 14, 16, 18 min, at 200^{o}C for 10, 12, 14, 16, 18 min, at 210^{o}C for 8, 9, 10, 11, 12 min, and at 220^{o}C for 6, 7, 8, 9, 10 min.

To test the calibration against recipe variations response surface methodology (RSM) was used. A 5-factor-3-level Central Composite Design (CCD) with six replicates at the center point was used to develop models for evaluating the effect of variables, namely non fat milk powder (NFMP) (0-0.8 g), salt (0-1.0 g), high fructose corn syrup (HFCS) (0.2-1.0 g), ammonium bicarbonate (NH_4HCO_3) (0-0.4 g) and flour (35-45 g), on color of biscuits. Experimental design was given in Table 2.1. Other ingredients, sucrose (16.8 g), shortening (16 g), and sodium bicarbonate (0.4 g), were fixed in the recipe. Water was added in variable amount in order to obtain same moisture content of 16.7% in dough. Mixing was performed as described in AACC method and biscuits were baked at 200^{o}C for 12 min.

Table 2.1 Experimental design to investigate the effect of recipe variations on color (NFMP: non-fat milk powder, HFCS: high-fructose corn syrup).

	Amount (g)					
#	NFMP	Salt	HFCS	NH_4HCO_3	Flour	Biscuit
1	0	0	0.2	0	45	
2	0	0	0.2	0.4	35	
3	0	0	1	0	35	
4	0	0	1	0.4	45	
5	0	1	0.2	0	35	
6	0	1	0.2	0.4	45	
7	0	1	1	0	45	
8	0	1	1	0.4	35	
9	0.8	0	0.2	0	35	
10	0.8	0	0.2	0.4	45	
11	0.8	0	1	0	45	
12	0.8	0	1	0.4	35	
13	0.8	1	0.2	0	45	
14	0.8	1	0.2	0.4	35	

15	0.8	1	1	0	35	
16	0.8	1	1	0.4	45	
17-22	0.4	0.5	0.6	0.2	40	

For five inputs, the equation of interaction response surface is:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{15} x_1 x_5 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{25} x_2 x_5 + \beta_{34} x_3 x_4 + \beta_{35} x_3 x_5 + \beta_{45} x_4 x_5$$

Outputs in this design are color, acrylamide and HMF content of biscuits.

2.3.3 Analysis of Acrylamide and HMF

Sample extraction. The samples were prepared for acrylamide and HMF analyses by multi-stage extraction strategy according to the procedure described before [164]. 1.0 g of ground biscuit sample was extracted with 20 mL of 10 mM formic acid in three stages (10, 5, and 5 mL). First extraction was carried out with 9 ml 10 mM formic acid and 0.5 ml Carrez I and 0.5 ml Carrez II solution. Each extract was centrifuged at 6080 x g for 10 min and combined for further centrifugation at 11,180 x g for 5 min. For acrylamide analysis, extract was cleaned up by Oasis MCX solid phase extraction cartridge that was previously conditioned by passing 1 ml of methanol and 1 ml of distilled water. After conditioning, 1 ml of the extract was introduced to preconditioned cartridge and the first 8-9 drops were discarded to avoid any dilution, and the rest was collected into an autosampler vial. For HMF analysis 1 ml of extract was filtered through 0.45 µm nylon filter into autosampler vial.

Acrylamide measurement. A Waters Acquity H Class UPLC system (Waters, Milford, MA, USA) coupled to a TQ detector with electrospray ionization operated in a positive mode was used to analyze acrylamide in biscuit extracts. The chromatographic separations were performed on the Acquity UPLC HSS T3 column using 10 mmol L^{-1} formic acid with

0.5% methanol as the mobile phase at a flow rate of 0.3 mL min^{-1}. The column equilibrated at 40°C and the Waters Acquity FTN autosampler was kept at 10°C during the analysis. The electrospray source had the following settings: capillary voltage 0.80 kV; cone voltage 21 V; extractor voltage 4 V; source temperature 120°C; desolvation temperature 450°C; desolvation gas (nitrogen) flow 900 L h^{-1}. The flow rate of the collision gas (argon) was set to 0.25 mL min^{-1}. Acrylamide was identified by multiple reaction monitoring (MRM) of two channels. The precursor ion $[M + H]^+$ 72 was fragmented and product ions 55 (collision energy 9 V) and 44 (collision energy 12 V) were monitored. The dwell time was 0.2 s for all MRM transitions. The concentration of acrylamide was calculated by means of a calibration curve built in a range between 1.0 and 50 ng mL^{-1}. Limit of detection (LOD) and limit of quantitation (LOQ) for acrylamide in biscuits were 3 and 10 ng g^{-1}, respectively.

HMF measurement. The filtered extract was injected onto a Shimadzu UFLC System (Kyoto, Japan) consisting of a quaternary pump, an autosampler, a diode array detector (DAD), 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. The concentration of HMF was calculated by means of a calibration curve built in a range between 1 and 10 µg ml^{-1}. LOD and LOQ for HMF in biscuits were 0.1 and 0.3 µg g^{-1}, respectively.

2.3.4 Color Measurement

As an alternative analytical tool, different computer vision based analysis algorithms were applied to biscuits in order to validate the potential of technique for online monitoring. These algorithms were applied by using MATLAB® software and method described previously [165]. Digital images of biscuits were taken from a digital image

acquisition system consisting of a color digital camera and illumination system with daylight fluorescent lamps. Images were captured, stored in a personal computer in JPEG format without compression. The CIE L*a*b* values from a region of interest, as well as brown and dark brown ratio of biscuits were measured using MATLAB® codes, given in Annex.

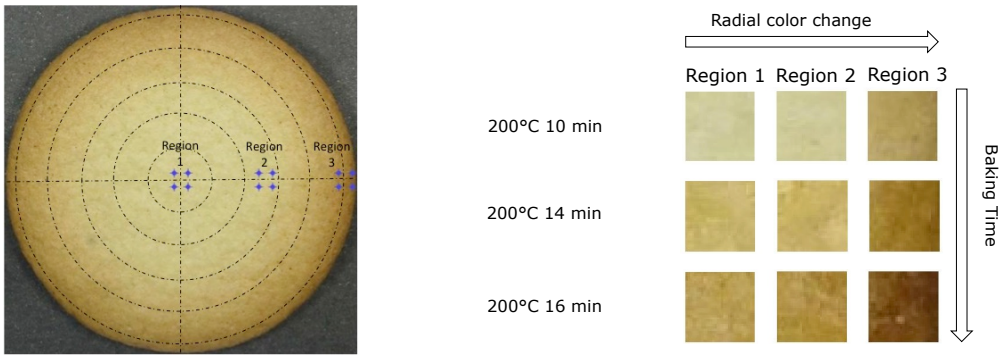
For proof of concept of online color measurement, video was recorded on a conventional oven having window-door while baking biscuit. Camera and daylight illumination were set on the door. Recorded video was used to capture photos at certain baking times and captured images were analyzed at the time. Used MATLAB® code was given in Annex.

2.4 Results and Discussion

The correlation between browning development and thermal process contaminants was investigated. In this part, data obtained from computer vision based image analysis were used to build calibration models to predict levels of acrylamide and/or hydroxymethylfurfural in biscuits.

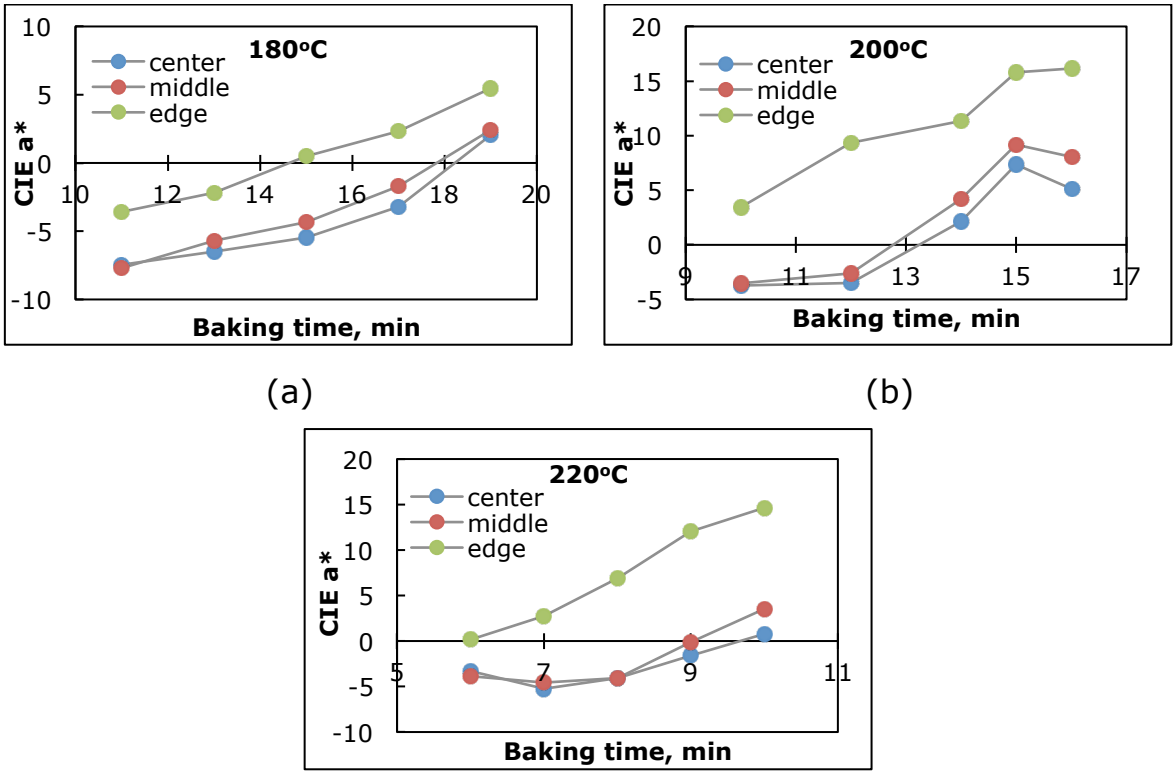
2.4.1 Mean Color Information

Browning develops as a circle during baking, so different color regions occur on biscuit surface. In the present analysis, digital cookie images were divided into three regions, namely center, middle, and edge along the radius as shown in Figure 2.4.



(a) (b)

Figure 2.4 (a) Color regions defined along the radius of circular biscuit image for mean color information and (b) color changes on these regions baked for different times.



(a) (b)

(c)

Figure 2.5 CIE a* values of the center, middle and edge region of biscuits

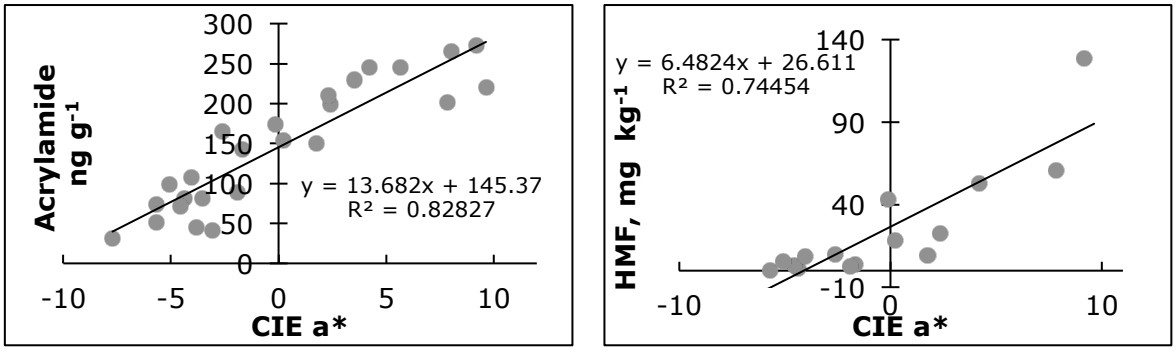
Among the color space coordinates, CIE a* value was better indicated the development of browning on biscuit surface during baking. As shown in Figure 2.5, change of CIE a* value with time was different in center, middle, and edge regions of biscuit. The edge of biscuit discs

became darker rapidly comparing to the middle and center regions expectedly.

Calculated a* values were correlated with acrylamide content of the biscuits. As shown in Figure 2.6, CIE a* values measured on the middle of biscuit surface correlated well with acrylamide concentrations of biscuits. There was a linear correlation between CIE a* value and acrylamide concentration with a high correlation coefficient (R^2=0.828). HMF was also correlated linearly with CIE a* values measured on the middle region of biscuits (R^2=0.745).

Based on this correlation, CIE a* value of 4 indicates an approximate acrylamide concentration of 200 ng g^{-1} in biscuits prepared from the basic recipe. Similarly, CIE a* value of 10 indicates an approximate acrylamide concentration of 280 ng g^{-1} in biscuits.

The preliminary results clearly indicated that mean color information taken from the digital image of biscuits could be used to predict acrylamide concentration in biscuits. This correlation reflects the changes in process parameters including temperature and baking time very well. However, its validity when the recipe of biscuit changed will be discussed later in this chapter.



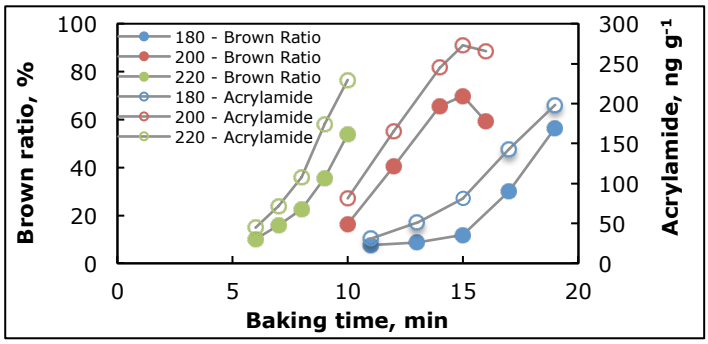
(a) (b)

Figure 2.6 Correlation of CIE a* values measured on the middle region with (a) acrylamide and (b) HMF content of biscuits prepared from the basic recipe at different temperature-time combinations

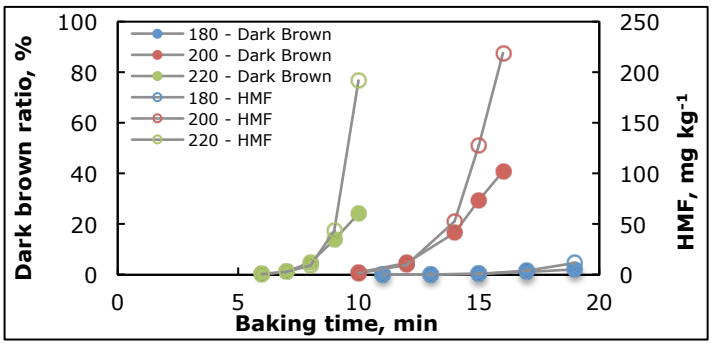
2.4.2 Featured Color Information

Besides mean color information, another algorithm developed for the determination of brown ratio and dark brown ratio was based on the color segmentation of digital biscuit images. In order to process this algorithm to calculate brown and dark brown ratios, reference values representing dough, brown and dark brown colors appeared in biscuits were defined preliminarily. Black color reference value was defined for background to eliminate it from the biscuit being analyzed. Color segmentation of digital images was performed according to predefined color reference values. The computer algorithm calculated brown ratio and dark brown ratio from the segmented images. Preliminary analyses performed on biscuits prepared from the basic recipe at different temperature-time combinations indicated that brown ratio and dark brown ratio were rational features that could be potentially correlated with acrylamide and HMF, respectively. This method requires an appropriately built calibration curve for the prediction of acrylamide level in heated foods such as bakery products.

Previous studies showed high linear correlation between acrylamide level and browning ratio of both potato crisps (R^2>0.97) and cookies (R^2>0.87)[165].



(a)

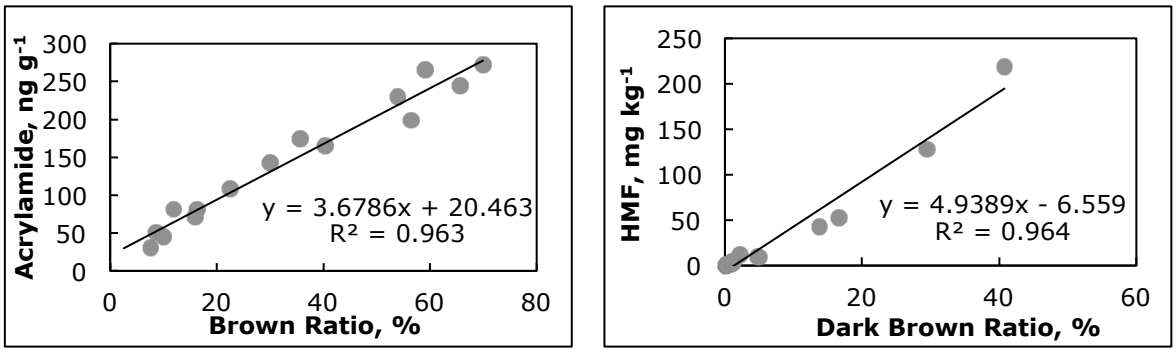


(b)

Figure 2.7 Formation kinetics of (a) brown ratio and acrylamide; (b) dark brown ratio and HMF in biscuits during baking.

As shown in Figure 2.7a-b, developments of brown and dark brown ratios, the new features defined here, had typical kinetic patterns resembling to acrylamide and HMF, respectively. These kinetic pattern similarities allowed to build a correlation between brown ratio and acrylamide concentration, and between dark brown ratio and HMF concentration for biscuits prepared at different temperature-time combinations. Data obtained from the biscuits baked at 180°C, 200°C and 220°C were used to set the calibration. As shown in Figure 2.8a, there was a linear correlation between brown ratio and acrylamide concentration with a high correlation coefficient (R^2=0.963). Based on this correlation, brown ratio value of 20% indicated an approximate acrylamide concentration of 100 ng g^{-1} in biscuits prepared from the basic recipe. Similarly, a correlation between dark brown ratio and HMF

concentration gave a high correlation coefficient (R^2=0.964) for biscuits prepared at different temperature-time combinations (Figure 2.8.b).



(a) (b)

Figure 2.8 Correlation between (a) acrylamide content and brown ratio %, and between (b) HMF and dark brown % of biscuits.

To test the model, these correlations were used to predict acrylamide and HMF content of biscuits baked at 190°C and 210°C from their brown ratio % and dark brown ratio %, respectively. Figure 2.9 shows the prediction capability of the model.

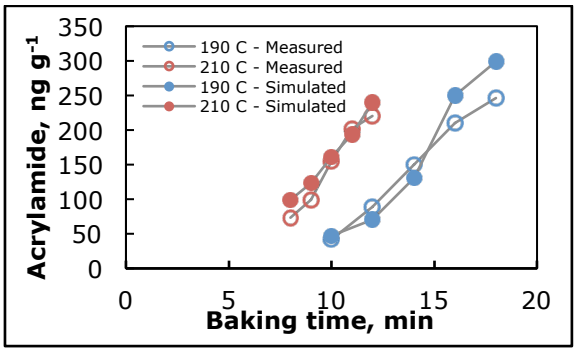


Figure 2.9 Testing the calibration by predicting acrylamide levels of biscuits baked at 190°C and 210°C

These results confirmed the potential of computer vision based image analysis algorithms for predictive monitoring of acrylamide and HMF during baking.

2.4.3 Online Monitoring of Baking Process

As an alternative analytical tool, computer vision based analysis algorithm was developed which could be applied to biscuits in order to validate the potential of technique for online monitoring. The algorithms can be applied for online process control in biscuit manufacturing process line at pilot scale. Doing so, success of the introduced image analysis technology can be tested under real processing conditions.

For this purpose, a calibration should be firstly built which gives the correlation between color information and acrylamide/HMF. Then, a camera installed at the processing line captures images at user-defined time intervals (seconds or minutes), and color is analyzed on these captured images by computer at the time.

In this context, a proof of concept was designed by using a recorded video of biscuit baking as image source. In real time analysis, video can be replaced by video stream, to which same algorithm can be applied. Developed algorithm was given in Annex. From a process control point of view, the potential use of computer vision technology is toward the classification of resulting product based on **pass/fail** manner. With this respect, the image analysis algorithms developed and validated can be adapted for online process control in biscuit manufacturing line. A digital camera placed to the end of tunnel oven can be used for baking biscuit. The biscuits moving on the band can be monitored online. The selected region or regions in viewing angle of the camera would be analyzed by means of developed algorithms. Online analyses can be based on the determination of both mean CIE a* value and brown ratio. For classification, a threshold value for thermal process contaminants should be defined. As example, a threshold level of 100 ng g^{-1} or 200 ng g^{-1} may be applicable for acrylamide in selected biscuit. The measured mean CIE a* value and brown ratio will be used to predict the concentration of process contaminant, in particular acrylamide. If predicted acrylamide level is higher than the threshold level, then the biscuits will be classified as “fail”, or vice-versa as “pass”.

Recorded video was analyzed at every minute by the algorithm and the L* a* b* values were shown in Figure 2.10.

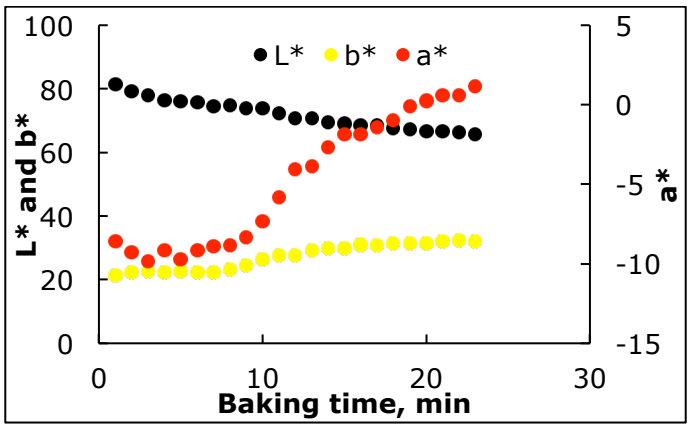


Figure 2.10 L*a*b* values calculated in biscuit streamed from video input

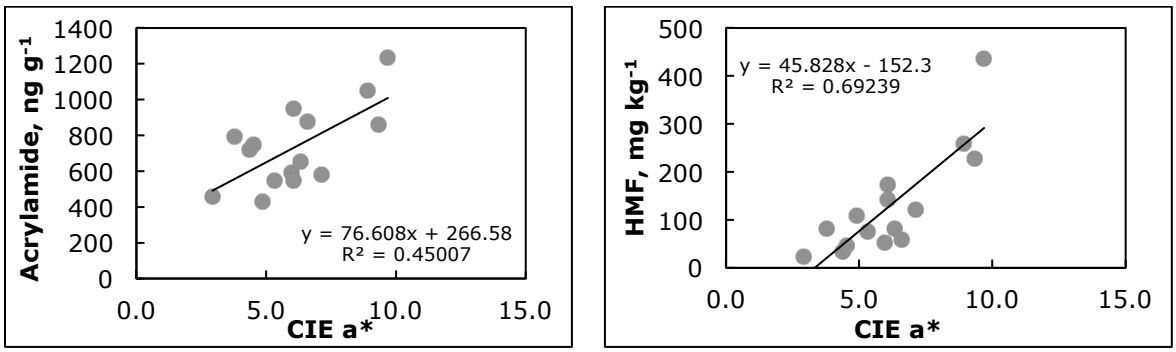
Typical baking behavior can be seen in Figure 2.10. L* value decreases, while a* and b* values increase due to browning reactions taking place during baking.

2.4.4 Effect of Recipe Variations on Color

Correlations obtained from computer vision based image analysis, either for CIE Lab values or brown/dark brown ratio is usually specific to the product. Some parameters, like the recipe and dimension of the biscuit, affect final color of the product. For example, when the concentration of reactants, involving browning reactions, is high in the recipe, browning rate would increase. Biscuit formulation could differ within a range of concentrations of certain ingredients. So, validity of the correlation between color information and acrylamide or HMF should be tested with varied concentration of certain ingredients having effect on color. For this purpose, NFMP, salt, HFCS, NH_4HCO_3 and flour were selected. Appearances of biscuits were given in Table 2.1. It is obvious that these ingredients affect browning reactions and consequently final color of biscuit.

Mean CIE a* value of 9 different regions on the biscuit surface were measured by means of computer vision based image analysis. Then, it

was correlated with acrylamide or HMF content of biscuits. Correlations are shown in Figure 2.11.



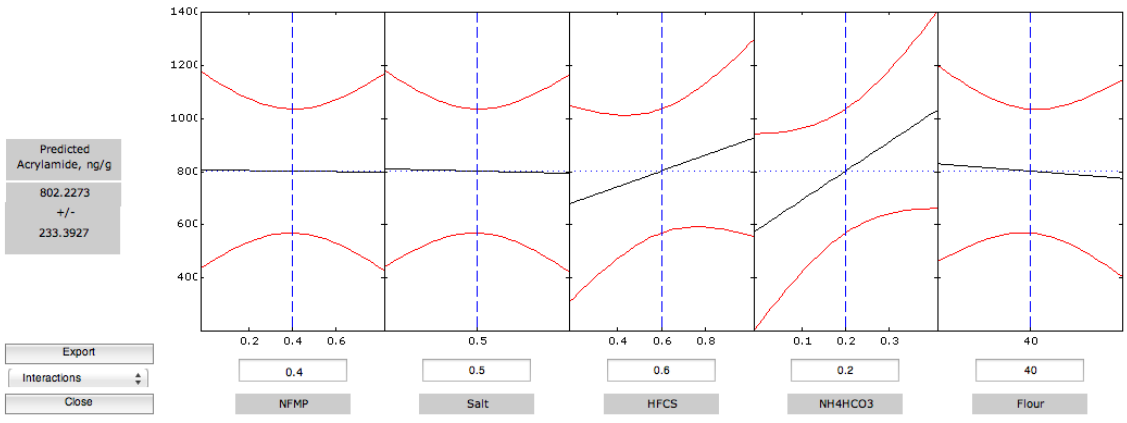
(a) (b)

Figure 2.11 Correlation of CIE a* values of biscuits with (a) acrylamide and (b) HMF.

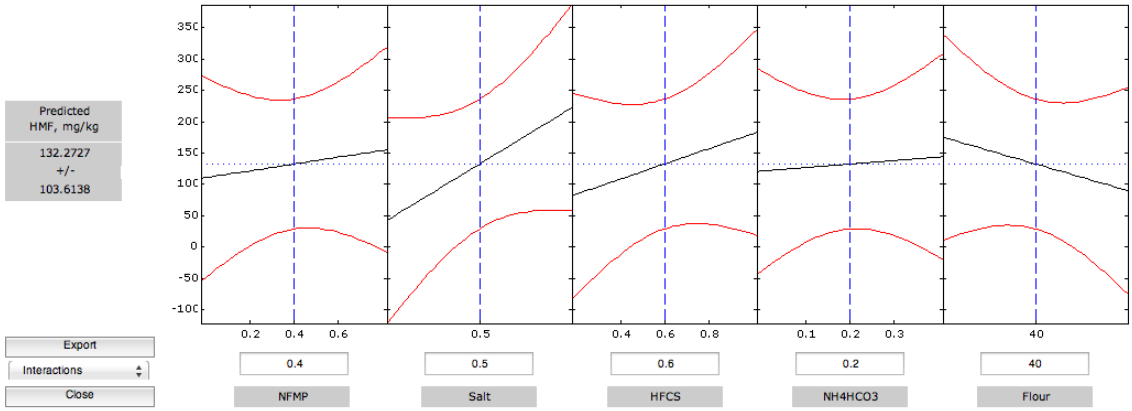
Correlation of CIE a* value of biscuits with acrylamide is not well (R^2<0.50), where it was found to be fairly good for HMF (R^2=0.69). It is noticeable that changing formulations affects the color in biscuits and it is correlated better with HMF than acrylamide. This could be explained that some ingredients, like HFCS, affect browning through both caramelisation and Maillard reaction. Both reactions are responsible for the HMF formation, while acrylamide formed only via Maillard reaction. It has been reported that salt increases sucrose decomposition consequently HMF formation [166]. The estimated regression coefficients, given in Table 2.2, confirmed that HFCS and salt affects HMF formation more than acrylamide. So, factors effecting browning could not be directly related with acrylamide content and its correlation could deviate at these circumstances. To conclude, correlation between CIE a* value and HMF content could be used in biscuits having modifications in formulation within a certain range, while correlation of CIE a* value with acrylamide should be evaluated as specific to product.

Table 2.2. Estimated regression coefficients by response surface analysis performed in MATLAB®.

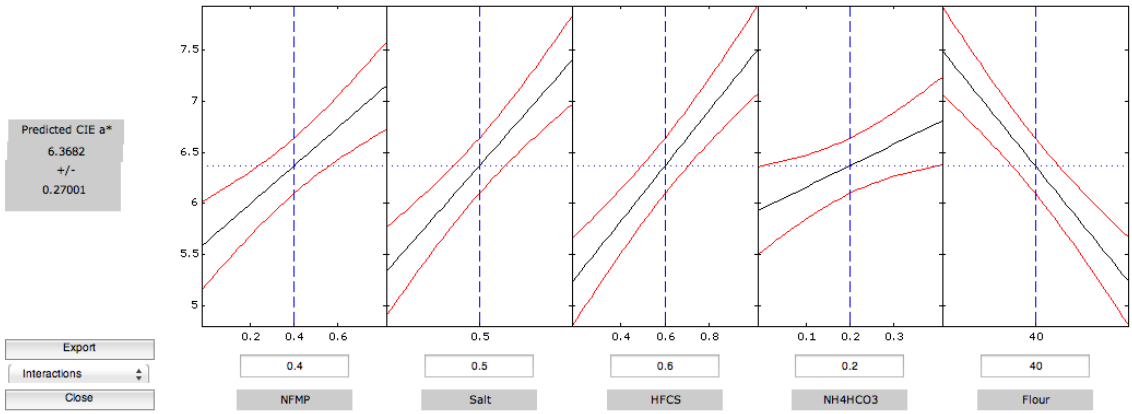
	Color	Acrylamide	HMF
β_0-Constant	10.79	466.41	-127.91
β_1-NFMP	-1.21	315.39	14.84
β_2-Salt	7.34	450.31	570.25
β_3-HFCS	-1.41	8.44	335.94
β_4-NH_4HCO_3	5.39	728.91	141.25
β_5-Flour	-0.19	-0.22	3.34
β_{12}	-0.41	-163.44	30.63
β_{13}	0.12	-55.08	-17.97
β_{14}	4.14	-24.22	-248.44
β_{15}	0.06	-5.16	2.12
β_{23}	-0.09	-159.06	202.50
β_{24}	1.69	135.63	167.50
β_{25}	-0.14	-8.33	-14.15
β_{34}	2.42	1017.97	40.63
β_{35}	0.09	4.66	-7.94
β_{45}	-0.18	-7.56	-2.38



(a)



(b)



(c)

Figure 2.12 Prediction plots of interactions model for (a) acrylamide, (b) HMF and (c) color.

2.4.5 Limitation

There are some limitations in the application of computer vision based image analysis. If image analysis is implemented to a processing line, then image analysis should be synchronized with process speed. This limitation can be easily overlooked with a computer having high computational power and an efficient algorithm structure that fits the purpose. Image resolution also limits analyzing speed. Higher the resolution, lower the analyzing speed. For that reason, image resolution should be optimized before analysis.

Computer vision based image analysis might be specified for a biscuit type. While using for prediction of certain contaminants, like acrylamide, the code needs to be recalibrated for any change in recipe (sugar type and concentration, lipid type and concentration, pH, etc) and/or in process (product dimension, etc) because all these factors affect surface browning. So, any change should be considered separately. In such situations, the code needs to be recalibrated according to requirements. However, correlation of HMF and CIE a* was found to be valid for certain recipe changes.

Another important point in image analysis is that it needs the image taken in standardized environment in terms of illumination, background, etc. For example, if the illumination changes in image acquisition environment frame by frame, than the algorithm would make a false decision. In processing line, a standard illumination utility is strictly needed. Light source should also be well positioned in order to avoid shadowing. Background color is important in terms of edge detection. It is difficult to define the region of interest in dark colored biscuit on a black colored surface because of color similarities.

2.5 Conclusion

As a decisive and informative quality indicator, color measurement using non-destructive computer vision based image analysis offers great advantages as an online process control tool. In fact, computer vision based image analysis has been widely established in the food industry for a rapid inspection of quality defects by means of color differences. However, there is a growing interest in the industry to expand its applications to improve food safety. One potential application of the computer vision based image analysis for this purpose could be online monitoring of thermal processing contaminants in bakery products. Nowadays thermal processing contaminants like acrylamide are one of the major concerns for consumers from a food safety point of view. Food industry has been looking for viable solutions not only to mitigate their formation during processing, but also to monitor by low

cost, rapid and reliable techniques. As exemplified above, color information such as degree of surface browning can be considered as a reliable indicator of acrylamide concentration in potato chips and biscuits. Therefore, a computer vision based image analysis system adapted to processing lines may be used to monitor online quality changes in these products.

3 MITIGATION OF ACRYLAMIDE AND HYDROXYMETHYLFURFURAL IN BISCUITS USING A COMBINED PARTIAL CONVENTIONAL BAKING AND VACUUM POST-BAKING PROCESS: PRELIMINARY STUDY AT THE LAB SCALE

3.1 Summary

The formation of acrylamide and HMF in biscuits depends on thermal load applied during baking. There are many studies indicating the effect of process on the formation of acrylamide and HMF (See **Chapter 1)**. However, the literature is lacking in investigation of the effects of low-pressure (vacuum) at elevated temperatures exceeding 150^{o}C on their formation in bakery products. This study aimed to develop a new baking technology combining conventional and vacuum process to mitigate acrylamide and HMF in biscuits.

Firstly, both of these processes were compared for acrylamide and HMF formations, drying rate, and browning development at different temperatures. Acrylamide concentrations in biscuits attained during vacuum baking were significantly lower than those attained during conventional baking at all temperatures studied ($p<0.05$). Besides, there was no HMF formation in vacuum baked biscuits. Comparing to conventional baking, heating under lower pressure provided lower time-temperature profile with slightly accelerated evaporation of water in dough. However, development of surface browning was lacking in vacuum baked biscuits. Secondly, combinations of conventional and vacuum processes were used to produce biscuits. The dough that was partially baked at 220^{o}C for 2-4 min under conventional conditions was post baked under vacuum for accelerated drying at 180^{o}C and 500 mbar for 4-6 min until the desired final moisture content was attained. Doing so, exposure of biscuits to higher temperatures for longer time, which was essential to facilitate the chemical reactions leading to thermal process contaminants, was prevented. There was no acrylamide or HMF (<LOQ) formation in biscuits baked by combined process.

This combined process was introduced for the first time as a new technology to mitigate certain undesired neo-formed compounds in biscuits. It was considered as a promising alternative to produce safer biscuits for targeted consumers like infants.

3.2 Experimental

3.2.1 Chemicals and Consumables

Chemicals and consumables used were given in Chapter 2. In addition, Springarom® GN 7001 flavor was kindly supplied by Bio Springer (France). Waters Atlantis HILIC silica column (150 × 2.1 mm, 3 µm particle size) was purchased from Waters Corporation (Milford, MA, USA).

3.2.2 Preparation of Biscuits

Biscuits were prepared according to the procedure given in **Chapter 2**. Biscuits were baked using three different processes, namely conventional baking, vacuum baking, and combined conventional-vacuum baking in order to determine their effects on acrylamide and HMF contents of biscuits. Conventional baking process was performed using an oven (Memmert, UNE 400, Germany) at 180, 190, 200^{o}C for different times up to 15 min. Vacuum baking process was performed using a vacuum oven (Memmert, VO 200) at 160, 180, 200^{o}C and at 500 mbar for different times up to 17 min. Lab-scale vacuum oven was used throughout experiments has a capacity of 100 g dough per baking. For combined conventional-vacuum baking process, a set of biscuits was first partially baked in the conventional oven at 220^{o}C for 2, 3, and 4 min, and then they were post baked in the vacuum oven set at 180^{o}C and 500 mbar for 6, 5, and 4 min, respectively, keeping a total baking time of 8 min for final products. Another set of biscuits was first baked in the conventional oven at 230^{o}C for 2 min, and then post baked in the vacuum oven set at 180^{o}C and 500 mbar for 6 min. Control biscuits were baked in the conventional oven at 220^{o}C for 8 min. In the combined conventional-vacuum process, the basic recipe was modified

by adding a brown-colored powder at different amounts (none, 0.5, and 1.0%). All baking experiments were performed in triplicate.

3.2.3 Analysis of Acrylamide and HMF

Acrylamide and HMF extraction and analysis of biscuits were performed as described in **Chapter 2**.

3.2.4 Analysis of Asparagine

Asparagine was extracted from 1.0 g of grinded biscuit with 20 ml of 10 mM formic acid by 2 steps (10ml + 10ml). Each extract was centrifuged at 6080 x g for 10 min and combined for further centrifugation at 11,180 x g for 5 min. 0.5 ml of extract was mixed with 0.5 ml of acetonitrile, then, filtered through 0.45 µm nylon filter into autosampler vial. Asparagine analysis were carried out according to Gökmen et al. [167]. Chromatographic separations were performed on a Waters Atlantis HILIC silica column (150 × 2.1 mm, 3 µm particle size). A gradient mixture of acetonitrile (A) and 0.1% formic acid in water (B) was used as the mobile phase at a flow rate of 400 µl/min at 30°C. The eluent composition starting with 75% of A linearly decreased to 50% in 4 min. Then, it was linearly increased to its initial conditions (75% of A) in 2 min. Doing so, the total chromatographic run was completed in 6 min. An ultra high-performance liquid chromatography (UHPLC) Accela system (Thermo Fisher Scientific, San Jose, CA, USA) consisting of a degasser, a quaternary pump, an auto sampler, and a column oven was used. The UHPLC was directly interfaced to an Exactive Orbitrap MS (Thermo Fisher Scientific, San Jose, CA, USA).

The Exactive Orbitrap MS equipped with a heated electrospray interface was operated in the positive mode, scanning the ions in *m/z* range of 60–220. The resolving power was set to 50,000 full width at half maximum resulting in a scan time of 0.5 s. Automatic gain control target was set into high dynamic range; maximum injection time was 100 ms. The interface parameters were as follows: the spray voltage of 3.5 kV, the capillary voltage of 25 V, the capillary temperature of

280°C, a sheath and auxiliary gas flow of 35. The instrument was externally calibrated by infusion of a calibration solution (*m/z* 138 to *m/z* 1822) by means of an automatic syringe injector (Chemyx Inc. Fusion 100 T, USA). The calibration solution (Sigma-Aldrich) contained caffeine, Met-Arg-Phe-Ala, Ultramark 1621, and acetic acid in the mixture of acetonitrile/methanol/water (2:1:1, v/v/v). Data were recorded using Xcalibur software version 2.1.0.1140 (Thermo Fisher Scientific). Asparagine concentration was calculated by means of external calibration.

3.2.5 Measurement of Moisture Content

Moisture content of biscuits was determined according to AACC Intl. Approved Method 44-15.02 [168].

3.2.6 Color Measurement

Color measurements were performed by means of computer vision based image analysis using MATLAB® software and method described previously [165]. Digital images of biscuits were taken from a digital image acquisition system mentioned in Chapter 2. Images were captured, stored in a personal computer in JPEG format without compression. The CIE L*a*b* values were measured from a region of interest using a MATLAB® code.

3.2.7 Temperature Measurement

The surface and center temperature profiles of biscuits were recorded during baking using thermocouples linked to a data acquisition system (Keithley Multimeter Data Acquisition System Model 2700).

3.2.8 Sensory Properties

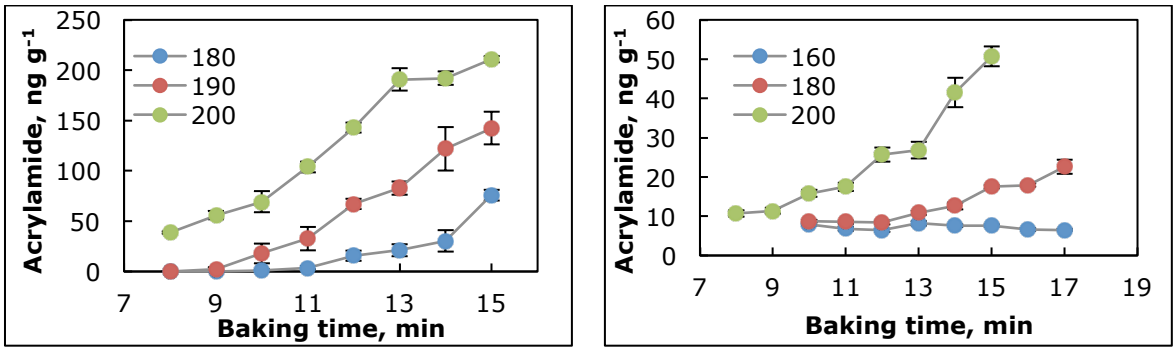
Untrained panel performed sensorial evaluation of biscuits. The taste, smell, color, texture characteristics of the biscuits were scored between 1 and 10. Overall acceptability of the biscuit was also evaluated.

3.2.9 Statistical Analysis

Acrylamide and HMF contents, moisture, color and sensory attributes of samples were statistically analyzed by ANOVA and *t*-test (α = 0.05) using the statistical software SPSS.

3.3 Results and Discussion

Figure 3.1a shows acrylamide formation in biscuits at different temperatures during conventional baking. Expectedly, increasing baking temperature or time significantly increased the amounts of acrylamide formed in biscuits during conventional baking. For example, acrylamide content of biscuits baked at 200°C for 8 min was 39 ng g^{-1}, while it increased to 211 ng g^{-1} when baked for 15 min. Similarly, acrylamide content of biscuit baked at 180°C for 13 min was found as 21 ng $g^{-1}$and increased to 83 ng g-1 and 191 ng g^{-1} at 190°C and 200°C, respectively. Previous researchers have repeatedly indicated that the amount of acrylamide increased with temperature or duration of thermal treatment in different food matrices and model systems [18, 21, 65, 69].



(a) (b)

Figure 3.1 Change of acrylamide concentration in biscuits with time during (a) conventional baking and (b) vacuum baking (500mbar) at different temperatures.

As shown in Figure 3.1b, similar kinetic trends were obtained in biscuits during vacuum baking at 180°C and 200°C. Interestingly, no significant

acrylamide formation (<LOQ) was observed in biscuits baked at 160°C. Obviously, the thermal load was limited to form acrylamide in biscuits under these conditions. At 200°C, mean acrylamide concentration ranged from 11 ng g^{-1} to 51 ng g^{-1} in biscuits baked under vacuum, while it ranged from 39 ng g^{-1} to 211 ng g^{-1} in biscuits baked in conventional oven. Biscuits baked in vacuum oven at 200°C for 15 min contain approx. 75 % less acrylamide than baked in conventional oven at same conditions. The results indicated that the levels of acrylamide attained during vacuum baking were significantly lower than those attained during conventional baking at all temperatures studied ($p<0.05$).

Asparagine, main precursor of acrylamide, was also monitored in biscuits during baking (Figure 3.2). There is an apparent decrease in asparagine content of biscuits baked in conventional oven, where asparagine decrease is very limited in biscuits baked in vacuum oven. This decrease explains higher acrylamide content of biscuits baked in conventional oven than that baked in vacuum oven. It is obvious that thermal load in vacuum oven was not sufficient for asparagine to react with carbonyls and form acrylamide during baking.

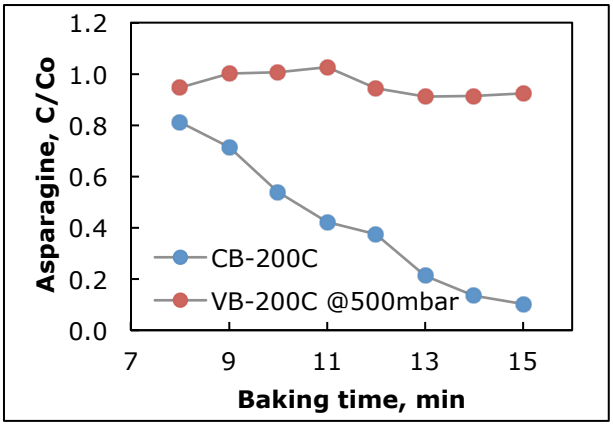


Figure 3.2 Asparagine content of biscuits baked with conventional (CB) and vacuum baking (VB) at 500 mbar.

Similar to acrylamide, HMF formation had also an increasing trend with increase of temperature and time (Figure 3.3).

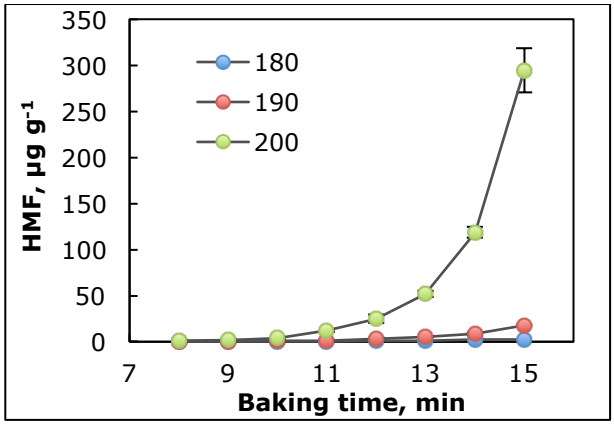


Figure 3.3 Change of HMF concentration in biscuits with time during conventional baking at different temperatures.

This exponential increase was remarkable at 200°C due to high thermal load. For example, mean HMF concentration in conventionally baked biscuits ranged between <LOD and 17.6 µg g^{-1} at 190°C, whereas it ranged between 1.2 µg g^{-1} and 294.4 µg g^{-1} for 200°C. There was no HMF formation (<LOD) in biscuits during vacuum baking at a temperature range of 160 and 200°C (data not shown). It is a fact that sucrose hydrolysis leading to the formation of HMF during baking requires higher thermal load at elevated temperatures.

The time-temperature profiles on the center of biscuits were recorded during conventional baking at 200°C under atmospheric pressure and vacuum baking at 500 mbar.

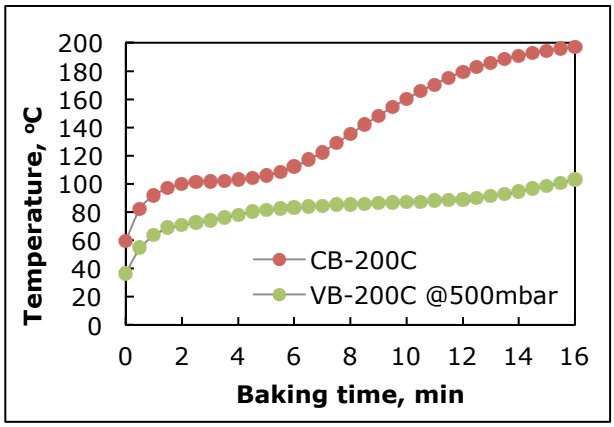


Figure 3.4 Time-temperature profiles of biscuits measured in the center during baking at 200°C under atmospheric pressure (CB) and vacuum (500 mbar) conditions (VB).

As shown in Figure 3.4, biscuits had a typical time-temperature profile during baking at 200°C under conventional baking conditions. The biscuit temperature rapidly rose to the boiling point of water (96.8°C in Ankara) within 2 min of baking and remained constant at the range of 97 and 103°C for 3 min until the moisture of biscuits largely evaporated. After a critically low moisture level was attained, the biscuit temperature began to rise again reaching to 200°C at the end of baking. The time-temperature profile of biscuits was different during vacuum baking at 200°C and at 500 mbar. The biscuit temperature rapidly rose to 71°C in 2 min, and then slowly to 81°C. It continued to rise very slowly reaching to 105°C at the end of baking. It is a fact that boiling point decreases as the pressure decreases. For example, the boiling point of water is 81.6°C at 500 mbar [169]. In comparison to conventional baking, noticeably low time-temperature profile of vacuum baked biscuits was the main reason of reduced formation of acrylamide and HMF.

Loss of moisture in biscuits gives also idea about the differences between conventional and vacuum baking processes. Expectedly, low pressure applied during vacuum baking accelerated the drying rate of biscuits. Drying rate of biscuits in conventional baking at 180°C was found to be similar to that in vacuum baking at 160°C. For example,

moisture contents of biscuits baked at 180°C for 8 and 11 min were 5.92% and 3.03% under the conventional conditions, respectively, whereas moisture contents of biscuits baked at 160°C for the same durations were found as 5.79% and 2.89% under vacuum at 500 mbar.

Regarding the surface color of biscuits, there were significant differences in the development of browning during conventional and vacuum baking processes ($p<0.05$). Surface browning was lacking in vacuum baked biscuits. In conventional baking, three modes of heat transfer, namely conduction, convection, and radiation are effective with certain contributions. Conduction is a heat transfer that takes place when the media is stationary, like the heat transfer from baking tray to bottom of biscuit dough, where convection occurs in a moving medium, like from heated air inside the oven to top of biscuit dough [170]. Since oven air was partially removed in vacuum baking, convective heating was limited, but conduction and radiation took place inside the oven. This was the main reason for lower thermal load, and so limited browning reactions in biscuits. It was reported that convective heat transfer coefficient depends on pressure of environment and it gradually decreases under vacuum [171].

In a recent study, lack of brown color on cookie surface due to lower thermal load during baking could be successfully solved by adding Maillard reaction products to the dough [172]. When dough is colored and flavored in the first instance, baking would be matter of drying to obtain final textural characteristics of biscuits. In the present study, a commercially available brown-colored powder, spray-dried (microgranulated) process flavor derived from yeast extract, was used in different amounts to modify recipe for biscuits prepared by means of a combined conventional and vacuum baking process. Firstly, the dough was partially baked at 220°C for short times (2-4 min) in the conventional oven. Then, partially baked biscuits were post baked in the vacuum oven for accelerated drying at 180°C and 500 mbar for 4-6 min until the desired final moisture content was attained. In the combined

process, exposure of biscuits to high temperature long time conditions, which were essential to facilitate the chemical reactions leading to the formation of thermal process contaminants, was prevented. There was no acrylamide or HMF formations (<LOD) in biscuits baked in the combined process. Control biscuits that were baked at 220^{o}C for 8 min in the conventional oven were found to contain acrylamide content of 40±3 ng g^{-1}. Addition of 0.5% and 1.0% of brown-colored powder increased significantly acrylamide content of control biscuits to 84±5 ng g^{-1} and 85±3 ng g^{-1}, respectively ($p<0.05$). This increase was attributed to the presence of reactive carbonyl species in the powder that could accelerate acrylamide formation in biscuits during conventional baking.

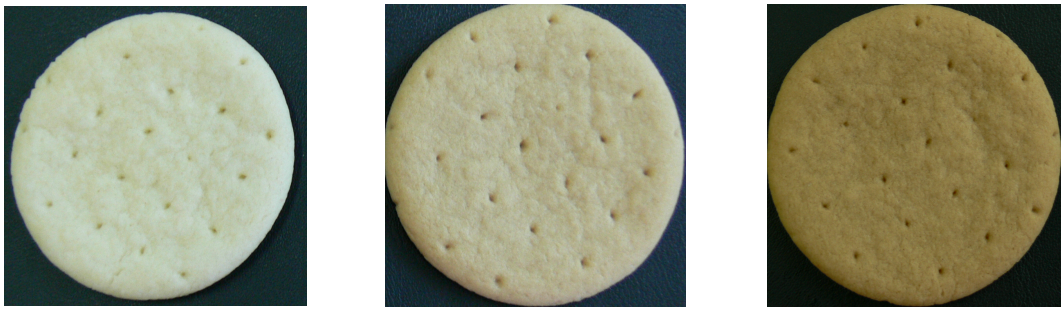
Table 3.1 summarizes the results of sensory analysis for biscuits. There were significant differences ($p<0.05$) between taste, smell, color and overall scores of biscuits baked by conventional and combined processes. However, no significant difference was observed between their texture scores. Adding brown-colored powder to the recipe improved the sensory scores of biscuits significantly. Overall acceptability score of the biscuit baked by combined process was similar to that baked by the conventional process when 1.0% of brown-colored powder was added to recipe.

As discussed in **Chapter 2**, considering CIE L*a*b* space, L* value typically decreases, where a* and b* values increases for biscuits baked in conventional process. As shown in Figure 3.5, combined baking process produced light colored biscuits from the basic recipe with mean L*, a*, and b* values of 81.5, -7.4, and 8.6, respectively. The L* value significantly decreased to 65.9 when 1.0% of brown powder was added to the recipe ($p<0.05$). Meanwhile, the a* and b* values significantly increased to -1.3 and 23.4, respectively. So, addition of brown-colored powder to recipe successfully simulated the color change of biscuit during baking.

Table 3.1 Sensory attributes of biscuits prepared by conventional baking and combined conventional - vacuum baking process*

	Conventional process[α]	Combined process[β]		
	Amount of brown powder added to basic recipe (%)			
	0	0	0.5	1.0
Taste	7.8±1.4[a]	5.3±1.9[b]	5.4±2.1[b]	6.6±2.3[a,b]
Smell	7.3±0.9[a]	3.9±0.8[c]	5.5±1.7[b,c]	6.5±2.2[b]
Color	5.6±1.4[a]	3.3±1.5[b]	8.3±1.4[c]	8.8±1.5[c]
Texture	8.0±0.9[a]	6.6±2.3[a]	7.6±2.2[a]	8.1±1.5[a]
Overall	7.9±1.8[a]	4.0±1.7[b]	6.8±2.1[a,b]	7.1±1.4[a]

* Values within rows having the same letter are not significantly different ($p > 0.05$)
[α] In conventional process, biscuits (control) were baked at 220°C for 8 min.
[β] In combined process, biscuits were partially baked at 220°C for 4 min in the conventional oven and post-baked at 180°C for 4 min in the vacuum oven (500 mbar).



L* : 81.5
a* : -7.4
b* : 8.6

(a)

L* : 72.8
a* : -3.6
b* : 16.7

(b)

L* : 65.9
a* : -1.3
b* : 23.4

(c)

Figure 3.5 Images of biscuits prepared by means of partial conventional baking at 220°C for 2 min and vacuum post baking at 180°C for 6 min. Amounts of brown-colored powder added to dough were as follows; (a) none, (b) 0.5%, (c) 1.0 %.

3.4 Conclusion

There have been many efforts to make thermally processed foods safer by controlling the chemical reactions responsible for the formation of process contaminants. Recently, application of radio frequency heating as a rapid post drying treatment in the last stage of baking process has been found promising for lowering acrylamide in bakery products [173, 174]. Beside these attempts, researchers have also introduced vacuum treatment to remove thermal process contaminants, such as furfural, HMF and acrylamide from biscuits, potato chips and coffee [175, 176].

Here, a new baking process in which partial conventional baking and vacuum post baking were used in combination was described for biscuits. Since it lowered the thermal load without extending total processing time, the combined process prevented the formations of acrylamide and HMF in biscuits. Lowering the thermal load would potentially reduce not only HMF and acrylamide formation, but also other processing contaminants in bakery products. Due to its potential carcinogenicity, acrylamide mitigation in foods is an important issue for consumers, health authorities, and industry. On the other hand, the authorities do not identify HMF mitigation as a priority for processed foods, even though HMF is considered as an indicator for heat damage.

Lack of browning development of biscuits appears as a disadvantage of the combined process. However, light colored biscuits may be particularly preferable for chocolate-coated products. Or, adding brown-colored powders to recipe can modify the color characteristics of biscuits baked in the combined process. As a promising alternative, the combined process may be of importance for the production of baby biscuits in which the highest level of product safety is required in terms of thermal process contaminants.

4 FORMATION OF MCPD AND ITS ESTERS IN BISCUITS DURING BAKING

4.1 Summary

Bakery products contain different amounts of lipids and table salt (sodium chloride). These ingredients have been reported to form chloropropanols in bakery products. Apparently, presence of NaCl as reactant creates food safety risk in bakery products that exposed to high temperature during baking. This study aimed to investigate the effects of temperature and sodium chloride on the formation kinetics of free chloropropanols and their esters in biscuits during baking. The effect of oil type on the formation of these contaminants was also investigated.

Kinetic examination of the data showed that increasing temperature led to an increase in the reaction rate constants for 3-MCPD, 2-MCPD and bound-MCPD. The activation energies of 3-MCPD and 2-MCPD were found to be 29 kJ mol^{-1}. Eliminating chloride from the recipe decreased 3-MCPD, 2-MCPD rate constants in biscuits by 57.5% and 85.4%, respectively, and bound-MCPD formation was prevented. Different oils were also used to test their effect on the 3-MCPD, 2-MCPD and bound-MCPD formation in biscuits. There was no significant difference on 3-MCPD concentrations of these biscuits, where 2-MCPD and bound-MCPD concentrations in biscuits prepared with refined olive oil was found to be the highest. Lowering thermal load or limiting chloride concentration should be considered a means to reduce or eliminate formation of these contaminants in biscuits.

4.2 Experimental

4.2.1 Chemicals and Consumables

Hexane and 2,2,4-Trimethylpentane, sodium chloride, sodium sulphate anhydrous, tert-butyl methyl ether, ethylacetate, and H_2SO_4 were purchased from Fisher Chemical (UK, Leicestershire). Diethyl ether was

purchased from Rathburn Chemicals (Walkerburn, Scotland). Chem-tube Hydromatrix was supplied by Varian (Agilent Technologies, Winnersh, UK) and 1-(heptafluorobutyryl)-imidazole (HFBI), 98+%was purchased from Alfa Aesar England (Great Britain). 3-chloro-1,2-propanediol (3-MCPD), Sodium methoxide, Filter Whatman No.4, and KBr were purchased from Sigma-Aldrich (UK). MCPD-*d5* and 3-MCPD-palmitate-*d5* were obtained from CDN isotopes (Canada) and Toronto Research Chemicals (Canada), respectively.

Raw material and ingredients other than oil for biscuit were kindly supplied by Kraft (Glattpark, Switwerland) and Eti (Eskişehir, Turkey). Refined corn, canola, hazelnut, olive and peanut oils were supplied by Zade (Konya, Turkey).

4.2.2 Preparation of Biscuits

The biscuits were prepared according to the American Association of Cereal Chemists Method 10–54 with some modifications [163]. The recipe contains 80.0 g standard wheat flour, 35.0 g grained sucrose, 20.0 g corn oil, 1.0 g NaCl, 0.4 g NH_4HCO_3, 0.8 g of $NaHCO_3$ and 17.6 ml water. All ingredients were thoroughly mixed in accordance with the AACC Method 10–54 procedure using a dough mixer Artisan Kitchen Aid 5KSM150 (MI, USA). Dough was rolled in 3 mm thickness and cut in three discs having 5 cm diameter and baked in a conventional oven (Memmert, UNE 400, Germany).

Two different recipes were prepared with corn oil, namely basic recipe and basic recipe without sodium chloride. Biscuits having basic recipe were baked at 180^{o}C, 200^{o}C and 220^{o}C for different times up to 19 min. The recipe without salt was baked at 220^{o}C for 7 to 11 min. Additionally, different refined oils, namely canola, nut, olive and peanut, were also used to determine the effect of oil type on MCPD formation by replacing corn oil in the recipe. These set of biscuits were baked at 220^{o}C for 10 min. All baking experiments were performed as duplicate. Regardless of the baking condition, all biscuits prepared contained less than 2 % of moisture.

4.2.3 Analysis of 3-MCPD and 2-MCPD

Extraction. The non-polar part of the biscuits was isolated by extraction of 4.00 g of the ground biscuit twice with 40 ml of hexane. The combined extracts were kept for MCPD-ester analysis. 100 µl of 10 µg/ml internal standard solution, 3-MCPD-d5, and 10 ml of 5 M NaCl were added to the defatted biscuits. Then, 7 g of diatomaceous earth sorbent, Hydromatrix, were added, mixed thoroughly and transferred to chromatography column. About 1 cm of sodium sulphate was added to the top of column. 3-MCPD and 2-MCPD were eluted over 15 min with 100 ml of diethyl ether. One spatula of anhydrous sodium sulphate was added to the eluent. The extract was then filtered through a Whatman No. 4 filter paper and the solvent removed by rotary evaporation to 1-5 ml. The concentrated extract was transferred to a 10 ml flask and made up to volume with diethyl ether.

Derivatization: Four ml of the final sample extract were transferred to a 4ml septum capped vial. The sample extracts were blown to dryness under a gentle stream of nitrogen before adding 1 ml of 2,2,4-trimethylpentane. A 50 µl aliquot of HFBI was added and the vial was capped, shaken and incubated at 70°C for 20 min. One ml of distilled water was added to the cooled mixture, the vial was shaken and the phases allowed separating. The upper 2,2,4-trimethylpentane phase was dried over anhydrous sodium sulfate, and carefully transferred to a 2 ml GC-MS vial.

For calibration, 1 ml of appropriate standard solutions were transferred to 4 ml septum cap vials and the same derivatization procedure was performed.

Quantitation. GC/MS determinations were made by using an Agilent 6890 Series GC with a Gerstel Multipurpose Sampler MPS2 injector interfaced with a 5973N mass selective detector (MSD) For chromatographic separation a fused silica capillary column, Rxi®-5ms, 30 m × 0.25 mm i.d., 0.25 µm film coated with Crossbond® diphenyl dimethyl polysiloxane (Restek, USA) was used with helium carrier at a

constant flow of 1.1 ml min^{-1}. The GC oven temperature program was: 50°C for 2 min, 2°C min^{-1} to 80°C, 50°C min^{-1} to 270°C, and hold for 2 min. The total run time was 22.8 min. Injections of 3 µl were made in the splitless mode. The MSD was operated in the selected ion-monitoring mode (SIM) using electron-impact ionization with an ionizing voltage of 70 eV. The MSD SIM acquisition parameters were *m/z* 253 (quantifier), 275, 289 (qualifiers) for 2-MCPD, *m/z* 253 (quantifier), 275, 289, 453 (qualifiers) for 3-MCPD and *m/z* 257 (quantifier) for 3-MCPD-d_5 with a dwell time of 80 ms. The LOQ was 0.1 mg kg^{-1}. The retention times of 3-MCPD, 2-MCPD, 3-MCPD-d_5 were 16.215 and 16.338 and 16.010 min, respectively.

Analysis of bound-MCPD. 3-MCPD bound as esters in biscuits was analyzed according to DGF Standard Methods C-VI 18(10) [177]. The non-polar extracts of biscuits, obtained by hexane extraction before 3-MCPD analysis, were evaporated until all the solvent phase was removed. 0.1 g of non-polar fraction was transferred to 4 ml vial. 100 µl MCPD-dipalmitate-d_5 was added and the mixture dissolved in 100 µl *tert*-butyl methyl ether. Then, 200µl CH_3ONa (25 g/l in methanol) was added, vortexed and mixture was left for 4 min at room temperature. 600 µl acidified chloride free salt solution (KBr 600 g/L, 35 ml H_2SO_4 (25%) to 1 L KBr solution) was added and aqueous phase was rinsed 2 times with 1ml n-hexane. The aqueous phase was extracted 2 times using 1 ml 60:40 ether:ethyl acetate. The combined extract was vortexed, evaporated to dryness under N_2 stream, dissolved again to 2,2,4-trimethylpentane (1 mL) and derivatized as for free MCPD.

4.2.4 Statistical Analysis

3-MCPD and 2-MCPD contents of samples were statistically analyzed by Duncan and *t*-test (α = 0.05) using the statistical software SPSS®.

4.3 Results and Discussion

The kinetics of 3-MCPD, 2-MCPD and bound-MCPD formations were examined in biscuits with a basic formulation baked at 180°C, 200°C, and 220°C for different baking times (Figure 4.1a-c).

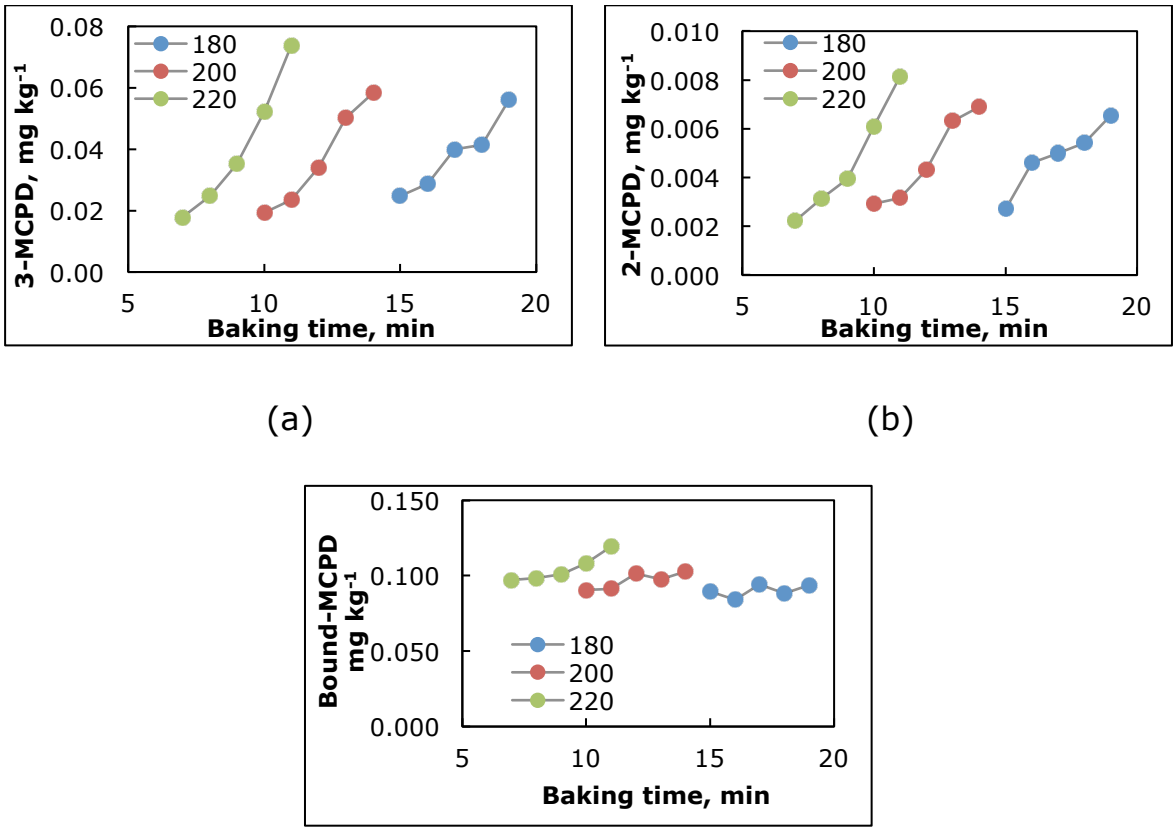


Figure 4.1 Effect of baking temperature and time on the formation of (a) 3-MCPD, (b) 2-MCPD and (c) bound-MCPD (mg kg^{-1} biscuit) in biscuits during baking

The free 3-MCPD content of biscuits was between 0.018 mg kg^{-1} and 0.074 mg kg^{-1}, whereas 2-MCPD ranged from 0.002 mg kg^{-1} to 0.008 mg kg^{-1}. As shown in Figure 4.1a and Figure 4.1b, both free 3-MCPD and 2-MCPD concentrations increased with increasing baking time. 3-MCPD and 2-MCPD concentrations in biscuits baked at 220°C increased by 4.1-fold and 3.7-fold, when the baking time increased from 7 to 11 min, respectively. Similarly, increased baking temperature led to

increase in 3-MCPD and 2-MCPD concentrations of biscuits. As the temperature increased from 200°C to 220°C, 3-MCPD and 2-MCPD concentrations increased to 3.2-fold and 2.6-fold in biscuits baked for 11 min, respectively.

The effect of process parameters on 3-MCPD and 2-MCPD formations could be related with the increased thermal load. During thermal process, water and steam hydrolyze triacylglycerols and phospholipids producing diacylglycerols, monoacylglycerols, glycerol and other products, acting as precursors of 3-MCPD and 2-MCPD [109]. Our results also showed that the relative proportions of the major chloropropanols (3-MCPD, 2-MCPD) in biscuits were approximately in the ratio of ranging from 6.24 to 13.57 in biscuits. The ratio of 10:1 has been reported in acid-HVPs [105].

Based on the assumption that precursors, i.e. lipid and chloride, were present in excess, Hamlet and Sadd reported that formation of 3-MCPD was consistent with zero order kinetics during the baking of wheat flour dough [106]. As the food system studied, i.e. biscuit, suits the same case, the data successfully fitted the zero order kinetic equation. The kinetic rate constants are summarized in Table 4.1.

Increase in temperature led to an increase in the reaction rate constants of both 3-MCPD and 2-MCPD, as the kinetic energy of the system increased. Each 20°C increase in temperature increased the reaction rate constants of both 3-MCPD and 2-MCPD approx. 1.4 times. The rate constant of 2-MCPD in biscuits during baking was found approx. 9.4-times less than that of 3-MCPD. This confirms a previous study, which reported that the rate of 2-MCPD formation is less than rate of 3-MCPD in breads prepared with wheat flour dough [106].

Table 4.1 Calculated reaction rate constants of 3-MCPD, 2-MCPD and bound-MCPD formations in biscuits (basic recipe and basic recipe without NaCl) baked at different temperatures according to zero order kinetic equation*.

Recipe / **Temperature**	**Rate constants, *k*, mmol kg^{-1} min^{-1}**		
	3-MCPD x 10^5	2-MCPD x 10^5	Bound-MCPD x 10^5
Basic recipe			
180°C	6.81±1.71 ^{c}	0.77±0.17 ^{c}	1.18±0.06 ^{c}
200°C	9.48±0.65 ^{b}	1.01±0.06 ^{b}	2.71±0.14 ^{b}
220°C	12.59±0.02 ^{a}	1.34±0.00 ^{a}	4.88±0.24 ^{a}
Basic recipe without NaCl			
220°C	5.35±0.91	0.20±0.08	ND

*Values within columns having the same letter are not significantly different ($p > 0.05$). ND: Not detected

The temperature dependence of simple chemical reactions was empirically described by Arrhenius' law, which is expressed as

$$k = A\, exp\left(-\frac{E_a}{RT}\right) \qquad \text{Eq. 1}$$

in which k (mmol kg^{-1} min^{-1}) is reaction rate constant, A (mmol kg^{-1} min^{-1}) is a pre-exponential factor, E_a is the activation energy (J mol^{-1}), R (8.314 J mol^{-1} K^{-1}) is the gas constant and T (K) is the absolute temperature. Rate constants obtained from both 3-MCPD and 2-MCPD kinetic data fitted to Arhenius' equation well (R^2=0.999). Finally, the activation energies of both 3-MCPD and 2-MCPD reactions were found to be equal to 29 kj mol^{-1}, which means that reactants need same amount of energy to start the reaction and carry on spontaneously for both formation reactions.

The initial bound-MCPD concentration of the refined corn oil used in the biscuit formulations was 0.5664 mg kg^{-1} oil. This was equivalent to 0.088 mg kg^{-1} biscuit, considering that the oil was present 15% in the biscuit dough. Figure 4.1-c shows bound-MCPD content of biscuits prepared, ranging between 0.084 mg kg^{-1} and 0.119 mg kg^{-1}. There was no significant difference between bound-MCPD concentrations of

the uncooked biscuit dough and biscuits baked for the least baking time, at all temperatures studied. The UK Food Standards Agency reported that both 2- and 3-MPCD could be released from MCPD-esters, identified in bread crust and toasted bread [119]. This might lead to a decrease in bound-MCPD concentration. However, after a certain thermal load, the concentration of bound-MCPD in biscuits started to increase. This increase could be related to a higher formation rate than degradation rate during baking. The reaction rate constants of bound-MCPD formation were also calculated. As given in Table 4.1, increased baking temperature has an increasing effect on the bound-MCPD formation rate constant in biscuits, which was found statistically significant ($p<0.05$).

It is known that chloride is one of the precursors of chloropropanols. Table salt (NaCl) used in food formulations is the main source of chloride in most foods. The effect of presence of chloride on the formation of 3-MCPD, 2-MCPD and bound-MCPD in biscuits was tested. The biscuit recipe described earlier was used without the incorporation of sodium chloride. The results were given in Figure 4.2a-c.

It was found that eliminating salt had a statistically significant effect on the formation of 3-MCPD and 2-MCPD ($p<0.05$). When the salt was removed from the recipe, the reaction rate constants of 3-MCPD and 2-MCPD formations in biscuits decreased 57.5% and 85.4%, respectively (Table 4.1). As discussed before, bound-MCPD content of biscuits baked for 7 min was same as of refined corn oil, i.e. 0.088 mg kg^{-1} biscuit. When salt was removed from the recipe, the concentration of bound-MCPD in biscuits baked for 7 min was found to be 0.085±0.003 mg kg^{-1} biscuit. There was no significant increase in bound-MCPD concentration of salt-free biscuits during baking ($p<0.05$), where presence of salt caused increase in formation of bound-MCPD.

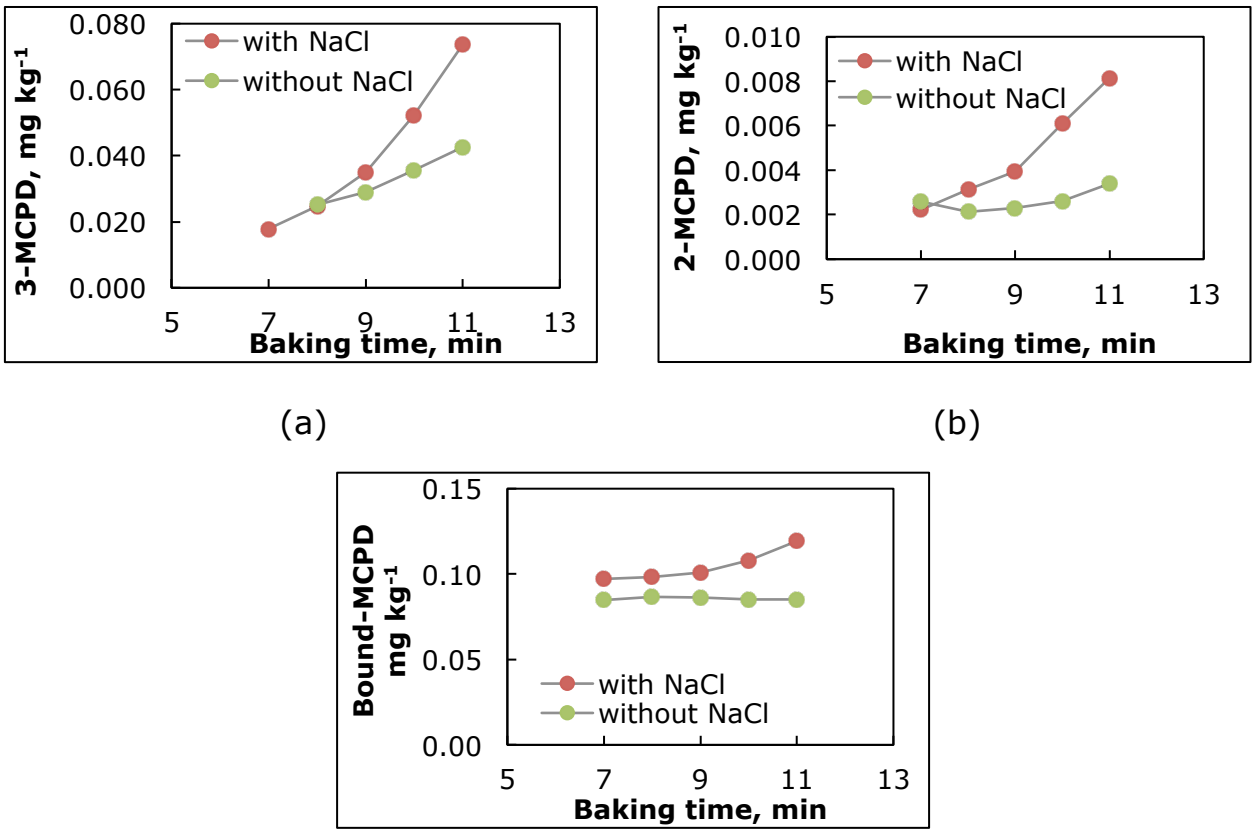
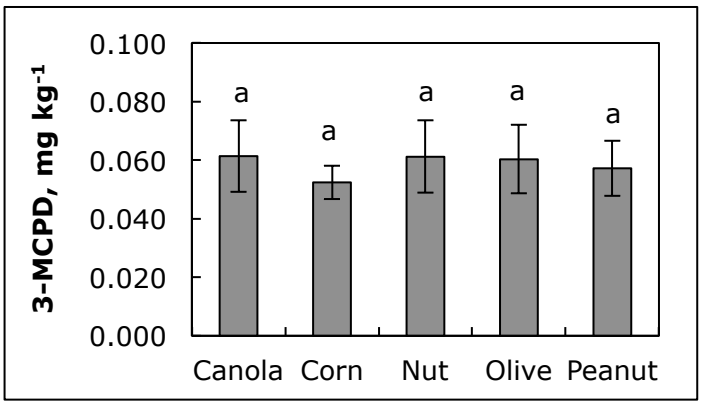
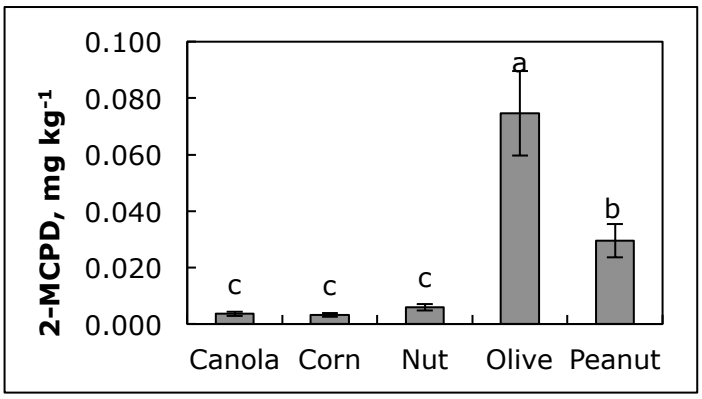


Figure 4.2 Effect of salt on (a) 3-MCPD, (b) 2-MCPD and (c) bound-MCPD (mg kg^{-1} biscuit) formation in biscuits during baking at 220°C

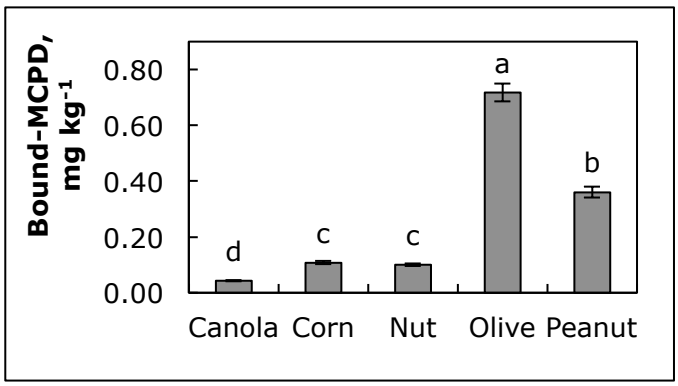
A range of different vegetable oils namely corn oil, canola, nut, olive and peanut, were used in the biscuit formulation in order to determine their effect on the formation of 3-MCPD, 2-MCPD and bound-MCPD (Figure 4.3a-c). The 3-MCPD content of all biscuits prepared with different oils was found approx. 0.06 mg kg^{-1}. Statistical analysis showed that there was no significant difference between 3-MCPD contents of these biscuits ($p<0.05$). Among the refined oils used in this study, 2-MCPD and bound-MCPD concentrations of the biscuit prepared with refined olive oil was found to be the highest, i.e. 0.075 mg kg^{-1} and 0.717 mg kg^{-1}, respectively. Zelinková also reported that bound-MCPD of refined olive oil is higher than that of other refined edible oils, namely soybean, sunflower, maize and rapeseed [178].



(a)



(b)



(c)

Figure 4.3 Effect of oil type on the formation of (a) 3-MCPD, (b) 2-MCPD and (c) bound-MCPD (mg kg^{-1} biscuit) in biscuits, baked at 220°C for 10 min. Values having the same letter are not significantly different ($p > 0.05$).

4.4 Conclusion

Removal of chloride from biscuit formulations controlled the 3-MCPD, 2-MCPD and bound-MCPD formation reactions, which could be an effective mitigation strategy without adverse effects on the biscuit flavor. Careful selection of the type of vegetable oil or fat and testing MCPD ester content prior to use in baking could also reduce the content of these processing contaminants in bakery products.

5 EFFECT OF SALT ON THE FORMATION OF HYDROXYMETHYLFURFURAL

5.1 Summary

Table sugar (sucrose) and salt (sodium chloride) are typical ingredients in bakery products. High temperature decomposes sucrose forming certain carbonyl compounds that lead to HMF through dehydration. Previous reports indicate the relevance of cations including sodium on the acceleration of sucrose decomposition during heating at elevated temperatures. As a reactive ingredient, presence of table salt in product formulations may pose safety risks in terms of HMF formation. Therefore, this study aimed to investigate the role of sodium chloride on the decomposition of sucrose. Main intermediates and HMF formed during heating sucrose were identified by orbitrap HRMS. In addition, the effect of sodium chloride on the formations of these intermediate compounds and HMF was determined.

The results revealed that the rate of HMF formation from sucrose increased 7.32 fold during heating sucrose at 200^{o}C in the presence of sodium chloride. In the meantime, the rate of sucrose decomposition increased 3,57 fold under the same conditions. This confirmed the catalytic role of sodium cation on the pyrolysis of sucrose leading to HMF.

5.2 Experimental

5.2.1 Chemicals and Consumables

Acetonitrile, water and methanol for HPLC and LC/MS/MS determination were of analytical grade and sodium chloride were obtained from Merck (Darmastadt, Germany). Formic acid (98%) was purchased from J.T. Baker (Deventer, Holland). 5-hydroxymethylfurfural (HMF) standards and sucrose were purchased from Sigma (St. Louis, MO). All the samples were filtered through nylon filters 25 mm 0.45 µm and 2.5 ml conventional syringes (BD,Franklin Lakes, NJ) equipped with a PTFE adapter (Phenomenex, Torrance,CA).

5.2.2 Preparation of Model Systems

A model system composed of sucrose and NaCl was used to determine the effect of salt on HMF formation. A total of 10 µmoles of sucrose and NaCl were transferred to 25 ml test tube (Pyrex, 25 ml) as their aqueous solutions. Total reaction volume was adjusted to 100 µl with deionized water. A total of 300 mg of silica gel was added to cover the reaction mixture and the tube was tightly closed with a screw cap. The reactions were performed in an oil bath at 200^{o} C for 5, 10 and 20 min. All reactions were performed in triplicate. The reaction mixtures after heating were suspended in 2 ml of 10 mM formic acid and the aqueous extract was obtained by vortexing the tube for 2 min. After centrifugation at 11180 g for 5 min, 1 ml of the supernatant was passed through a 0.45 µm nylon syringe filter into a vial.

5.2.3 High Resolution Mass Spectrometry Analysis (HRMS) of Reaction Products Formed in Model System

Extracts of model systems were analyzed by HRMS in order to identify the reaction intermediates and products. A Thermo Scientific Accela UHPLC system (San Jose, CA) coupled to a Thermo Scientific Exactive Orbitrap HRMS was used. The HRMS system was operated in positive electrospray ionization mode. The chromatographic separations were performed on Atlantis T3 Column (250 mm x 4.6 mm id; 5 cm) (Waters Corporation, Milford, USA) using 0.05% aqueous formic acid and methanol isocratically (70:30) at a flow rate of 0.5 mL/min (30^{o}C) for 15 min. The scan analyses were performed in an *m/z* range between 50 and 600 at ultra-high resolving power (R=100.000). The data acquisition rate, the automatic gain control target and maximum injection time were set to 1 Hz, 1x10^{6} and 100 ms, respectively. The source parameters were as follows: sheath gas flow rate 45 (arbitrary units), auxiliary gas flow rate 20 (arbitrary units), sweep gas flow 3 (arbitrary units) spray voltage 3 kV, capillary temperature 300^{o}C, capillary voltage 25 V, tube lens voltage 55 V and vaporizer temperature 300^{o}C. To confirm the reaction path leading to HMF,

possible forms of sucrose decomposition products were extracted from the total ion chromatograms.

5.3 Results and Discussion

Effect of NaCl on HMF formation in biscuits was previously reported [89, 166]. NaCl promoted the formation of HMF in biscuits and the presence of 0.5% NaCl, which is the usual concentration of salt used in many commercial biscuits, significantly increased HMF formation up to 75% [166].

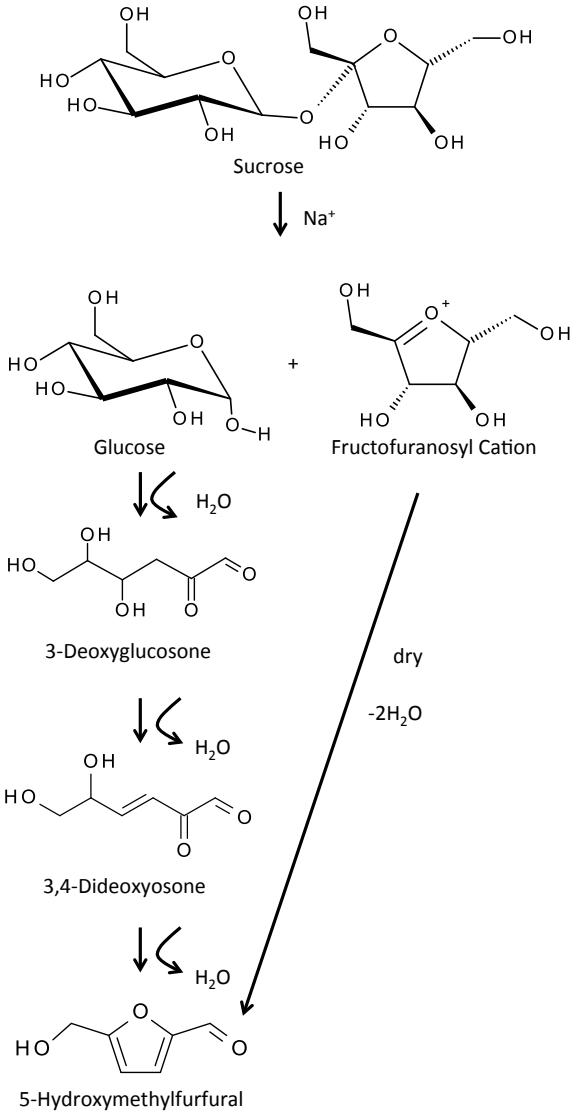


Figure 5.1 Sucrose pyrolysis pathway adapted from Perez Locas and Yaylayan [75]

The mechanisms leading to conversion of sucrose into HMF through the fructofuranosyl cation at elevated temperatures have been previously

described [75]. As shown in Figure 5.1, both glucose and fructofuranosyl cation can generate HMF by the elimination of three and two moles of water, respectively. The initial rate of HMF was found to be 1.11 nmol min^{-1} in the model sucrose system heated at 200°C. With NaCl, the rate of HMF formation from sucrose increased to 8.13 nmol min^{-1}. This confirmed the catalytic role of sodium on the pyrolysis of sucrose leading to HMF. It was a fact that the presence of NaCl accelerated the pyrolytic decomposition of sucrose during heating at 200°C. The rate of sucrose decomposition increased from 2.85 μmol min^{-1} to 10.18 μmol min^{-1} when NaCl was present in the reaction mixture during heating. It is thought that NaCl as a metal cation acts as Lewis acid in the reaction mixture that accelerates the decomposition of sucrose. It is known that organic acids, inorganic acids, salts, and Lewis acids catalyze dehydration of hexoses [179].

Formations of key intermediates and HMF in the heated model reaction mixtures were determined to better understand the role of NaCl in sucrose decomposition. Scan HRMS analyses of sucrose pyrolyzates with and without NaCl confirmed the presence of 3-deoxyglucosone, 3,4-dideoxyosone, and HMF having *m/z* of 163.0601, 145.0495 and 127.0390, respectively, with a very high mass accuracy (Δ<2.0 ppm). Extracted ion chromatograms of these compounds in the pyrolyzate of sucrose heated with NaCl at 200°C for 10 min are shown in Figure 5.2. The rates of the formation of 3-deoxyglucosone and 3,4-dideoxyosone from sucrose increased by a factor of 4.3 and 23.5 times in the presence of NaCl during heating as shown in Figure 5.3.

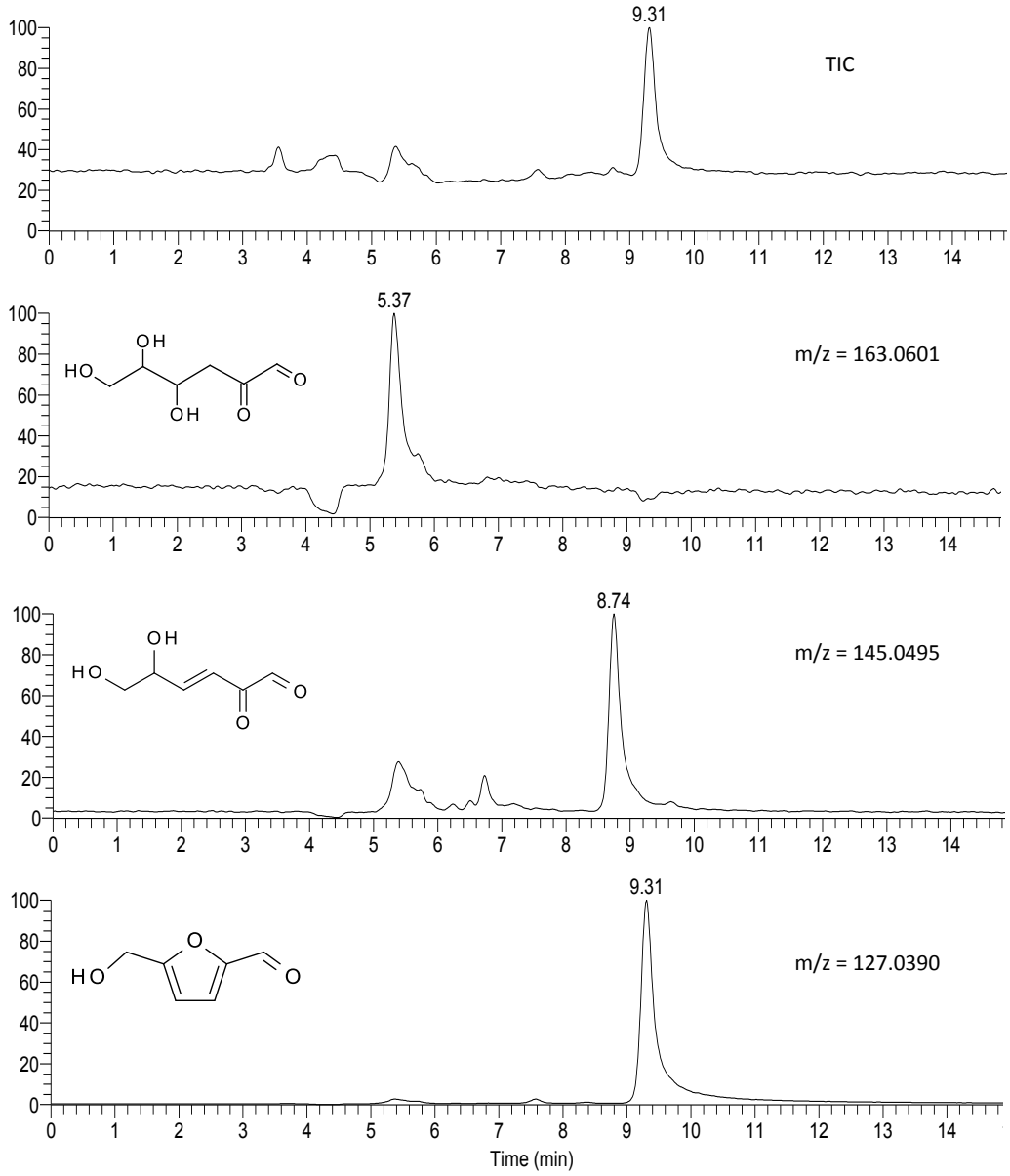


Figure 5.2 Extracted ion chromatograms of 3-deoxyglucosone, 3,4-dideoxyosone, and HMF formed in the model system heated at 200°C for 10 min

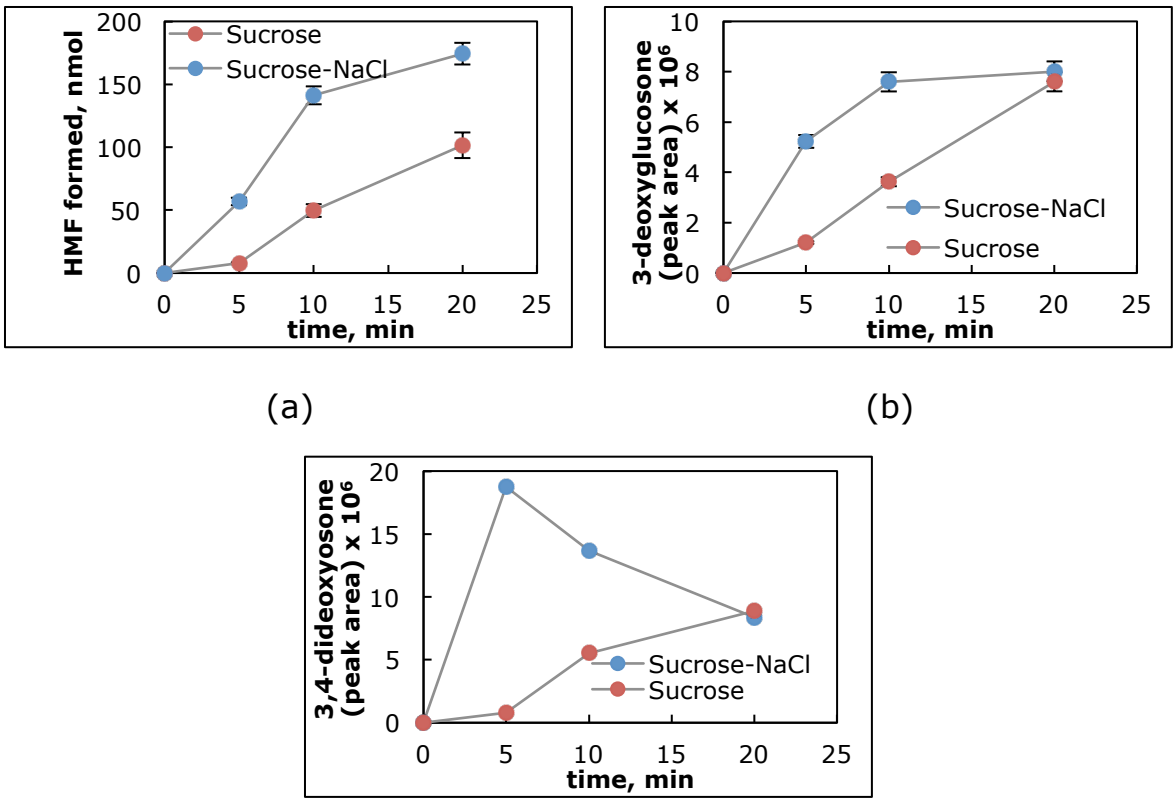


Figure 5.3 Amounts of (a) 3-deoxyglucosone and (b) 3,4-dideoxyosone formed during heating of sucrose with and without NaCl at different time points.

5.4 Conclusion

In conclusion, sodium chloride acts as Lewis acid and accelerates the decomposition of sucrose, as well as dehydration of intermediate compounds formed in further steps. Therefore, elimination of sodium chloride, catalyzing the HMF formation, can be an effective mitigation strategy to prevent its formation in bakery products containing sucrose in the formulation. In addition, encapsulation of sodium chloride can be a possible approach for the mitigation of HMF. Blocking NaCl inside the microparticles would reduce the time of its participation to the reaction converting sucrose into HMF. However, melting point of the coating material should be considered, as the increase of the melting point of the coating delays sodium chloride release and reaction during the thermal process. Based on these data, incorporation of encapsulated

NaCl to biscuit dough was successfully performed and HMF formation in these biscuits was reduced [166].

6 KINETICS OF FURAN FORMATION FROM ASCORBIC ACID DURING HEATING UNDER REDUCING AND OXIDIZING CONDITIONS

6.1 Summary

Oxidation-reduction potential is one of the main intrinsic factors of food, which may affect the reactions occurring during thermal process. It is known that oxidation-reduction potential affects ascorbic acid (AA) degradation [180]. But its effect on the formation of furan through AA degradation has not been reported. Therefore, this study aimed to investigate the effect of oxidizing and reducing agents on the formation of furan through AA degradation during heating at elevated temperatures (≥100^{o}C) under low moisture conditions. To obtain these conditions, oxidizing agent, ferric chloride (Fe), or reducing agent, cysteine (Cys) was added to reaction medium. Kinetic constants, estimated by multiresponse modeling, stated that adding Fe significantly increased furan formation rate constant, namely 369-fold higher than that of control model at 100^{o}C. Rate-limiting step of furan formation was found as the reversible reaction step between Intermediate (Int) and diketogluconic acid (DKG). Additionally, Fe decreased activation energy of ascorbic acid (AA) hydration and furan formation steps by 28.6% and 60.9%, respectively. Results of this study are important for heated foods, fortified by ferric ions and vitamins, which targets specific consumers, e.g infant formulations.

6.2 Experimental

6.2.1 Chemicals and Consumables

Solvents, HPLC-grade water, and methanol used for chromatographic analysis were purchased from Sigma-Aldrich (Steinheim, Germany) and formic acid (98%) was purchased from J.T. Baker (Deventer, Holland). L-(+)-Ascorbic acid (min >99.7%), L-(+)Dehydroascorbic acid, cysteine (min 99%), furan (99.9%), silica gel 60GF (for thin-layer chromatography) were obtained from Merck and Fe(III)-chloride anhydrous was obtained from Riedel-de Haën (Seelze, Germany). d4-

Furan (for NMR 99% atom) was purchased from Acros Organics (New Jersey, USA). Stock solutions of furan and spiking d_4-furan were prepared in methanol at a concentration of 1000 mg/ml. All solutions were prepared and kept at 4°C.

6.2.2 Preparation of Model Systems

Three model systems were prepared to monitor furan formation from AA under different conditions. Reaction mixtures were prepared containing 100 µmol/ml AA in water. Oxidizing or reducing agents were added to the reaction mixtures, specifically 10 µmol Fe^{3+} equivalent ferric chloride or 10 µmol Cys, respectively. 100 µl of these reaction mixtures, containing 10 µmol AA, 1 µmol Fe^{3+} or 1µmol Cys, mixed with 30 mg silica gel in 20-ml headspace vials, and then covered with additional 270 mg of silica gel. The vials were sealed with crimp cap, immediately, and then heated in a temperature-controlled oven (Memmert, Schwabach, Germany) at 100°C, 120°C, and 140°C for 5, 10, 15, 20, 30, 60, 120, 180, and 360 min. All reactions were performed in duplicate. The reaction conditions and response variables for the model systems used for kinetic modeling are given in Table 6.1.

Table 6.1. The range of reaction conditions and response variables used for multiresponse kinetic modeling

Model	Range of reaction conditions		Response variables
	T (°C)	t (min)	
Control	100 - 140	0 - 360	Furan, AA, DHAA, DKG
Fe	100 - 140	0 - 360	Furan, AA, DHAA, DKG
Cys	100 - 140	0 - 360	Furan, AA, DHAA, DKG

6.2.3 Analysis of Furan

After reaction, the vials containing reactants and reaction products were spiked through septa with 1.0 nmol of d_4-furan. Determination of furan was carried out using Agilent 6890N series GC coupled with Agilent 5973 mass selective detector. Furan was extracted by 75 mm carboxen-polydimethylsiloxane Solid Phase Micro Extraction (SPME)

fiber (Supelco, Bornem, Belgium). Before use, the fiber was conditioned in the GC injection port under helium flow in accordance with the temperature and time recommended by the manufacturer. Fiber was then incubated in headspace of vials in a temperature-controlled oven at 30^{o}C for 30 min. The vials were gently mixed in every 5 min. Thermal desorption of analytes was carried out by exposing the fiber in the GC injector port at 200^{o}C for 5 min and splitless injection was used. Separation was performed on a 24 m x 0.32 µm, 20 µm HP-PLOTQ column. The MS was operated in electron ionization mode. Working conditions were as follows: injector 2 mL/min; oven temperature, 100°C (5 min), with a temperature ramp of 10°C/min to 200°C and held for 15 min. The MS source temperature was 230°C, and the MS quad temperature was 150°C, with a dwell time of 100 ms. Furan was detected using single-ion monitoring of the fragments *m/z* 68 and 39. The internal standard d_4-furan was detected by monitoring the fragments *m/z* 72 and 42. The concentration of furan in the reaction mixtures was calculated by means of a calibration curve built in a range of 0-15 nmol (0, 0.01, 0.15, 1.5, 3.0, 7.5, 15.0 nmol). The limit of quantification was 0.01 nmol per reaction mixture for furan under the stated analytical conditions. All analytical determinations were performed in duplicates.

6.2.4 Analysis of AA, DHAA, and Reaction Intermediates by High-Resolution Mass Spectrometer

AA, DHAA, and other intermediates were extracted by adding 5 ml of 10 mM formic acid in water to vial in two steps (2x2.5 ml). After mixing thoroughly, the extracts were transferred to tube, which was then centrifuged at 5000 g for 10 min. Supernatants were filtered through 0.45 µm nylon filter to HPLC vials.

An ultra high-performance liquid chromatography (UHPLC) Accela system (Thermo Fisher Scientific, San Jose, CA, USA) consisting of a degasser, a quaternary pump, an auto sampler, and a column oven was used. The UHPLC was directly interfaced to an Exactive Orbitrap MS

(Thermo Fisher Scientific, San Jose, CA, USA). Chromatographic separations were performed on a HIBAR Purospher-STAR RP-18e column (150 × 4.6 mm, 5 µm particle size) (Merck, Darmstadt, Germany). An isocratic mixture (95:5, v/v) of 0.1% formic acid in water and 0.1% formic acid in methanol was used as the mobile phase at a flow rate of 500 µl/min at 30 °C. The total run time was 10 min. The Exactive Orbitrap MS equipped with a heated electrospray interface was operated in the negative mode, scanning the ions in *m/z* range of 50–300. The resolving power was set to 100,000 full width at half maximum resulting in a scan time of 0.5 s. Automatic gain control target was set into balanced; maximum injection time was 50 ms. The interface parameters were as follows: the spray voltage of 4 kV, the capillary voltage of 25 V, the capillary temperature of 350°C, a sheath gas flow 45 and auxiliary gas flow of 20. The instrument was externally calibrated by infusion of a calibration solution (*m/z* 138 to *m/z* 1822) by means of an automatic syringe injector (Chemyx Inc. Fusion 100 T, USA). The calibration solution (Sigma-Aldrich) contained caffeine, Met-Arg-Phe-Ala, Ultramark 1621, and acetic acid in the mixture of acetonitrile/methanol/water (2:1:1, *v/v/v*). Data were recorded using Xcalibur software version 2.1.0.1140 (Thermo Fisher Scientific). The concentrations of AA and DHAA in the reaction mixtures were calculated by means of calibration curves (0.0, 0.05, 0.10, 0.25, 0.50, 1.0 µmol). All analytical determinations were performed in duplicates.

6.2.5 Statistical Analysis

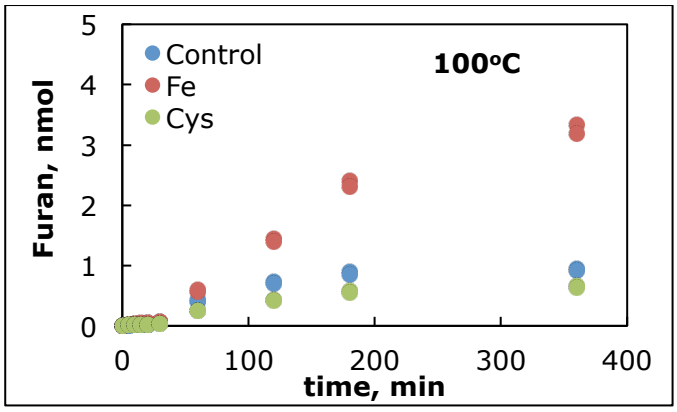
Data were analyzed by one-way analysis of variance (ANOVA) using Duncan test with the SPSS program (SPSS 16.0). Significance was defined as $p<0.05$.

6.3 Results and Discussion

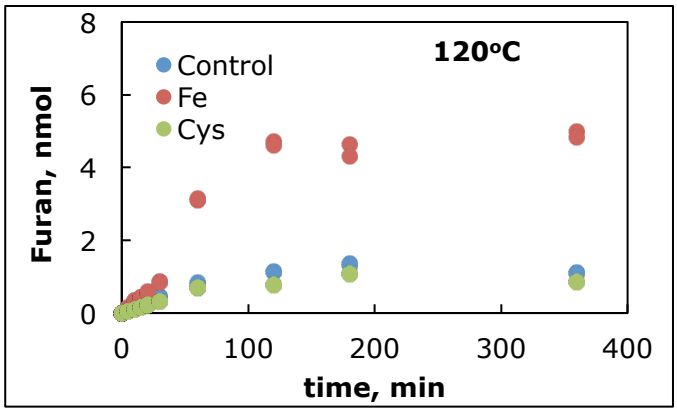
Changes in furan concentrations in three models (control, Fe, and Cys) were given in Figure 6.1 indicating that presence of reducing or

oxidizing agents in the reaction medium affected furan formation. Both Cys and Fe^{3+} ions may be naturally present in the foods. Ferric ions might also migrate from metal containers used in processing [181], or foods might be fortified by ferric ions and/or AA targeting specific consumers, e.g infant formulations. Adding ferric chloride to the model significantly accelerated furan formation from AA during heating at temperatures exceeding 100°C ($p<0.05$). On the other hand, presence of Cys did not have significant effect on furan formation ($p>0.05$).

Figure 6.1 shows that increasing temperature lead to increase in furan concentration regardless from the composition of reaction medium. Likewise, reaction time also affected the furan formation. Increased thermal load, either increasing temperature or time, also increased the amount of furan formed to a certain extend. At 100°C and 120°C, furan concentration reached to a steady apparent maximum. However, at 140°C, furan concentration after reaching to its apparent maximum level within 2 h of heating began to decrease slightly in the presence of ferric chloride. This could be the result of increased furan degradation rate, becoming more dominant than its formation rate at those conditions.



(a)



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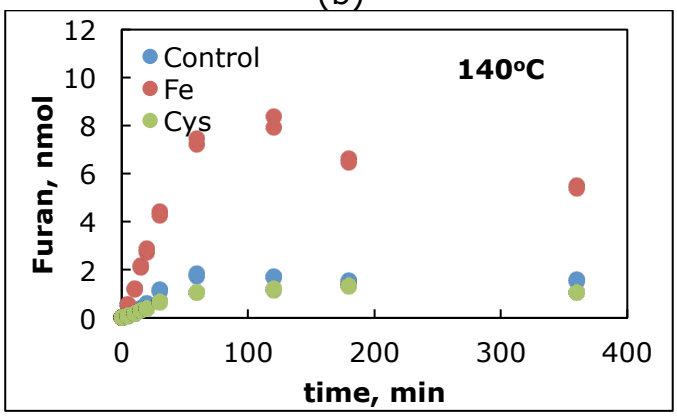


(c)

Figure 6.1 Amount of furan formed in different model systems (control, Fe, Cys) during heating at different temperatures. a) 100°C b) 120°C c) 140°C

Formation of furan has been studied in simple model systems containing precursors in order to understand their contributions in the formation mechanism. In a previous study, Perez Locas and Yaylayan proposed a mechanism describing that AA might be transformed under non-oxidative pyrolytic conditions to 2-deoxyaldotetrose as key intermediate leading directly to furan [132].

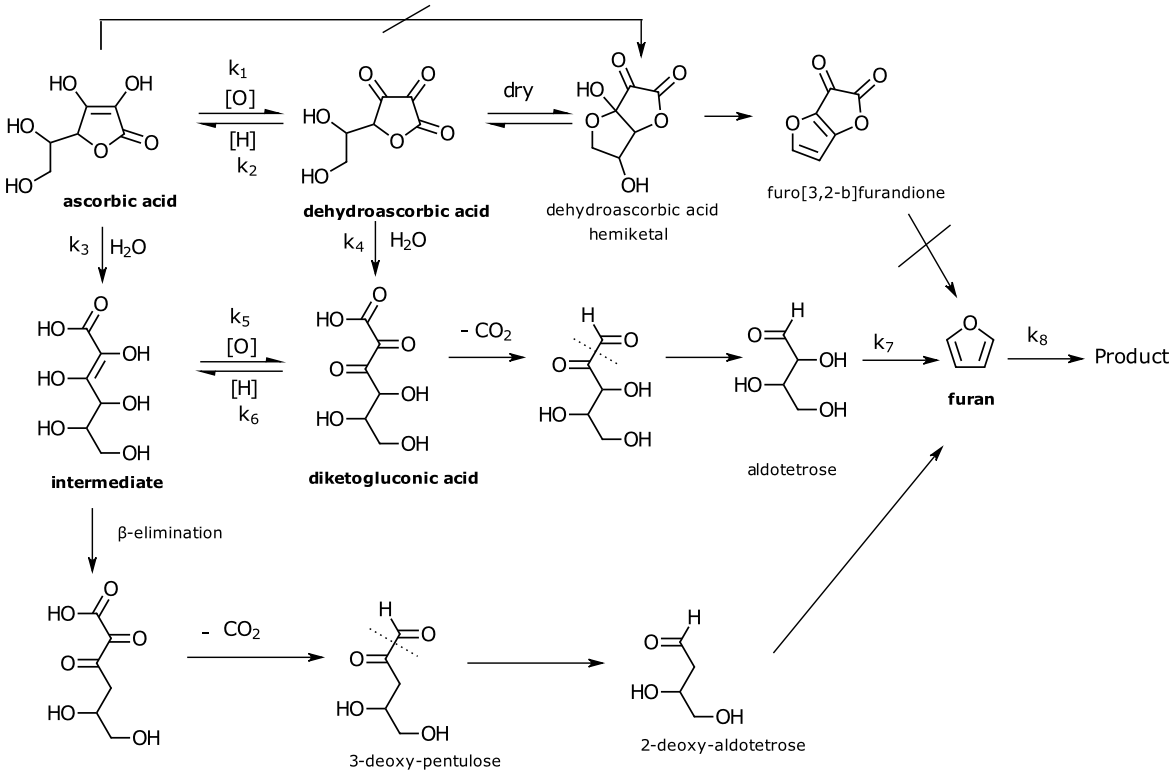


Figure 6.2 Mechanism of furan formation from AA adapted from Perez Locas and Yaylayan [132]. Compounds indicated bold was used as response variables in multiresponse kinetic modeling. [O]: oxidation, [H]: reduction.

In this study, furan formation was modeled using multiresponse approach from the kinetic data obtained at different temperatures by Athena Visual Studio software (Version 14.2). Reaction mechanisms in foods are challenging, as key compounds involve in many simultaneous and successive steps. In this sense, multiresponse modeling is a useful approach to understand such mechanisms. Multiresponse modeling, which is based on measurement of reactants and products simultaneously, provides the possibility to estimate parameters more accurately than with uniresponse modeling in which only one reactant or only one product is analyzed [182]. The modeling of the formation of compounds derived from kinetic data is a great tool to estimate kinetic parameters, which facilitate to understand the reaction mechanism.

Initially, a proposed reaction mechanism describing the change in the concentration of key reaction compounds is needed to develop a mechanistic model [182]. The mechanism of furan formation from AA proposed by Perez Locas and Yaylayan was used for this purpose [132]. The reaction network shown in Figure 6.2 is a representation of the possible oxidative and non-oxidative degradation pathways. Among these pathways, some were selected to simplify the reaction network for modeling purposes. Such selections were based on experimental measurements of the reactants and certain products (compounds indicated in **bold**). Proposed mechanism was slightly modified by adding a reversible reduction step to oxidation of Int to diketogluconic acid (DKG). The reason was a reduction step could take place, if there would be an oxidation step in these model systems having different oxidation-reduction conditions.

The model was formed with differential equations (1), (2), (3), (4), (5), and (6) which were derived according to the proposed mechanism. The model was then fitted to experimental data and rate constants (k_1 to k_8) were estimated for each model system and temperature. The proposed model successfully described the experimental data, as given in the example in Figure 6.3.

$$\frac{d[AA]}{dt} = -k_1[AA] - k_3[AA] + k_2[DHAA] \quad (1)$$

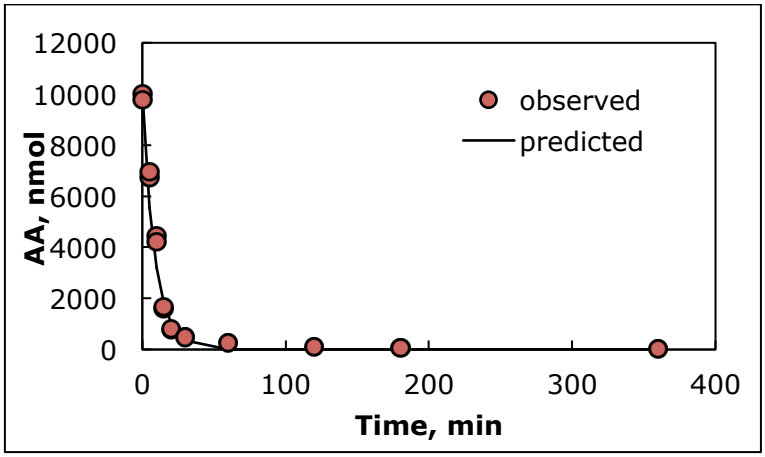
$$\frac{d[DHAA]}{dt} = k_1[AA] - k_2[DHAA] - k_4[DHAA] \quad (2)$$

$$\frac{d[Int]}{dt} = k_3[AA] - k_5[Int] + k_6[DKG] \quad (3)$$

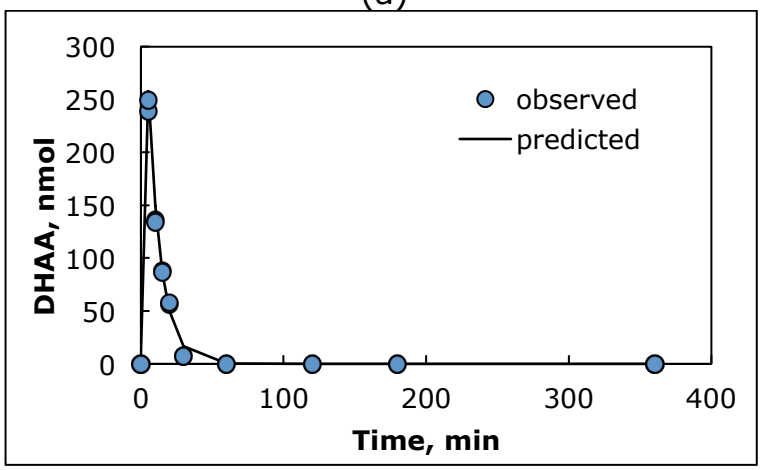
$$\frac{d[DKG]}{dt} = k_4[DHAA] + k_5[Int] - k_6[DKG] - k_7[DKG] \quad (4)$$

$$\frac{d[Furan]}{dt} = k_7[DKG] - k_8[Furan] \quad (5)$$

$$\frac{d[Product]}{dt} = k_8[Furan] \qquad (6)$$



(a)



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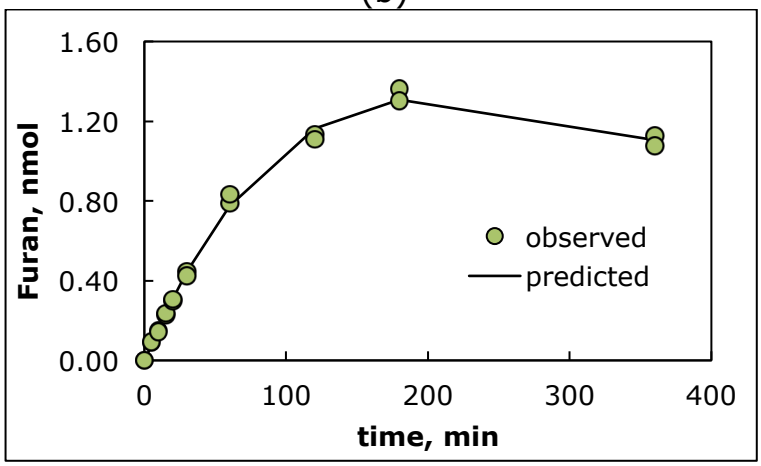

(c)

Figure 6.3.Change of the amounts of AA, DHAA and furan with time in model system (control) during heating at 120°C (solid lines indicate model fit)

The rate constants for the degradation of AA to Int (k_3), and the formation of furan (k_7) are given in Table 6.2. Adding ferric chloride significantly increased furan formation rate constant at all temperatures studied ($p<0.05$). Moreover, k_7 increased with the increase of temperature in control, Fe, and Cys containing model systems. Rate constant of the degradation of AA to Int was higher than that of AA to DHAA. For example, k_1 and k_3 for control model system heated at 140°C were calculated as 0.042 min^{-1} and 0.182 min^{-1}, respectively. Rate constant of the degradation of AA to Int (k_3) increased in the presence of ferric chloride at all temperatures studied while presence of Cys showed no significant effect ($p>0.05$). Moreover, increasing the heating temperature caused to an increase in k_3 rate constant.

High-resolution MS offers advantages to help identifying the structure of compounds in complex reaction systems. In this study, formation of reaction intermediates, proposed by Perez Locas and Yaylayan, were confirmed by high resolution MS [132]. Two compounds in this reaction scheme, $[Int]^-$ (*m/z* 193) and $[DKG]^-$ (*m/z* 191), were successfully extracted from the total ion chromatograms. As they could not be quantified, peak areas were considered to compare as given in Table 6.3.

Table 6.2. The rate constants calculated for the degradation of AA to Int (k_3) and the formation of furan (k_7)

T(°C)	Model	k_3, min^{-1}	k_7, min^{-1}
100	Control	5.34E-02 ± 2.98E-03[a]	2.13E-05 ± 8.71E-06[a]
	Fe	2.69E-01 ± 2.52E-02[b]	7.86E-03 ± 2.16E-03[b]
	Cys	5.77E-02 ± 6.17E-03[a]	7.99E-07 ± 1.08E-07[a]
120	Control	1.10E-01 ± 4.72E-03[a]	5.58E-03 ± 7.56E-04[a]
	Fe	4.15E-01 ± 5.38E-02[b]	2.97E-02 ± 5.93E-03[b]
	Cys	1.04E-01 ± 1.40E-02[a]	2.14E-05 ± 4.38E-06[a]
140	Control	1.82E-01 ± 1.52E-02[a]	4.22E-02 ± 2.60E-02[a,b]
	Fe	6.47E-01 ± 1.09E-01[b]	1.58E-01 ± 4.71E-02[b]
	Cys	2.04E-01 ± 3.56E-02[a]	3.85E-04 ± 9.37E-05[a]

Table 6.3. Effects of oxidizing and reducing agents on the formation of Int and DKG during heating the models systems containing AA at different temperatures for 5 min. Signal intensities are given as peak area of corresponding compounds detected by high resolution MS.

T (°C)	Model	Peak Area	
		Int ***m/z* 193**	**DKG** ***m/z* 191**
100	Control	ND	ND
	Fe	5.21E4±3.42E3	1.13E4±6.50E2
	Cys	ND	ND
120	Control	1.31E4±7.46E2	6.45E3±3.38E2
	Fe	1.22E5±9.36E3	4.12E4±2.48E3
	Cys	0.62E4±3.51E2	3.14E3±2.23E2
140	Control	2.18E4±1.40E3	1.00E4±6.88E2
	Fe	1.17E5±7.70E3	7.87E4±3.93E3
	Cys	1.43E4±8.98E2	7.50E3±7.05E2

ND: Not detected.

Results showed that presence of ferric chloride promoted the formation of both intermediates. Although there was no formation of Int and *DKG* in control and Cys models heated at 100°C for 5 min, presence of ferric chloride induced both compounds to be formed. Peak area of Int was found to be higher than DKG at all temperatures indicating that the main reaction pathway to form furan was through Int formed from AA. Based on these results it could be concluded that rate-limiting step of furan formation reaction mechanism was the reversible reaction step between Int and DKG.

The results of present study revealed that furan formation was strongly affected by ferric chloride. However, Becalski and Seaman was reported that adding ferric chloride to AA model did not increase furan formation in their model system [181]. This conflict may result from the differences in reactants' concentration or in the state of the model system. They used relatively low amount of ferric chloride compared with AA (approx. 50 μmol AA and 0.062 μmol $FeCl_3$) in aqueous model system, while we used (10 μmol AA and 1 μmol Fe^{3+}) in low moisture model system.

Temperature dependence of simple chemical reactions was empirically described by Arrhenius' law, which is expressed as

$$k = A\, exp\left(-\frac{E_a}{RT}\right) \qquad (7)$$

in which k (s^{-1}) is reaction rate constant, A (s^{-1}) is a pre-exponential factor, E_a is the activation energy (J mol^{-1}), R (8.314 J mol^{-1} K^{-1}) is the gas constant and T (K) is the absolute temperature. The Arrhenius' equation gives a quantitative account [182].

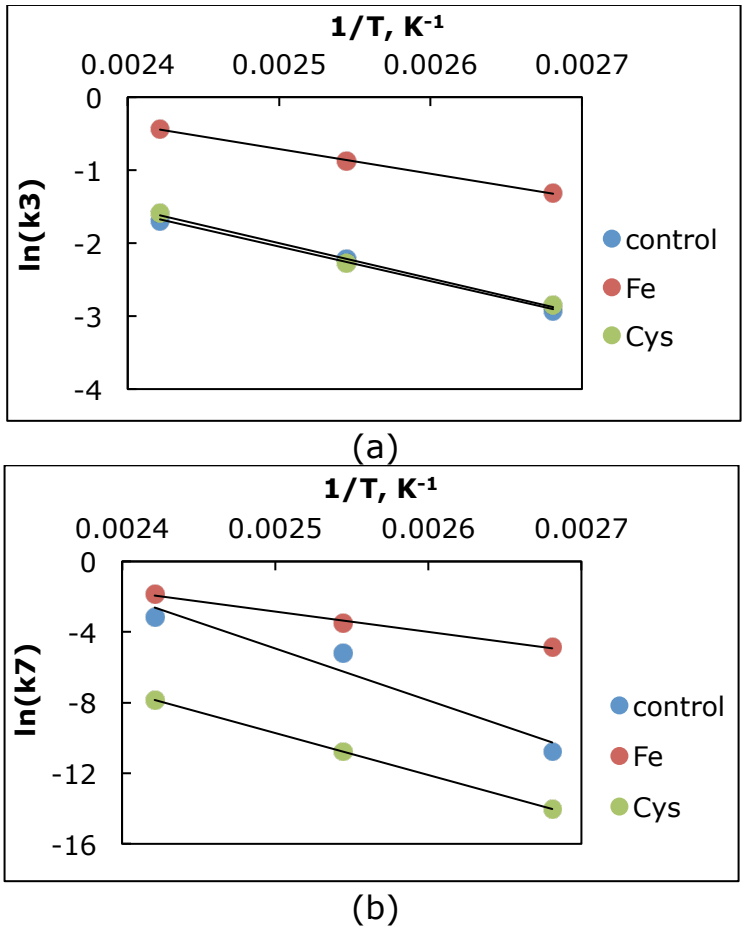

Figure 6.4.Arrhenius plots for (a) the degradation of AA into Int (k_3), and (b) the formation of furan (k_7)

In the present study, linear Arrhenius dependence was obtained for the degradation of AA to Int (k_3), and the formation of furan (k_7) (Figure 6.4). For k_3, the activation energies were calculated as 39.40, 28.14,

and 40.28 kJ mol^{-1} for control, Fe, and Cys models, respectively. Moreover the activation energies for k_7 were calculated as 244.93, 95.79, and 197.95 kJ mol^{-1} for control, Fe, and Cys models, respectively. It is clear that both reaction steps were affected by ferric chloride in terms of decreasing activation energy, which means that reactants need less energy to start the reaction and carry on spontaneously. Furthermore, it was previously reported that activation energy of the degradation of AA ranges between 20 to 167 kJ mol^{-1} in aqueous systems, which is comparable with the results of this study [183].

6.4 Conclusion

Composition of food is important, as it constitutes the reaction medium. Each constituent might affect the reactions occurring in foods during heating. As a conclusion, oxidation-reduction potential was found to be one of the main intrinsic factors to consider for furan formation through the degradation of AA. Oxidation-reduction potential should be taken into account in heated foods while developing a mitigation strategy for furan formation. The results indicated that oxidation-reduction potential should be kept low to limit furan formation in these foods. The results are considered to be relevant for low moisture foods like infant biscuits enriched with vitamins and minerals including AA and Fe^{3+}.

CONCLUSION AND GENERAL DISCUSSION

From a food safety point of view, occurrence of thermal process contaminants in foods is still one of the major concerns for consumers, health authorities and industry. Their mitigation, therefore, remains a challenging task for both food scientists and food industry. This thesis describes some potential applications to limit the formation of these contaminants including acrylamide, HMF, chloropropanols, and furan.

Color is an indicator of the degree of browning in thermally processed foods. Previous findings revealed the fact that color end point could be a practical measure to control the amounts of acrylamide formed in foods during thermal processing. In this thesis, a camera prototype was developed for online color measurement to monitor acrylamide and HMF formation in biscuits during baking. Using the calibration models for a fixed biscuit recipe, surface color could be monitored online by means of the camera prototype to predict processing contaminants under real processing conditions. This color measurement tool is important, as food industry has been looking for viable solutions not only to mitigate their formation during processing, but also their monitoring by means of low cost, rapid and reliable techniques. The camera prototype can be adapted to baking lines as a process control tool to monitor quality and safety of biscuits. The calibration models described in the thesis are specific to the recipe used. Any changes or modifications in the recipe would require revalidating these calibration models.

Decreasing thermal load during processing is a valid strategy to limit the formation of process contaminants in foods. However, lowering the temperature is not viable, as it requires longer time to finish the process at lower temperatures in order to achieve desired final moisture content. In this thesis, a combined baking process based on partial conventional baking followed by vacuum post baking was developed for

biscuits. It is a fact that high temperature and low moisture conditions attained during the later stages of baking favors the formation of these process contaminants in biscuits. Since it does not allow increasing the temperature of biscuit to critical levels when moisture becomes low, the combined process prevents the formations of acrylamide and HMF in biscuits. Lowering the pressure during vacuum post baking accelerates moisture evaporation enabling faster drying of biscuits. So, it is possible to achieve desired final moisture levels in shorter time. Depending on lowered thermal load, the development of surface browning is limited in the biscuits baked by the combined process. This could be considered as a disadvantage, but the biscuits produced by this process can be preferably used for chocolate-coated products. Another option would be adding brown-colored powder to simulate the browning in the dough. As a promising alternative, the combined process may be of importance for the production of baby biscuits in which the highest level of product safety is required in terms of thermal process contaminants.

The results of this thesis revealed **the role of table salt** (sodium chloride), a typical ingredient of bakery products, on the formation of certain process contaminants, namely HMF and chloropropanols (3-MCPD, 2-MCPD and bound-MCPD) during heating. Sodium chloride increases the rate of sucrose decomposition, hence the formation of HMF during heating. In addition, it is responsible for the formation of free and bound MCPD derivatives in biscuits during baking. Therefore, the results suggest that its elimination from the formulations could be an effective strategy to mitigate both chloropropanols (free and bound) and furfurals in biscuits. Or, it could be used as encapsulated in a coating material to limit its reactivity during the baking process.

Finally, the results indicated **the importance of oxidation-reduction potential** on the formation of furan from ascorbic acid during heating at elevated temperatures under low moisture conditions. Enrichment of

baby biscuits with vitamins and minerals including ascorbic acid and iron is a usual practice in the food industry. In such foods, presence of added nutrients may pose an increased risk in terms of furan formation during baking, because presence of oxidizing agents like ferric ions accelerate significantly the formation of furan from ascorbic acid during heating. As a potential mitigation strategy, the results suggest keeping the oxidation-reduction potential low during thermal processing of foods rich in ascorbic acid.

In overall, this PhD study contributed greatly to understanding new strategies to mitigate thermal process contaminants that could be effectively used in common heated foods.

REFERENCES

[1] Zhou, W.; Therdthai, N., Heat and Mass Transfer during Baking of Sweet Goods. *Food Engineering Aspects of Baking Sweet Goods*, (eds:Sumnu, S.G., Sahin, S.), CRC Press, Florida, 173-190, **2008**.

[2] Manley, D., Technology of Biscuits, Crackers, and Cookies. Third (eds: Woodhead Publishing Limited and CRC Press LLC: England 398, **2000**.

[3] Tireki, S., Technology of Cookie Production. *Food Engineering Aspects of Baking Sweet Goods*, (eds: CRC Press: 159-172, **2008**.

[4] Sablani, S. S., Physical and Thermal Properties of Sweet Goods. *Food Engineering Aspects of Baking Sweet Goods*, (eds: Sumnu, S.G., Sahin, S.), CRC Press, Florida, 191-213, **2008**.

[5] Mowbray, W. R., Technology of the "hot box", *Food Manufacture*, October, **1981**.

[6] Maillard, L., Action of amino acids on sugars. Formation of melanoidins in a methodical way, *Comptes rendus de l'Académie des sciences*, 154, 66-68, **1912**.

[7] Hodge, J. E., Chemistry of browning reactions in model systems, *Journal of Agricultural and Food Chemistry*, 1, 928-943, **1953**.

[8] Reynolds, T. M., Chemistry of nonenzymatic browning II, *Advances in Food Research*, 14, 167-283, **1965**.

[9] McWeeny, D. J.; Knowels, M. E.; Hearne, J. F., The chemistry of non-enzymic browning and its control by sulphites, *Journal of Agricultural and Food Chemistry*, 25, 735-746, **1974**.

[10] Ghiron, A. F.; Quack, B.; Mawhinney, T. P.; Feather, M. S., Studies on the role of 3-deoxy-D-erythro-glucosulose (3-deoxyglucosone) in nonenzymic browning. Evidence for involvement in a Strecker degradation, *Journal of Agricultural and Food Chemistry*, 36, 677-680, **1988**.

[11] Huber, B.; Ledl, F., Formation of 1-amino-1,4-dideoxy-2,3-hexodiuloses and 2-aminoacetylfurans in the Maillard reaction, *Carbohydrate Research*, 204, 215-220, **1990**.

[12] Lingnert, H.; Eriksson, C. E., Antioxidative Maillard reaction products. I. Products from sugars and free amino acids, *Journal of Food Processing and Preservation*, 4, 161-172, **1980**.

[13] Aeschbacher, H. U., Anticarcinogenic effect of browning reaction products. *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, (eds: Finot, P. A.; Aeschbacher, H. U.; Hurrel, R. F.; Liardon, R.), Birkhäuser Verlag, Basel, 335–348, **1990**.

[14] Hayase, F.; Hirashima, S.; Okamoto, G.; Kato, H., Scavenging of active oxygens by melanoidines, *Agricultural Biology and Chemistry*, 53, 3383-3385, **1989**.

[15] Henle, T.; Walter, H.; Krause, I.; Klostermeyer, H., Efficient determination of individual maillard compounds in heat-treated milk products by amino acid analysis, *International Dairy Journal*, 1, 125-135, **1991**.

[16] Lineback, D. R.; Stadler, R. H., Introduction to food process toxicants. *Process-Induced Food Toxicants: Occurence, Formation, Mitigation and Health Risks*, (eds: Stadler, R. H.; Lineback, D. R.), A John Wiley & Sons, Inc, Publications, 3-19, **2009**.

[17] IARC, *Acrylamide*, International Agency for Research on Cancer Monographs, Lyon, France, **1994**.

[18] Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T., Food chemistry: Acrylamide is formed in the Maillard reaction, *Nature*, 419, 448-449, **2002**.

[19] Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M. C.; Riediker, S., Food chemistry: Acrylamide from Maillard reaction products, *Nature*, 419, 449-450, **2002**.

[20] Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M., Acrylamide: A Cooking Carcinogen?, *Chemical Research in Toxicology*, 13, 517-522, **2000**.

[21] Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Tornqvist, M., Analysis of acrylamide, a carcinogen formed in heated foodstuffs, *Journal of Agricultural and Food Chemistry*, 50, 4998-5006, **2002**.

[22] Stadler, R. H.; Robert, F.; Riediker, S.; Varga, N.; Davidek, T.; Devaud, S.; Goldmann, T.; Hau, J.; Blank, I., In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the maillard reaction, *Journal of Agricultural and Food Chemistry*, 52, 5550-5558, **2004**.

[23] Yaylayan, V. A.; Wnorowski, A.; Perez Locas, C., Why asparagine needs carbohydrates to generate acrylamide, *Journal of Agricultural and Food Chemistry*, 51, 1753-7, **2003**.

[24] Zyzak, D. V.; Sanders, R. A.; Stojanovic, M.; Tallmadge, D. H.; Eberhart, B. L.; Ewald, D. K.; Gruber, D. C.; Morsch, T. R.; Strothers, M. A.; Rizzi, G. P.; Villagran, M. D., Acrylamide formation mechanism in heated foods, *Journal of Agricultural and Food Chemistry*, 51, 4782-7, **2003**.

[25] Blank, I.; Robert, F.; Goldmann, T.; Pollien, P.; Varga, N.; Devaud, S.; Saucy, F.; Huynh-Ba, T.; Stadler, R. H., Mechanisms of acrylamide formation - Maillard-induced transformation of asparagine. *Chemistry and Safety of Acrylamide in Food*, (eds: Friedman, M.; Mottram, D. S.), Springer, New York, 171-189, **2005**.

[26] Granvogl, M.; Schieberle, P., Thermally generated 3-aminopropionamide as a transient intermediate in the formation of acrylamide, *Journal of Agricultural and Food Chemistry*, 54, 5933-8, **2006**.

[27] Wedzicha, B. L.; Mottram, D. S.; Elmore, J. S.; Koutsidis, G.; Dodson, A. T., Kinetic models as a route to control acrylamide formation in food. *Chemistry and Safety of Acrylamide in Food*, (eds: Friedman, M.; Mottram, D. S.), Springer, New York, 235-253, **2005**.

[28] Schieberle, P.; Köhler, P.; Granvogl, M., New aspects on the formation and analysis of acrylamide. *Chemistry and Safety of Acrylamide in Food*, (eds: Friedman, M.; Mottram, D. S.), Springer, New York, 205-222, **2005**.

[29] Blank, I., Current status of acrylamide research in food: measurement, safety assessment, and formation, *Annals of the New York Academy of Sciences*, 1043, 30-40, **2005**.

[30] Yasuhara, A.; Tanaka, Y.; Hengel, M.; Shibamoto, T., Gas chromatographic investigation of acrylamide formation in browning model systems, *Journal of Agricultural and Food Chemistry*, 51, 3999-4003, **2003**.

[31] Stadler, R. H.; Verzegnassi, L.; Varga, N.; Grigorov, M.; Studer, A.; Riediker, S.; Schilter, B., Formation of vinylogous compounds in model Maillard reaction systems, *Chemical Research in Toxicology*, 16, 1242-50, **2003**.

[32] Yaylayan, V. A.; Locas, C. P.; Wnorowski, A.; O'Brien, J., The role of creatine in the generation of N-methylacrylamide: a new toxicant in cooked meat, *Journal of Agricultural and Food Chemistry*, 52, 5559-65, **2004**.

[33] Yaylayan, V. A.; Stadler, R. H., Acrylamide formation in food: a mechanistic perspective, *Journal of AOAC International*, 88, 262-7, **2005**.

[34] Granvogl, M.; Schieberle, P., Quantification of 3-aminopropionamide in cocoa, coffee and cereal products, *European Food Research and Technology*, 225, 857-863, **2007**.

[35] Granvogl, M.; Jezussek, M.; Koehler, P.; Schieberle, P., Quantitation of 3-Aminopropionamide in PotatoesA Minor but Potent Precursor in Acrylamide Formation, *Journal of Agricultural and Food Chemistry*, 52, 4751-4757, **2004**.

[36] Doerge, D. R.; Young, J. F.; McDaniel, L. P.; Twaddle, N. C.; Churchwell, M. I., Toxicokinetics of acrylamide and glycidamide in B6C3F1 mice, *Toxicology and Applied Pharmacology*, 202, 258-267, **2005**.

[37] Doerge, D. R.; Young, J. F.; McDaniel, L. P.; Twaddle, N. C.; Churchwell, M. I., Toxicokinetics of acrylamide and glycidamide in

Fischer 344 rats, *Toxicology and Applied Pharmacology*, 208, 199-209, **2005**.

[38] Calleman, C. J.; Bergmark, E.; Costa, L. G., Acrylamide is metabolized to glycidamide in the rat: evidence from hemoglobin adduct formation, *Chemical Research in Toxicology*, 3, 406-412, **1990**.

[39] Mills, C.; Mottram, D. S.; Wedzicha, B. L., Acrylamide. *Process-Induced Food Toxicants: Occurence, Formation, Mitigation and Health Risks*, (eds: Stadler, R. H.; Lineback, D. R.), John Wiley & Sons, Inc: New Jersey, USA, 23-50, **2009**.

[40] Shipp, A.; Lawrence, G.; Gentry, R.; McDonald, T.; Bartow, H.; Bounds, J.; Macdonald, N.; Clewell, H.; Allen, B.; Van Landingham, C., Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects, *Critical Reviews in Toxicology*, 36, 481-608, **2006**.

[41] Doerge, D. R.; Gamboa da Costa, G.; McDaniel, L. P.; Churchwell, M. I.; Twaddle, N. C.; Beland, F. A., DNA adducts derived from administration of acrylamide and glycidamide to mice and rats, *Mutation Research*, 580, 131-141, **2005**.

[42] Sumner, S. C. J.; Fennell, T. R.; Moore, T. A.; Chanas, B.; Gonzalez, F.; Ghanayem, B. I., Role of Cytochrome P450 2E1 in the Metabolism of Acrylamide and Acrylonitrile in Mice, *Chemical Research in Toxicology*, 12, 1110-1116, **1999**.

[43] Boettcher, M. I.; Schettgen, T.; Kütting, B.; Pischetsrieder, M.; Angerer, J., Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population, *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 580, 167-176, **2005**.

[44] Fennell, T. R.; Friedman, M., Comparison of acrylamide metabolism in humans and rodents. *Chemistry and Safety of Acrylamide in food*, (eds: Friedman, M.; Mottram, D. S.), Springer, New York, 109-116, **2005**.

[45] Sumner, S. C.; Williams, C. C.; Snyder, R. W.; Krol, W. L.; Asgharian, B.; Fennell, T. R., Acrylamide: a comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure, *Toxicological Science*, 75, 260-70, **2003**.

[46] Sumner, S. C. J.; MacNeela, J. P.; Fennell, T. R., Characterization and quantitation of urinary metabolites of [1,2,3-13C]acrylamide in rats and mice using carbon-13 nuclear magnetic resonance spectroscopy, *Chemical Research in Toxicology*, 5, 81-89, **1992**.

[47] Rice, J. M., The carcinogenicity of acrylamide, *Mutation Research*, 580, 3-20, **2005**.

[48] JECFA, *Summary and Conclusions of the Sixty-Fourth Meeting. Food Contaminants*, Rome, **2005**.

[49] Tardiff, G. T.; Gargas, M. L.; Kirman, C. R.; Carson, M. L.; Sweeney, L. M., Estimation of safe dietary intake levels of acrylamide for humans, *Food and Chemical Toxicology*, 48, 658-667, **2010**.

[50] E.C., European Union Acrylamide Monitoring Data- base. **2006**.

[51] E.C., Commision recommendation on investigations into the levels of acrylamide in food. In *2013/647/EU*, **2013**.

[52] EFSA, *Update on acrylamide levels in food from monitoring years 2007 to 2010*, EFSA Journal, 10, 2938 – 2976, **2012**.

[53] FoodDrinkEurope, Acrylamide Toolbox. **2011**.

[54] Brathen, E.; Kita, A.; Knutsen, S. H.; Wicklund, T., Addition of Glycine Reduces the Content of Acrylamide in Cereal and Potato Products, *Journal of Agricultural and Food Chemistry*, 53, 3259-3264, **2005**.

[55] Mestdagh, F.; Maertens, J.; Cucu, T.; Delporte, K.; Van Peteghem, C.; De Meulenaer, B., Impact of additives to lower the formation of acrylamide in a potato model system through pH reduction and other mechanisms, *Food Chemistry*, 107, 26-31, **2008**.

[56] Low, M. Y.; Koutsidis, G.; Parker, J. K.; Elmore, J. S.; Dodson, A. T.; Mottram, D. S., Effect of Citric Acid and Glycine Addition on Acrylamide and Flavor in a Potato Model System, *Journal of Agricultural and Food Chemistry*, 54, 5976-5983, **2006**.

[57] Kim, C. T.; Hwang, E.-S.; Lee, H. J., Reducing Acrylamide in Fried Snack Products by Adding Amino Acids, *Journal of Food Science*, 70, C354-C358, **2005**.

[58] Gökmen, V.; Şenyuva, H. Z., Acrylamide formation is prevented by divalent cations during the Maillard reaction, *Food Chemistry*, 103, 196-203, **2007**.

[59] Gökmen, V.; Açar, Ö. Ç.; Köksel, H.; Acar, J., Effects of dough formula and baking conditions on acrylamide and hydroxymethylfurfural formation in cookies, *Food Chemistry*, 104, 1136-1142, **2007**.

[60] Capuano, E.; Oliviero, T.; Açar, Ö. Ç.; Gökmen, V.; Fogliano, V., Lipid oxidation promotes acrylamide formation in fat-rich model systems, *Food Research International*, 43, 1021-1026, **2010**.

[61] Cieserova, Z.; Kiss, E.; Boegl, P., Impact of L-asparaginase on acrylamide content in potato product., *Journal of Food and Nutrition Research*, 45, 141-146, **2006**.

[62] Anese, M.; Quarta, B.; Frias, J., Modelling the effect of asparaginase in reducing acrylamide formation in biscuits, *Food Chemistry*, 126, 435-440, **2011**.

[63] Pedreschi, F.; Kaack, K.; Granby, K., The effect of asparaginase on acrylamide formation in French fries, *Food Chemistry*, 109, 386-392, **2008**.

[64] Kukurova, K.; Ciesarova, Z.; Mogol, B. A.; Acar, O. C.; Gökmen, V., Raising agents strongly influence acrylamide and HMF formation in cookies and conditions for asparaginase activity in dough, *European Food Research and Technology*, 237, 1-8, **2013**.

[65] Rydberg, P.; Eriksson, S.; Tareke, E.; Karlsson, P.; Ehrenberg, L.; Törnqvist, M., Investigations of Factors That Influence the Acrylamide Content of Heated Foodstuffs, *Journal of Agricultural and Food Chemistry*, 51, 7012-7018, **2003**.

[66] Lindsay, R. C.; Jang, S. J., Chemical intervention strategies for substantial suppression of acrylamide formation in fried potato products. *Chemistry and Safety of Acrylamide in Food*, (eds: Friedman, M.; Mottram, D. S.), Springer, New York, 393-404, **2005**.

[67] Pedreschi, F.; Granby, K.; Risum, J., Acrylamide Mitigation in Potato Chips by Using NaCl, *Food and Bioprocess Technology*, 3, 917-921, **2010**.

[68] Amrein, T. M.; Schönbächler, B.; Escher, F.; Amadò, R., Acrylamide in Gingerbread: Critical Factors for Formation and Possible Ways for Reduction, *Journal of Agricultural and Food Chemistry*, 52, 4282-4288, **2004**.

[69] Becalski, A.; Lau, B. P. Y.; Lewis, D.; Seaman, S. W., Acrylamide in Foods: Occurrence, Sources, and Modeling, *Journal of Agricultural and Food Chemistry*, 51, 802-808, **2003**.

[70] Ames, J. M., The Maillard Reaction. *Biochemistry of Food Proteins*, (eds: Hudson, B. J. F.), Elsevier Applied Science: London, 99-153, **1992**.

[71] Kroh, L. W., Caramelisation in food and beverages, *Food Chemistry*, 51, 373-379, **1994**.

[72] Morales, F. J., Hydroxymethylfurfural (HMF) and related compounds. *Process-Induced Food Toxicants: Occurence, Formation, Mitigation and Health Risks*, (eds: Stadler, R. H.; Lineback, D. R.), A john Wiley & Sons, Inc, Publications, 135, **2009**.

[73] Antal Jr, M. J.; Mok, W. S. L.; Richards, G. N., Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from d-fructose and sucrose, *Carbohydrate Research*, 199, 91-109, **1990**.

[74] Capuano, E.; Fogliano, V., Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies, *LWT-Food Science and Technology*, 44, 793-810, **2011**.

[75] Locas, C. P.; Yaylayan, V. A., Isotope labeling studies on the formation of 5-(hydroxymethyl)-2-furaldehyde (HMF) from sucrose by

pyrolysis-GC/MS, *Journal of Agricultural and Food Chemistry*, 56, 6717-6723, **2008**.

[76] Lee, H. S.; Nagy, S., Relative Reactivities of Sugars in The Formation Of 5-Hydroxymethylfurfural in Sugar-Catalyst Model Systems, *Journal of Food Processing and Preservation*, 14, 171-178, **1990**.

[77] Gökmen, V.; Açar, Ö. Ç.; Serpen, A.; Morales, F. J., Effect of leavening agents and sugars on the formation of hydroxymethylfurfural in cookies during baking, *European Food Research and Technology*, 226, 1031-1037, **2008**.

[78] Gökmen, V.; Şenyuva, H. Z., Improved method for the determination of hydroxymethylfurfural in baby foods using liquid chromatography-mass spectrometry, *Journal of Agricultural and Food Chemistry*, 54, 2845-2849, **2006**.

[79] Surh, Y. J.; Liem, A.; Miller, J. A.; Tannenbaum, S. R., 5-Sulfooxymethylfur- fural as a possible ultimate mutagenic and carcinogenic metabolite of the Maillard reaction product, 5-hydroxymethylfurfural., *Carcinogenesis*, 15, 2375-2377, **1994**.

[80] Lee, Y. C.; Shlyankevich, M.; Jeong, H. K.; Douglas, J. S.; Surh, Y. J., Bioactivation of 5-Hydroxymethyl-2-Furaldehyde to an Electrophilic and Mutagenic Allylic Sulfuric Acid Ester, *Biochemical and Biophysical Research Communications*, 209, 996-1002, **1995**.

[81] Surh, Y.-J.; Tannenbaum, S. R., Activation of the Maillard Reaction Product 5-(Hydroxymethyl)furfural to Strong Mutagens via Allylic Sulfonation and Chlorination, *Chemical Research in Toxicology*, 7, 313-318, **1994**.

[82] EFSA, *Scientific Opinion: Furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms from chemical group 14*, The EFSA Journal, 8, 1403, **2010**.

[83] Abraham, K.; Gürtler, R.; Berg, K.; Heinemeyer, G.; Lampen, A.; Appel, K. E., Toxicology and risk assessment of 5-Hydroxymethylfurfural in food, *Molecular Nutrition & Food Research*, 55, 667-678, **2011**.

[84] EFSA, *Scientific opinion on the re-evaluation of caramel colours (E 150 a,b,c,d) as food additives. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)*, The EFSA Journal, 9, 2004, **2011**.

[85] Ibarz, A.; Pagán, J.; Garza, S., Kinetic models of non-enzymatic browning in apple puree, *Journal of the Science of Food and Agriculture*, 80, 1162-1168, **2000**.

[86] Rufián-Henares, J.; García-Villanova, B.; Guerra-Hernández, E., Occurrence of furosine and hydroxymethylfurfural as markers of

thermal damage in dehydrated vegetables, *European Food Research and Technology*, 228, 249-256, **2008**.

[87] Alimentarius, C., Codex Standard for Honey. In 1981; Vol. 12-1981.

[88] AIJN, Association of the Industry of Juices and Nectars of the European Economic Community Code of Practice for Evaluation of Fruit and Vegetable Juices. In AIJN: Brussels, 1996.

[89] Gökmen, V.; Şenyuva, H. Z., Effects of some cations on the formation of acrylamide and furfurals in glucose–asparagine model system, *European Food Research and Technology*, 225, 815-820, **2007**.

[90] Akıllıoglu, H. G.; Mogol, B. A.; Gökmen, V., Degradation of 5-hydroxymethylfurfural during yeast fermentation, *Food Additives & Contaminants. Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment*, 28, 1629-1635, **2011**.

[91] Hamlet, C. G.; Sadd, P. A., Chloropropanols and Chloroesters. *Process-Induced Food Toxicants. Occurrence, Formation, Mitigation and Health Risks*, (eds: Stadler, R. H.; Lineback, D. R.), Hoboken, John Wiley & Sons, Inc., 175-214, **2009**.

[92] Velíšek, J.; Davídek, J.; Kubelka, V.; Bartošova, J.; Tucková, A.; Hajšlová, J.; Janíček, G., Formation of volatile chlorohydrins from glycerol triacetin, tributyrin and hydrochloric acid, *Lebensmittel Wissenschafts Technologie*, 12, **1979**.

[93] Hamlet, C. G.; Sadd, P. A.; Crews, C.; Velisek, J.; Baxter, D. E., Occurrence of 3-chloro-propane-1,2-diol (3-MCPD) and related compounds in foods: a review, *Food Additives and Contaminants*, 19, 619-31, **2002**.

[94] RSC, ChemSpider, http://www.chemspider.com/Chemical-Structure.7018.html?rid=c0777caf-64a9-484c-b82b-397a1be65c6a (April **2014**).

[95] Abraham, K.; Appel, K. E.; Berger-Preiss, E.; Apel, E.; Gerling, S.; Mielke, H.; Creutzenberg, O.; Lampen, A., Relative oral bioavailability of 3-MCPD from 3-MCPD fatty acid esters in rats, *Archives of Toxicology*, 87, 649-659, **2013**.

[96] Barocelli, E.; Corradi, A.; Mutti, A.; Petronini, P. G., *Comparison between 3-MCPD and its palmitic esters in a 90-day toxicological study*, Parma, Italy, **2011**.

[97] Hamlet, C.; Asuncion, L.; Velíšek, J.; Doležal, M.; Zelinkova, Z.; Crews, C., Formation and occurrence of esters of 3-chloropropane- 1,2-diol (3-CPD) in foods: What we know and what we assume, *European Journal of Lipid Science and Technology*, 113, 279-303, **2011**.

[98] Velíšek, J.; Davídek, J.; Hajšlová, J.; Kubelka, V.; Janíček, G.; Mánková, B., Chlorohydrins in protein hydrolysates., *Zeitschrift für Lebensmittel-untersuchung und -Forschung*, 167, 241-244, **1978**.

[99] Davídek, J.; Velíšek, J.; Kubelka, V.; G., J., *New chlorine-containing compounds in protein hydrolysates:* Euro Food Chem I., Vienna, Austria, 1981;., (eds: Baltes, W., Czedik-Eysenberg, P. B., Pfannhauser, W.), 322-325, **1981**.

[100] Collier, P. D.; Cromie, D. D. O.; Davies, A. P., Mechanism of formation of chloropropanols present in protein hydrolysates, *Journal of the American Oil Chemists Society*, 68, 785-790, **1991**.

[101] Doležal, M.; Velíšek, J., Kinetics of 3-chloro-1,2-propanediol degradation in model systems., *Proceedings of Chemical Reactions in Foods II*, 24-26 September, 1992, Prague, Czech Republic, 297-302, **1992**.

[102] Doležal, M.; Calta, P.; Velíšek, J., Formation and decomposition of 3-chloropropane-1,2-diol in model systems, *Czech Journal of Food Sciences*, 22, 263-266, **2004**.

[103] Calta, P.; Velíšek, J.; Doležal, M.; Hasnip, S.; Crews, C.; Réblová, Z., Formation of 3-chloropropane-1,2-diol in systems simulating processed foods, *European Food Research and Technology*, 218, 501-506, **2004**.

[104] Crews, C.; Brereton, P.; Davies, A., The effects of domestic cooking on the levels of 3-monochloropropanediol in foods, *Food Additives and Contaminants*, 18, 271-80, **2001**.

[105] Reece, P., *The origin and formation of 3-MCPD in foods and food ingredients (final project report)*, London **2005**.

[106] Hamlet, C. G.; Sadd, P. A.; Gray, D. A., Influence of composition, moisture, pH and temperature on the formation and decay kinetics of monochloropropanediols in wheat flour dough, *European Food Research and Technology*, 216, 122-128, **2003**.

[107] Hamlet, C. G.; Sadd, P. A.; Gray, D. A., Generation of monochloropropanediols (MCPDs) in model dough systems. 1. Leavened doughs, *Journal of Agricultural and Food Chemistry*, 52, 2059-66, **2004**.

[108] ILSI, *3-MCPD esters in food products. Summary report of a workshop held in February 2009,* ILSI Europe Report Series *in International Life Sciences Institute, Brussels, Belgium*, **2009**.

[109] Svejkovska, B.; Doležal, M.; Velíšek, J., Formation and decomposition of 3-Chloropropane-1,2-diol esters in models simulating processed foods, *Czech Journal of Food Sciences*, 24, 172-179, **2006**.

[110] Sunhara, G.; Perrin, L.; Marchessini, M., *Carcinogenicity study on 3-monochloro propane 1,2-diol(3- MCPD) administered in drinking water to Fischer 344 rats.*, Nestec Ltd., Research and Development, RE-SR93003, Switzerland, **1993**.

[111] FAO/WHO, *Discussion paper on chloropropanols derived from the manufacture of acid-HVP and the heat processing of food*, Proc 1st

Session of Codex Committee on Contaminants in Foods, Beijing, China, **2007**.

[112] JECFA, Safety evaluation of certain food additives and contaminants, Vol. 48, pp 401-432, **2002**.

[113] EC, Setting maximum levels for certain contaminants in foodstuffs. In Official Journal of the European Communities ed.; Commission, E., Ed. Office for Official Publications of the European Communities: Luxembourgh, 2001; Vol. Regulation (EC) No. 466/2001, pp 1-13.

[114] Porter, K. E.; Jones, A. R., The effect of the isomers of alpha-cholorohydrin and racemic beta-chlorolactate on the rat kidney, *Chemico-Biological Interaction*, 41, 95-104, **1982**.

[115] Ford, W. C.; Waites, G. M., Activities of various 6-chloro-6-deoxysugars and (S) alpha-chlorohydrin in producing spermatocoeles in rats and paralysis in mice and in inhibiting glucose metabolism in bull spermatozoa in vitro, *Journal of Reproduction and Fertility*, 65, 177-83, **1982**.

[116] Bakhiya, N.; Abraham, K.; Gürtler, R.; Appel, K. E.; Lampen, A., Toxicological assessment of 3-chloropropane-1,2-diol and glycidol fatty acid esters in food, *Molecular Nutrition and Food Research*, 55, 509-521, **2011**.

[117] Robert, M. C.; Oberson, J. M.; Stadler, R. H., Model studies on the formation of monochloropropanediols in the presence of lipase., *Journal of Agricultural and Food Chemistry*, 52, 5102-5108, **2004**.

[118] EC *E.C., 2001, Opinion of the Scientific Committee on Food on 3-Monochloro-propane-1,2-diol (3-MCPD) Updating the SCF Opinion of 1994 (adopted on 30 May 2001)*, **2001**.

[119] FSA, *Survey of 3-monochloropropane-1,2-diol (3-MCPD) in selected food groups*, London, **2001**.

[120] Crews, C.; Hough, P.; Brereton, P.; Harvey, D.; MacArthur, R.; Matthews, W., Survey of 3-monochloropropane-1,2-diol (3-MCPD) in selected food groups, 1999-2000, *Food Additives and Contaminants*, 19, 22-27, **2002**.

[121] Massey, R.; Hamlet, C. G., Chloropropanol contaminants in food: the story continues, *Food Science and Technology*, 21, 32-34, **2007**.

[122] Massey, R., Fight against 3-MCPD, *Food Manufacture*, 82, 35-36, **2007**.

[123] Hamlet, C. G.; Jayaratne, S. M.; Matthews, W., 3-Monochloropropane-1,2-diol (3-MCPD) in food ingredients from UK food producers and ingredient suppliers, *Food Additives and Contaminants*, 19, 15-21, **2002**.

[124] Breitling-Utzmann, C. M. 3-MCPD: Untersuchungen in Lebensmitteln. http://www.lebensmittel.org/wissenswertes/lebensmittel-kosmetika-und-bedarfsgegenstaende/67-3-mcpd-untersuchungen-in-lebensmitteln.html (01 April 2014).

[125] Breitling-Utzmann, C. M.; Bler, H.; Herzbolzheimer, D.; Maier, A., *3-MCPD: Occurrence in bread crust and various food groups as well as formation in toast*, Behr's Verlag: Hamburg, ALLEMAGNE, Vol. 99, 6. **2003.**

[126] Baer, I.; de la Calle, B.; Taylor, P., 3-MCPD in food other than soy sauce or hydrolysed vegetable protein (HVP), *Analytical and Bioanalytical Chemistry*, 396, 443-456, **2010**.

[127] Hamlet, C. G., Sadd, P.A, Chloropropanols and chloroesters. *Process-Induced Food Toxicants: Occurence, Formation, Mitigation and Health Risks*, (eds: Stadler, R. H.; Lineback, D. R.), A john Wiley & Sons, Inc, Publications, 135, **2009**.

[128] IARC, *Furan, Dry cleaning, some chlorinated solvents and other industrial chemicals.* , International Agency for Research on Cancer (IARC) Monograph, 63, 393, **1995**.

[129] Moro, S.; Chipman, J. K.; Wegener, J. W.; Hamberger, C.; Dekant, W.; Mally, A., Furan in heat-treated foods: Formation, exposure, toxicity, and aspects of risk assessment, *Molecular Nutrition & Food Research*, 56, 1197-1211, **2012**.

[130] Maga, J. A., Furans in Foods, *Crc Critical Reviews in Food Science and Nutrition*, 11, 355-400, **1979**.

[131] US-FDA, *Exploratory Data on Furan in Food*, **2004**.

[132] Perez Locas, C.; Yaylayan, V. A., Origin and mechanistic pathways of formation of the parent furan - A food toxicant, *Journal of Agricultural and Food Chemistry*, 52, 6830-6836, **2004**.

[133] EFSA, *Update on furan levels in food from monitoring years 2004-2010 and exposure assessment*, The EFSA Journal, 9, 2347, **2011**.

[134] Burka, L. T.; Washburn, K. D.; Irwin, R. D., Disposition of [14C]furan in the male F344 rat, *Journal of Toxicology and Environmental Health*, 34, 245-257, **1991**.

[135] NTP, *Toxicology and Carcinogenesis Studies of Furan (CAS No. 110-00-9) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*, NTP Technical Report No. 402, Research Triangle Park, NC., **1993**.

[136] FAO/WHO, *Summary and conclusions report of the seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*, 1-16, **2010**.

[137] Guenther, H.; Hoenicke, K.; Biesterveld, S.; Gerhard-Rieben, E.; Lantz, I., Furan in coffee: pilot studies on formation during roasting and losses during production steps and consumer handling, *Food Additives and Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment*, 27, 283-290, **2010**.

[138] Van Lancker, F.; Adams, A.; Owczarek, A.; De Meulenaer, B.; De Kimpe, N., Impact of various food ingredients on the retention of furan in foods, *Molecular Nutrition & Food Research*, 53, 1505-1511, **2009**.

[139] Zoller, O.; Sager, F.; Reinhard, H., Furan in food: Headspace method and product survey, *Food Additives and Contaminants*, 24, 91-107, **2007**.

[140] EFSA, *Update of results on the monitoring of furan levels in food*, The EFSA Journal, 8, 1702-1720, **2010**.

[141] Mariotti, M. S.; Granby, K.; Rozowski, J.; Pedreschi, F., Furan: a critical heat induced dietary contaminant, *Food & Function*, 4, 1001-1015, **2013**.

[142] Jestoi, M.; Jarvinen, T.; Jarvenpaa, E.; Tapanainen, H.; Virtanen, S.; Peltonen, K., Furan in the baby-food samples purchased from the Finnish markets – Determination with SPME-GC-MS, *Food Chemistry*, 117, 522-528, **2009**.

[143] Lachenmeier, D. W.; Maser, E.; Kuballa, T.; Reusch, H.; Kersting, M.; Alexy, U., Detailed exposure assessment of dietary furan for infants consuming commercially jarred complementary food based on data from the DONALD study, *Maternal & Child Nutrition*, 8, 390-403, **2012**.

[144] Altaki, M. S.; Santos, F. J.; Galceran, M. T., Occurrence of furan in coffee from Spanish market: Contribution of brewing and roasting, *Food Chemistry*, 126, 1527-1532, **2011**.

[145] Kim, T.-K.; Lee, Y.-K.; Kim, S.; Park, Y. S.; Lee, K.-G., Furan in Commercially Processed Foods: Four-Year Field Monitoring and Risk Assessment Study in Korea, *Journal of Toxicology and Environmental Health, Part A*, 72, 1304-1310, **2009**.

[146] Hasnip, S.; Crews, C.; Castle, L., Some factors affecting the formation of furan in heated foods, *Food Additives and Contaminants*, 23, 219-227, **2006**.

[147] Crews, C.; Castle, L., A review of the occurrence, formation and analysis of furan in heat-processed foods, *Trends in Food Science & Technology*, 18, 365-372, **2007**.

[148] Du, C.-J.; Sun, D.-W., Learning techniques used in computer vision for food quality evaluation: a review, *Journal of Food Engineering*, 72, 39-55, **2006**.

[149] Gunasekaran, S., Computer vision technology for food quality assurance, *Trends in Food Science and Technology*, 7, 245-256, **1996**.

[150] Wu, D.; Sun, D.-W., Colour measurements by computer vision for food quality control – A review, *Trends in Food Science and Technology*, 29, 5-20, **2013**.

[151] Solomon, C.; Breckon, T., *Fundamentals of Digital Image Processing, A Practical Approach with Examples in Matlab*, A John Wiley & Sons, Ltd.: NJ, USA, **2011**.

[152] Gonzales-Barron, U.; Butler, F., A comparison of seven thresholding techniques with the k-means clustering algorithm for measurement of bread-crumb features by digital image analysis, *Journal of Food Engineering*, 74, 268-278, **2006**.

[153] Gonzales, R. C.; Woods, R. E., *Digital Image Processing*, Prentice Hall: NJ, USA, **2002**.

[154] Gökmen, V.; Süğüt, İ., A Non-Contact Computer Vision Based Analysis of Color in Foods, *International Journal of Food Engineering*, 3, Article 5, **2007**.

[155] Papadakis, S. E.; Abdul-Malek, S.; Kamdem, R. E.; Yam, K. L., A Versatile and Inexpensive Technique for Measuring Color of Foods, *Food Technology*, 54, 48-51, **2000**.

[156] Segnini, S.; Dejmek, P.; Öste, R., A low cost video technique for colour measurement of potato chips, *LWT - Food Science and Technology*, 32, 216-222, **1999**.

[157] Yam, K. L.; Papadakis, S. E., A simple digital imaging method for measuring and analyzing color of food surfaces, *Journal of Food Engineering*, 61, 137-142, **2004**.

[158] Hunt, R. W. G., *Measuring colour*, 2nd ed.; Ellis Horwood Limited: Chichester, England, **1991**.

[159] Socaciu, C.; Diehl, H. A., Instruments to Analyze Food Colors. In *Handbook of Food Analysis Instruments*, (ed: Ötleş, S.), Taylor & Francis Group: Florida, USA, 14, **2009**.

[160] Pedreschi, F.; Moyano, P.; Kaack, K.; Granby, K., Color changes and acrylamide formation in fried potato slices, *Food Research International*, 38, 1-9, **2005**.

[161] Serpen, A.; Gökmen, V., Evaluation of the Maillard reaction in potato crisps by acrylamide, antioxidant capacity and color, *Journal of Food Composition and Analysis*, 22, 589-595, **2009**.

[162] Gökmen, V.; Açar, Ö. Ç.; Arribas-Lorenzo, G.; Morales, F. J., Investigating the correlation between acrylamide content and browning

ratio of model cookies, *Journal of Food Engineering*, 87, 380-385, **2008**.

[163] AACC, Approved methods of the American Association of Cereal Chemists. In Association of Cereal Chemists: Minnesota, Vol. 10-54, **2000**.

[164] Gökmen, V.; Morales, F. J.; Ataç, B.; Serpen, A.; Arribas-Lorenzo, G., Multiple-stage extraction strategy for the determination of acrylamide in foods, *Journal of Food Composition and Analysis*, 22, 142-147, **2009**.

[165] Gökmen, V.; Mogol, B. A., Computer vision-based image analysis for rapid detection of acrylamide in heated foods, *Quality Assurance and Safety of Crops & Foods*, 2, 203-207, **2010**.

[166] Fiore, A.; Troise, A. D.; Mogol, B. A.; Roullier, V.; Gourdon, A.; Jian, S. E. M.; Hamzalıoğlu, B. A.; Gökmen, V.; Fogliano, V., Controlling the Maillard Reaction by Reactant Encapsulation: Sodium Chloride in Cookies, *Journal of Agricultural and Food Chemistry*, 60, 10808-10814, **2012**.

[167] Gökmen, V.; Serpen, A.; Mogol, B. A., Rapid determination of amino acids in foods by hydrophilic interaction liquid chromatography coupled to high-resolution mass spectrometry, *Analytical and Bioanalytical Chemistry*, 403, 2915-2922, **2012**.

[168] AACC, AACC International Approved methods of analysis Method, 44-15.02. In 2009.

[169] Wolfram|Alpha, In 2014.

[170] Wong, K.-F. V., Fundamentals of Heat Transfer. *Intermediate Heat Transfer*, CRC Press, **2003**.

[171] Saidi, M.; Abardeh, R. H. In *Air Pressure Dependence of Natural-Convection Heat Transfer*, World Congress on Engineering London, U.K., June 30 - July 2, 2010, London, U.K., **2010**.

[172] Kocadağlı, T.; Palazoğlu, T. K.; Gökmen, V., Mitigation of acrylamide formation in cookies by using Maillard reaction products as recipe modifier in a combined partial conventional baking and radio frequency post-baking process, *European Food Research and Technology*, 235, 711-717, **2012**.

[173] Anese, M.; Sovrano, S.; Bortolomeazzi, R., Effect of radiofrequency heating on acrylamide formation in bakery products, *European Food Research and Technology*, 226, 1197-1203, **2008**.

[174] Palazoğlu, T. K.; Coşkun, Y.; Kocadağlı, T.; Gökmen, V., Effect of Radio Frequency Postdrying of Partially Baked Cookies on Acrylamide Content, Texture, and Color of the Final Product, *Journal of Food Science*, 77, E113-E117, **2012**.

[175] Anese, M.; Suman, M.; Nicoli, M. C., Acrylamide removal from heated foods, *Food Chemistry*, 119, 791-794, **2010**.

[176] Quarta, B.; Anese, M., Furfurals removal from roasted coffee powder by vacuum treatment, *Food Chemistry*, 130, 610-614, **2012**.

[177] Deutsche Einheitsmethoden zur Unter-suchung von Fetten, F., Tensiden und verwandten Stoffen, Wissenschaftliche Verlagsgesellschaft, Fatty-acid-bound 3-chloropropane-1,2-diol (3-MCPD) and 2,3-epoxi-propane-1-ol (glycidol). In Stuttgart, Germany, **2011**.

[178] Zelinkova, Z.; Svejkovska, B.; Velisek, J.; Dolezal, M., Fatty acid esters of 3-chloropropane-1,2-diol in edible oils, *Food Additives and Contaminants*, 23, 1290-8, **2006**.

[179] Xu, H.; Templeton, A. C.; Reed, R. A., Quantification of 5-HMF and dextrose in commercial aqueous dextrose solutions, *Journal of Pharmaceutical and Biomedical Analysis*, 32, 451-459, **2003**.

[180] Serpen, A.; Gökmen, V., Reversible degradation kinetics of ascorbic acid under reducing and oxidizing conditions, *Food Chemistry*, 104, 721-725, **2007**.

[181] Becalski, A.; Seaman, S., Furan precursors in food: A model study and development of a simple headspace method for determination of furan, *Journal of AOAC International*, 88, 102-106, **2005**.

[182] Van Boekel, M. A. J. S., Kinetic Modeling of Food Quality: A Critical Review, *Comprehensive Reviews in Food Science and Food Safety*, 7, 144-158, **2008**.

[183] Villota, R.; Hawkes, J. G., Reaction kinetics in food system. *Handbook of Food Engineering*, (eds: Heldman, D. R.; Lund, D. B.), New York, NY, **1992**.

ANNEX

Code for CIE L*a*b* color measurement

```
RGB=im2double(RGB);
RGB=imresize(RGB, 0.1);
Z=roipoly(RGB);
[d1,d2]=size(Z);
c=0;
L=[];
for a=1:d1
    for b=1:d2
        if Z(a,b)==1
n=1;
c=c+1;
L(n,c)=a;
n=2;
L(n,c)=b;
        end
    end
end
cform = makecform('srgb2lab');
lab = applycform(RGB,cform);
P=[];
for n=1:c
P(n,:)=impixel(lab,L(2*n),L(2*n-1));
end
roil=[];roia=[];roib=[];
sum_l=0;sum_a=0;sum_b=0;
for n=1:c
    sum_l=sum_l+P(n,1);
    sum_a=sum_a+P(n,2);
    sum_b=sum_b+P(n,3);
end
roil=sum_l/c;
roia=sum_a/c;
roib=sum_b/c;
Lab__value=[roil roia roib]
close all
```

Code for brown % and dark brown % measurement

```
% File VectorQuantize.m
% ====================
function [seg_im] = VectorQuantize(im_seg,u);
u=im2double(u);
[r c h]=size(im_seg);
% reduce from 3 dimensions to 2 dimensions for easy handling
of data
im=reshape(im2double(im_seg),r*c,h)';
% compute the distance from cluster centers for all pixels
for i=1:4
    dist(i,:)=sum((im-repmat(u(:,i),[1 r*c])).^2);
end
% find and store the location of minimum distance cluster
for each pixel
[y loc]=min(dist);
seg_im=zeros(r*c,h);
% change pixels values with their representative cluster
means for displaying purposes
for i=1:4
    pos=find(loc==i);
    seg_im(pos,:)=repmat(u(:,i)',[length(pos) 1]);
end
% restore the image back to its original dimensions
seg_im=reshape(seg_im,[r c h]);
% display the segmented image in new window
figure(2); imshow(seg_im,[]);
% compute AN2 ratio from segmented image
ratio=length(find(loc==2))/length(find(loc~=4)); %brown
ratio
ratio=length(find(loc==3))/length(find(loc~=4)); %dark brown
ratio
% display this ratio in command prompt
disp(ratio);
```

Code for color measurement on a selected area from video streaming

```
clear all
%Read Video file
obj=VideoReader('cam2-2.mov');
%Define frame per second
framepers=obj.FrameRate;
%Define frame per minute for analysis
frameperm=uint16(framepers*60);
%Total frame number
framenumber=obj.NumberOfFrames;
%Dimension of Video frame
height=obj.Height;
width=obj.Width;

for i=1:frameperm:12*frameperm %frames until 12th min.
maxAreaComponentIndex=0;
maxArea=0;
video=read(obj,i); %Take frame from video
BW=im2bw(video,0.60); %Convert it into binary image
Z=imcrop(BW,[320 200 220 130]); %Crop the right cookie
cc=bwconncomp(Z,8); %connected components with 8
neighbours

objnumber=cc.NumObjects; %Numbers of connected components
cookiedata = regionprops(cc, 'basic');
cookie=false(size(Z));
        for j=1:objnumber
            if cookiedata(j).Area > maxArea
                maxArea = cookiedata(j).Area;
                maxAreaComponentIndex = j;
            end

        end

cookie(cc.PixelIdxList{maxAreaComponentIndex})=true;
        cookie_edges=edge(cookie,'canny',0.7);

            min_i=1000;
            max_i=0;
            min_j=1000;
            max_j=0;
            for k=1:131
            for j=1:221
                if cookie(k,j)==1
                    if k > max_i
                        max_i = k;
                    end
                    if k<min_i
                        min_i=k;
                    end
```

```
                    if j > max_j
                        max_j = j;
                    end
                    if j<min_j
                        min_j=j;
                    end
                end
            end
            end
subplot(1,4,1);imshow(cookie_edges);
middle=min_i+(((max_i-20)-min_i)/2);
x1=min_j+7;
y1=middle-15;
croppedarea=imcrop(cookie_edges,[x1 y1 30 30]);
subplot(1,4,2);imshow(croppedarea);
Z2=imcrop(video,[320 200 220 130]);
subplot(1,4,3);imshow(Z2);
croppedarea2=imcrop(Z2,[x1 y1 30 30]);
subplot(1,4,4);imshow(croppedarea2);
croppedarea2=im2double(croppedarea2);

    for a=1:30
    for b=1:30
        X(a,b)=1;
    end
    end

    [d1,d2]=size(X);
    c=0;
    L=[];
    for a=1:d1
        for b=1:d2
            if X(a,b)==1
    n=1;
    c=c+1;
    L(n,c)=a;
    n=2;
    L(n,c)=b;
            end
        end
    end
    cform = makecform('srgb2lab');
    lab = applycform(croppedarea2,cform);
    P=[];
    for n=1:c
    P(n,:)=impixel(lab,L(2*n),L(2*n-1));
    end
    roil=[];roia=[];roib=[];
    sum_l=0;sum_a=0;sum_b=0;
    for n=1:c
        sum_l=sum_l+P(n,1);
        sum_a=sum_a+P(n,2);
```

```
        sum_b=sum_b+P(n,3);
    end
    roil=sum_l/c;
    roia=sum_a/c;
    roib=sum_b/c;
    Lab__value=[roil roia roib]
    if roia>-8.0
        sprintf('%s','FAIL')
    else
        sprintf('%s','PASS')
    end

end
```

CURRICULUM VITAE

Credentials

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Education

High School: Ankara Anatolian High School (1994-2001)

BSc :Hacettepe University, Department of Food Engineering (2001-2006)

MSc : Hacettepe University, Department of Food Engineering (2006-2009)

PhD : Hacettepe University, Department of Food Engineering (2009-2014)

Foreign Languages

English (Fluent); German (Elementary)

Work Experience

Research Assistant, Hacettepe University, Department of Food Engineering (2007-Current)

Areas of Experiences

Food Engineering, Food Chemistry, Food Safety

Projects and Budgets - Involved as researcher

International projects:

[1] FP7-KBBE-2010-4 "PROMETHEUS – PROcess contaminants: Mitigation and Elimination Techniques for High food quality and their

Evaluation Using Sensors & Simulation" 2011-2014 (Project No: 265558)

[2] FP7-SME-2007-1 "NANOFOODS – Development of foods containing nano-encapsulated ingredient" 2008-2010 (Project No: 222006)

[3] COST 928 "Investigation of the Inhibition Possibilities of Oxidative Enzymes in Foods by Maillard Reaction Products" 2006-2010 (Project No: 105O716)

National Project:

[1] BAP "Mitigation of Acrylamide Formation in Bread during Baking by Adding Amino Acids and Minerals" 2007-2009 (Project No: 07.01.602.010)

Publications

Published within this PhD thesis are indicated as **[bold]**

[1] Mogol, B.A., Pye, C., Anderson, W., Crews, C., Gökmen, V. (2014) Formation of MCPD and its esters in biscuits during baking. Journal of Agricultural and Food Chemistry (submitted).

[2] Mogol, B. A.; Gökmen, V., Computer vision-based analysis of foods: A non-destructive colour measurement tool to monitor quality and safety, *Journal of the Science of Food and Agriculture*, 94, 1259-1263, **2014**.

[3] Zilic, S.; Mogol, B. A.; Akıllıoğlu, G.; Serpen, A.; Delic, N.; Gökmen, V., Effects of extrusion, infrared and microwave processing on Maillard reaction products and phenolic compounds in soybean, *Journal of the Science of Food and Agriculture*, 94, 45-51, **2014**.

[4] Van Der Fels-Klerx, H. J.; Capuano, E.; Nguyen, H. T.; Ataç Mogol, B.; Kocadağlı, T.; Göncüoğlu Taş, N.; Hamzalıoğlu, A.; Van Boekel, M. A. J. S.; Gökmen, V., Acrylamide and 5-hydroxymethylfurfural formation during baking of biscuits: NaCl and

temperature–time profile effects and kinetics, *Food Research International*, 57, 210-217, **2014**.

[5] Truong, V. D.; Pascua, Y. T.; Reynolds, R.; Thompson, R. L.; Palazoğlu, T. K.; Mogol, B. A.; Gökmen, V., Processing Treatments for Mitigating Acrylamide Formation in Sweetpotato French Fries, *Journal of Agricultural and Food Chemistry*, 62, 310-316, **2014**.

[6] Mogol, B. A.; Gökmen, V., Mitigation of Acrylamide and Hydroxymethylfurfural in Biscuits Using A Combined Partial Conventional Baking and Vacuum Post-Baking Process: Preliminary Study at the Lab Scale, *Innovative Food Science & Emerging Technologies*, **2014**.

[7] Zilic, S.; Mogol, B. A.; Akıllıoğlu, G.; Serpen, A.; Babic, M.; Gökmen, V., Effects of infrared heating on phenolic compounds and Maillard reaction products in maize flour, *Journal of Cereal Science*, 58, 1-7, **2013**.

[8] Mogol, B. A.; Gökmen, V.; Shimoni, E., Nano-encapsulation improves thermal stability of bioactive compounds Omega fatty acids and silymarin in bread, *Agro Food Industry Hi-Tech*, 24, 62-65, **2013**.

[9] Mogol, B. A.; Gökmen, V., Kinetics of Furan Formation from Ascorbic Acid during Heating under Reducing and Oxidizing Conditions, *Journal of Agricultural and Food Chemistry*, 61, 10191-10196, **2013**.

[10] Kukurova, K.; Ciesarova, Z.; Mogol, B. A.; Açar, O. C.; Gökmen, V., Raising agents strongly influence acrylamide and HMF formation in cookies and conditions for asparaginase activity in dough, *European Food Research and Technology*, 237, 1-8, **2013**.

[11] Hamzalıoğlu, A.; Mogol, B. A.; Lumaga, R. B.; Fogliano, V.; Gökmen, V., Role of curcumin in the conversion of asparagine into acrylamide during heating, *Amino Acids*, 44, 1419-1426, **2013**.

[12] Alasalvar, C.; Pelvan, E.; Özdemir, K. S.; Kocadağlı, T.; Mogol, B. A.; Pasli, A. A.; Özcan, N.; Özçelik, B.; Gökmen, V.,

Compositional, Nutritional, and Functional Characteristics of Instant Teas Produced from Low- and High-Quality Black Teas, *Journal of Agricultural and Food Chemistry*, 61, 7529-7536, **2013**.

[13] Serpen, A.; Pelvan, E.; Alaşalvar, C.; Mogol, B. A.; Yavuz, H. T.; Gökmen, V.; Özcan, N.; Özçelik, B., Nutritional and Functional Characteristics of Seven Grades of Black Tea Produced in Turkey, *Journal of Agricultural and Food Chemistry*, 60, 7682-7689, **2012**.

[14] Serpen, A.; Gökmen, V.; Mogol, B. A., Effects of different grain mixtures on Maillard reaction products and total antioxidant capacities of breads, *Journal of Food Composition and Analysis*, 26, 160-168, **2012**.

[15] Gökmen, V.; Serpen, A.; Mogol, B. A., Rapid determination of amino acids in foods by hydrophilic interaction liquid chromatography coupled to high-resolution mass spectrometry, *Analytical and Bioanalytical Chemistry*, 403, 2915-2922, **2012**.

[16] Gökmen, V.; Kocadağlı, T.; Göncüoğlu, N.; Mogol, B. A., Model studies on the role of 5-hydroxymethyl-2-furfural in acrylamide formation from asparagine, *Food Chemistry*, 132, 168-174, **2012**.

[17] Fiore, A.; Troise, A. D.; Mogol, B. A.; Roullier, V.; Gourdon, A.; Jian, S. E. M.; Hamzalıoğlu, B. A.; Gökmen, V.; Fogliano, V., Controlling the Maillard Reaction by Reactant Encapsulation: Sodium Chloride in Cookies, *Journal of Agricultural and Food Chemistry*, 60, 10808-10814, **2012**.

[18] Gökmen, V.; Mogol, B. A.; Lumaga, R. B.; Fogliano, V.; Kaplun, Z.; Shimoni, E., Development of functional bread containing nanoencapsulated omega-3 fatty acids, *Journal of Food Engineering*, 105, 585-591, **2011**.

[19] Ataç, B.; Gökmen, V., Adsorption of dark colored compounds in apple juice - Effects of initial soluble solid concentration on adsorption kinetics and mechanism, *Journal of Food Process Engineering*, 34, 108-124, **2011**.

[20] Akıllıoğlu, H. G.; Mogol, B. A.; Gökmen, V., Degradation of 5-hydroxymethylfurfural during yeast fermentation, *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment*, 28, 1629-1635, **2011**.

[21] Gökmen, V.; Mogol, B. A., Computer vision-based image analysis for rapid detection of acrylamide in heated foods, *Quality Assurance and Safety of Crops & Foods*, 2, 203-207, **2010**.

[22] Ataç, B. M.; Yıldırım, A.; Gökmen, V., Inhibition of enzymatic browning in actual food systems by the Maillard reaction products, *Journal of the Science of Food and Agriculture*, 90, 2556-2562, **2010**.

[23] Gökmen, V.; Morales, F. J.; Ataç, B.; Serpen, A.; Arribas-Lorenzo, G., Multiple-stage extraction strategy for the determination of acrylamide in foods, *Journal of Food Composition and Analysis*, 22, 142-147, **2009**.

[24] Serpen, A.; Ataç, B.; Gökmen, V., Adsorption of Maillard reaction products from aqueous solutions and sugar syrups using adsorbent resin, *Journal of Food Engineering*, 82, 342-350, **2007**.

Oral Presentations

Presented within this PhD thesis are indicated as **[bold]**

[1] Mogol, B.A., Gökmen, V., (Invited Speaker) Combined conventional – vacuum baking as a novel process for safer bakery products. 27th VH Yeast Conference, Istanbul, Turkey.

[2] Mogol, B.A., Gökmen, V., Combined conventional – vacuum baking as a novel process for safer bakery products, iFood2013 Innovation Food Conference, 8-10 October 2013, Hannover, Germany

[3] Mogol, B.A., Gökmen, V., 2013, Kinetics of furan formation from ascorbic acid under reducing and oxidizing conditions, EuroFoodChem XVII, 7-10 May 2013, Istanbul, Turkey

[4] Mogol, B.A., Gökmen, V., 2012, Online Imaging as a Tool to Monitor Neo-Formed Compounds in Biscuits during Baking, CEFood 2012: 6th Central European Congress on Food, 23-26 May 2012, Novi Sad, Serbia

[5] Mogol, B.A., Gökmen, V., 2010, Inhibition of Enzymatic Browning by the Maillard Reaction Products, CEFood 2010 – 5th Central European Congress on Food, 19-22 May, 2010, Bratislava, Slovak Republic

[6] Mogol, B.A., Gökmen, V., 2008, Effect of Maillard Reaction Products on Inhibition of Apple Polyphenol Oxidase, COST 928 2nd Annual Meeting, 15-17 October 2008, Istanbul, Turkey

[7] Ataç, B., Serpen, A., Gökmen, V., 2008, Analysis of Acrylamide in Cereal Products, Bosphorus 2008 – ICC International Congress, 24-26 April 2008, Istanbul, Turkey

Poster Presentations

[1] Truong, V.D., Pascua, Y.T., Reynolds, R., Thompson, R.L., Palazoğlu, T.K., Mogol, B.A., Gökmen, V., 2013, Processing treatments for low acrylamide formation in sweet potato French fries, IFT Annual Meeting, 13-16 July 2013, Chicago, Illinois USA

[2] Mogol, B.A., Gökmen, V., 2012, 4-Methylimidazole formation from sugars and amino acids, 11th International Symposium on the Maillard Reaction "Centenary of the Maillard Reaction Discovery (1912-2012)", 16-20 September 2012, Nancy, France (Best Poster Award)

[3] Mogol, B.A., Göncüoğlu, N., Kocadağlı, T., Gökmen, V., 2011, High Resolution Mass Spectrometry Analysis of Reaction Products and Intermediates Formed in Carbonyl-Asparagine Model System during heating, RAFA 2011, 1-4 November 2011, Prague, Czech Republic

[4] Gökmen, V., Serpen, A., Mogol, B.A., 2011, Determination of Amino Acids in tea by Hydrophilic Interaction Liquid

Chromatography Coupled to High Resolution Mass Spectrometry, RAFA 2011, 1-4 November 2011, Prague, Czech Republic

[5] Akıllıoğlu, G., Mogol, B.A., Gökmen, V., 2011, Degradation of 5-hydroxymethylfurfural in malt during fermentation of beer, ICEF 2011 International Congress on Engineering and Food, 22-26 May 2011, Athens, Greece

[6] Kocadağlı, T., Mogol, B.A., Gökmen, V., 2011, Removal of phenolic compounds from olive mill wastewater by adsorbent resins, ICEF 2011 International Congress on Engineering and Food, 22-26 May 2011, Athens, Greece

[7] Göncüoğlu, N., Mogol, B.A., Gökmen, V., 2011, Regeneration of frying oils by using adsorbent resins, ICEF 2011 International Congress on Engineering and Food, 22-26 May 2011, Athens, Greece

[8] Göncüoğlu, N., Mogol, B.A., Gökmen, V., 2010, Removal of Hydroxymethylfurfural from Frying Oil by Using Adsorbent Resin, 9-10 December 2010, Istanbul, Turkey

Patents

Number: 30801.03, 2013/00673, Baked Product Without Acrylamide And The Production Method Thereof.