Abdullatif Albouchi (MSc)

Hydroxymethy-Substituted Furan Derivatives in Coffee: Its Analysis, Formation Kinetics, Exposure, and Mitigation.

DOCTORAL THESIS

to achieve the university degree of
Doktor der Naturwissenschaften

submitted to

Graz University of Technology

Supervisor

Ao.Univ.-Prof. Dipl.-Ing. Dr.techn. Michael Murkocvic

Institute of Biochemistry

Graz, April 2018

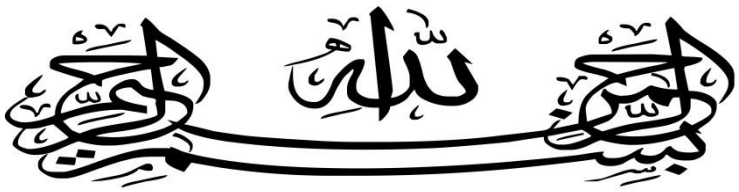
AFFIDAVIT

I declare that I have authored this thesis independently, that I have not used other than the declared sources/resources, and that I have explicitly indicated all material which has been quoted either literally or by content from the sources used. The text document uploaded to TUGRAZonline is identical to the present doctoral thesis.

Date

Signature

Table of contents



My praise and gratitude be to Allah the Most kind, who by his favour the good deeds are accomplished. Also, I would like to express my deepest acknowledgments to my supervisor Ao.Univ.-Prof. Dipl.-Ing. Dr. techn. Michael Murkovic for giving me the opportunity to carry out my doctoral research and be part of his research group. His research ideas, continuous scientific support, the provided free and friendly atmosphere in the laboratory, and the warm conversations along delightful coffee cups are some of many things that I am thankful for.

I would like to pass my sincere appreciation to the Erasmus Mundus Avempace Plus program for granting me the full-degree scholarship to pursue my PhD degree at Graz University of Technology. I would like to thank the coordination office of the scholarship in Berlin University of Technology and the office for International Relations and Mobility Programmes in Graz University of Technology for their step by step support through the entire program. I want to acknowledge the International University for Science and Technology in Syria for backing me in the application process for this scholarship.

I am thankful to my thesis committee members, Ao.Univ.-Prof. Dipl.-Ing. Dr.techn. Hansjörg Weber and Ao.Univ.-Prof. Dipl.-Ing. Dr.techn. Arnold Stütz from the Institute of Organic Chemistry for lending me part of their precious time to discuss multiple aspects of my research and for their highly valued suggestions. My thanks are also for Ao.Univ.-Prof. Dipl.-Ing. Dr.nat.techn. Ebrahim Razzazi-Fazeli from the University of Veterinary Medicine of Vienna for approving to be an expert referee for the defence of this PhD dissertation.

No words I can say can pay enough tribute to my entire family for their care and support. To my mother, whose prayers, sacrifices and unlimited support are like a boat that carries me through the waves of life to the safe shores. To my father, whose advice, care and compassion are the biggest reasons for being the person I am today. To the unconditional love of my brothers and my sister. To my love, colleague,

companion and wife that she spared no effort on all levels, from scientific to emotional, in helping me achieve my goals. To the delight, purity and innocence of my life and to the hope of my future, to my son and daughter. To my father-in-law Univ.-Prof. Ing. Dr. Mohammad Hashem Abolkhair for his support and care about my progress.

Deep regards are to all the colleagues and staff of the Functional foods research group and the Institute of Biochemistry namely Majd Lahham, Marini Damanik, Oliver Wollner, and Christopher Hartl for helping me on the personal and scientific levels during my stay in Graz. My thanks also go to my dear friend Dr. Ahmed Hassan from the Medical University of Graz and to the Arabic and Muslim communities in Graz as they allowed me to find home away from home due to their overwhelming support and wonderful gatherings.

Thanks to all those who contributed to this work, who were not named here due to space restrictions.

Abdullatif Albouchi

°C	Degree Celsius
1/2 D NMR	One/Two-Dimensional Nuclear Magnetic Resonance
2-FA	2-Furoic Acid
ADI	Acceptable Daily Intake
Arabica	*Coffea arabica*
C	Carbon
DAD	Diode Array Detector
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-Acetic Acid
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FFA	Furfuryl alcohol
Fig.	Figure
GC	Gas Chromatography
H	Hydrogen
HMF	Hydroxymethyl Furaldehyde
HMFA	Hydroxymethyl Furoic Acid
HPLC	High Performance Liquid Chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	Kilogram
L	Levo
LD 50	The Median Lethal Dose
mL	Millilitre
N	Nitrogen
pH	Potential of Hydrogen
Robusta	*Coffea canephora*
S. typhimurium	*Salmonella typhimurium*
SCE	Sister Chromatid Exchange
SULT	Sulfotransferase
UPLC	Ultra-High-Performance Liquid Chromatography
UV-Vis	Ultraviolet-Visible Detector
WHO	World Health Organisation

Hydroxymethyl-substituted furan derivatives contribute significantly to the total furan derivatives of roasted coffee; furthermore, they receive a lot of attention due to their mutagenic properties after conversion by intracellular sulfotransferases. Their analysis, formation kinetics, exposure and mitigation in coffee are of special importance and thus were the focus of this dissertation.

A validated simple and selective HPLC-DAD method was developed for the simultaneous separation and quantitation of furfuryl alcohol, 2-furoic acid, 5-hydroxymethyl furaldehyde and 5-hydroxymethyl furoic acid in roasted coffee. Different extraction solvents brought about various extraction efficacies, with water being the most suitable while methanol resulted in lacking extraction. Conversely, no significant differences were observed between tested extraction techniques.

Coffee produces more furfuryl alcohol compared to other beans or seeds roasted at the same conditions with observed formation kinetics resembling those of other process contaminants (e.g. HMF, acrylamide). The temperature and duration of applied roasting as well as the species of coffee used affected the level of furfuryl alcohol to be produced, whereas the moisture content of the green coffee had limited effect. Great amounts of furfuryl alcohol (up to 57 %) are evaporating and get released to the atmosphere during roasting of coffee.

An eight weeks storage at varying temperatures did not affect the level of furfuryl alcohol in the ground coffee matrix. On the other hand, the use of different brewing techniques affected the concentration of furfuryl alcohol in the brew and its extraction

efficiency from the ground coffee. Furfuryl alcohol was at least 2-fold the amounts of 5-hydroxymethyl furaldehyde and 5-fold the amounts of 2-furoic acid or 5-hydroxymethyl-2-furoic acid in the same prepared brew with an observed variation of up to 22-fold in their concentrations. Stressing furfuryl alcohol with simulated gastrointestinal fluids points out a significant decrease under simulated gastric fluid conditions only, although the same effect could not be reproduced when mimicking a regular coffee ingestion situation.

Fourteen different mitigation agents were incorporated into two dry model systems mimicking important coffee natural constituents and an actual coffee system afore a controlled roasting process to evaluate their mitigation capacity of hydroxymethyl substituted furan derivatives. The agents can be chemically categorized to hydroxy cinnamates and their esters, hydroxy benzoates, flavonoids, as well as other non-phenolic agents. An especially high mitigation capacity could be shown with polyphenolics (hydroxy cinnamates or benzoates derivatives), quinic acid and EDTA. The number and availability of phenolic groups affected the mitigation capacity of polyphenolics. Certain agents exhibited a furan derivative-specific reducing capacity while others exerted a generalized effect. In the coffee system, a comparable mitigation trend to that in chemically-set model systems was observed with taurine and sodium sulfite exerting the highest capacity. The mitigation efficacy decreased gradually by the increasing chemical complexity of the tested model.

Hydroxymethyl-substituierte Furan Derivate tragen signifikant zu den gesamten Furan-Derivaten von geröstetem Kaffee bei, darüber hinaus erhalten sie aufgrund ihrer mutagenen Eigenschaften nach Umwandlung durch intrazelluläre Sulfotransferasen viel Aufmerksamkeit. Ihre Analyse, Bildungskinetik, Exposition und Reduktion in Kaffee sind von besonderer Bedeutung und standen daher im Mittelpunkt dieser Dissertation.

Eine validierte einfache und selektive HPLC-DAD-Methode wurde für die simultane Trennung und Quantifizierung von Furfuryl Alkohol, 2-Furan Carbonsäure, 5-Hydroxymethyl furaldehyd und 5-Hydroxymethyl Furan Carbonsäure in Röstkaffee entwickelt. Verschiedene Extraktionslösungsmittel führten zu verschiedenen Extraktionseffizienzen, wobei Wasser am besten geeignet war. Mit Methanol konnten diese Substanzen nicht extrahiert werden. Die Extraktionseffizienz war unabhängig von der angewandten Extraktionsmethode.

Kaffee produziert wesentlich mehr Furfuryl Alkohol als andere Bohnen oder Samen, die unter den gleichen Bedingungen geröstet wurden, mit beobachteten Bildungskinetiken, die denen anderer Kontaminanten (z.B. HMF, Acrylamid) ähneln. Die Temperatur und die Dauer der angewandten Röstung sowie die verwendeten Kaffeesorten beeinflussten das Niveau des zu produzierenden Furfuryl Alkohols, während der Feuchtigkeitsgehalt des Rohkaffees eine begrenzte Wirkung hatte. Große Mengen von Furfuryl Alkohol (bis zu 57%) verdampfen beim Rösten.

Eine achtwöchige Lagerung bei unterschiedlichen Temperaturen beeinflusste die Menge des Furfuryl Alkohols in der gemahlenen Kaffeematrix nicht. Auf der anderen Seite beeinflusste die Verwendung verschiedener Brühtechniken die Konzentration von Furfuryl Alkohol im Gebräu und seine Extraktionseffizienz aus dem gemahlenen Kaffee. Furfuryl Alkohol war mindestens das Doppelte der Mengen des 5-Hydroxymethylfuraldehyds und 5-fach die Mengen der 2-Furosäure oder 5-Hydroxymethyl-2-furansäure im gleichen gebrühten Gebräu mit einer beobachteten Variation ihrer Konzentrationen bis zum 22-fachen. Die Belastung von Furfuryl Alkohol mit simulierten gastrointestinalen Flüssigkeiten weist auf eine signifikante Abnahme nur unter simulierten Magensaftbedingungen hin, obwohl der gleiche Effekt nicht reproduziert werden konnte, wenn eine normale Kaffeeaufnahme-Situation nachgeahmt wird.

Vierzehn verschiedene Substanzen zur Reduktion wurden in zwei trockene Modellsysteme eingearbeitet, die wichtige natürliche Kaffeebestandteile nachahmten, und ein tatsächliches Kaffeesystem vor einem kontrollierten Röstprozess, um das Potential zur Reduktion diesen hydroxymethyl-substituierten Furan Derivaten zu bewerten. Diese Substanzen können chemisch in Hydroxycinnamate und deren Ester, Hydroxybenzoate, Flavonoide sowie andere nichtphenolische Substanzen eingeteilt werden. Eine besonders hohe Mitigationskapazität konnte mit Polyphenolen (Hydroxycinnamate oder Benzoatderivate), Chinasäure und EDTA nachgewiesen werden. Die Anzahl und Verfügbarkeit von phenolischen Gruppen beeinflusste die Abschwächungskapazität von Polyphenolen. Bestimmte Substanzen zeigten eine Furan

Derivat-spezifische Reduktionskapazität, während andere eine generalisierte Wirkung ausübten. Im Kaffee-System wurde eine vergleichbare Abschwächungstendenz zu dem in chemisch eingestellten Modellsystemen beobachtet, wobei Taurin und Natriumsulfit die höchste Wirkung hatte. Die Minderungseffizienz nahm durch die zunehmende chemische Komplexität des getesteten Modells allmählich ab.

تشكل مشتقات هيدروكسي ميثيل فوران جزءاً معتداً من إجمالي مشتقات الفوران في القهوة المحمصة، وعلاوةً على ذلك فإنها تلقى الكثير من الاهتمام بسبب خصائصها المطفرة الناتجة عن تحويلها بواسطة الإنزيم داخل الخلوي الناقل للسلفات، وتتمتع دراسات تحليل هذه المركبات وحرائك تشكلها ومدى التعرض لها ومحاولات التخفيف من انتاجها في القهوة بأهميةٍ خاصةٍ، لذا كانت هذه الدراسات محور هذه الرسالة.

طورت طريقة استشرابٍ سائلٍ رفيعة الإنجاز مرفقةٌ بمكشاف مصفوفة الديودات بسيطٌ وانتقائيةٌ وموثوقة المصداقية للفصل والتعيين الكمي المتزامن لكحول فورفوريل و2-حمض فورويك و5-هيدروكسي ميثيل فورمالديهايد و5-هيدروكسي ميثيل حمض فورويك في القهوة المحمصة، وقد نتجت كفاءات استخلاصٍ متغايرةٌ باستخدام مذيبات استخلاصٍ مختلفةٍ مع كون الماء الأنسب بينها في حين أن الكحول الميثيلي أدى إلى استخلاصٍ غير تام، وعلى العكس من ذلك لم تلاحظ فروقٌ ذات دلالةٍ إحصائيةٍ بين تقنيات الاستخلاص المختبرة.

تنتج القهوة كميةً أكبر من كحول فورفوريل مقارنةً بالحبوب أو البذور الأخرى المحمصة تحت نفس الظروف بحرائك تشكلٍ مشابهةٍ لتلك المرصودة لملوثات العمليات التصنيعية الأخرى (مثل 5-هيدروكسي ميثيل فورمالديهايد وأكريلاميد)، حيث أثرت درجة الحرارة ومدة التحميص المطبقة وكذلك الأنواع المستخدمة من البن على كمية كحول فورفوريل المنتجة، في حين أظهر محتوى الرطوبة في البن الأخضر تأثيراً محدوداً، وقد لوحظ أيضا بأن كمياتٍ كبيرةً من كحول فورفوريل (ما يصل إلى 57 % منها) تتبخر وتطلق في الجو أثناء تحميص القهوة.

لم يؤثر التخزين لمدة ثمانية أسابيعٍ بدرجات حرارةٍ متفاوتةٍ على كمية كحول فورفوريل في مطرس القهوة المطحونة، ومن ناحيةٍ أخرى فقد أثر استخدام تقنيات تحضيرٍ مختلفةٍ على تركيز كحول فورفوريل في الشراب وعلى كفاءة استخلاصه من القهوة المطحونة، وبلغت كمية كحول فورفوريل مثلي كمية 5-هيدروكسي ميثيل فورمالديهايد على الأقل وخمسة أمثال كميات كل من 2-حمض فورويك أو 5-هيدروكسي ميثيل حمض فورويك على الأقل في نفس الشراب المحضر مع اختلافٍ مشاهدٍ في تراكيزهم يصل إلى 22 ضعفاً، وأشارت دراسة إجهاد كحول فورفوريل بتعريضه لسوائلٍ معديةٍ معويةٍ محاكاةٍ إلى انخفاضٍ كبيرٍ في تركيزه في حالة السائل

المعدي المحاكى فقط، على الرغم من أنه لا يمكن تكرار مثل هذا التأثير عند تقليد تناول القهوة تحت شروطٍ منتظمةٍ.

تم تضمين أربعة عشر عاملاً من عوامل التخفيف المختلفة في منظومتين نموذجيتين جافتين تحاكيان المكونات الطبيعية الهامة في القهوة وأيضاً في منظومة قهوةٍ فعليةٍ وذلك قبل عملية تحميصٍ خاضعةٍ للرقابةٍ بهدف تقييم قدرة عوامل التخفيف على التقليل من مشتقات هيدروكسي ميثيل فوران، ويمكن تصنيف هذه العوامل المستخدمة كيميائياً إلى مشتقات هيدروكسي سينامات وإستراتها ومشتقات هيدروكسي بنزوات وفلافونوئيدات بالإضافة إلى عواملٍ أخرى غير فينوليةٍ، حيث يمكن ملاحظة قدرة تخفيفٍ عاليةٍ بشكلٍ خاصٍ عند استخدام متعددات الفينول (مشتقات هيدروكسي سينامات وهيدروكسي بنزوات) بالإضافة إلى حمض كينيك واثيلين ثنائي الأمين رباعي حمض الخل، و من الملاحظ تأثير عدد وتوافر مجموعات الفينول على قدرة التخفيف الخاصة بمتعددات الفينول، ويشار بأن هناك عواملٌ قد امتلكت سعة تخفيفٍ خاصةٍ بأحد مشتقات الفوران في حين مارس البعض الآخر تأثيراً معمماً، وقد لوحظ بأن نزعة التخفيف في منظومة القهوة مماثلةٌ لتلك في المنظومات النموذجية المحددة كيميائياً بحيث امتلك كبريتات الصوديوم وتورين السعة الأعلى، وقد انخفضت فعالية التخفيف تدريجياً مع زيادة التعقيد الكيميائي للمنظومة المختبرة.

1. Introduction

1.1. Coffee:

Coffee is one of the most popular beverages worldwide with more than 60% of adults in America consuming it daily on an average of 3.1 cups (Ding 2014). The annual consumption was estimated in 2010 to reach 8.1 million tons resulting in more than 500 billion cups distributed mainly in the United States, Brazil, Germany, Japan, and Italy while the consumption in North European countries such as Finland, Norway, Denmark, and Sweden are double that of the United States or Brazil (Farah 2012). The seeds of an evergreen shrub of several species from the genus Coffea were roasted and brewed to produce the drink as early as 1000 years ago. The word "coffee" itself originated from the Arabic word "QAHWAH" and the Ethiopian Province of Kaffa is considered the origin of the plant. The first people to identify the effects of coffee consumption are believed to be the Oromo people after its consumption by their goats. Muslims in Mocha in Yemen have been reported to roast and brew coffee (Garg 2016), and the first coffee house was established in Mecca at the end of the fifteenth century. Thereafter coffee spread to the Middle East, Persia, Turkey, and North America around the sixteenth century to become nowadays the most commercialized food product in the world (Farah 2012).

1.1.1. Composition of green coffee:

The aroma and flavour of coffee are produced from its pre-roasting constituents. These natural constituents are not only important for the formation of flavour, taste and colour, but also help deter insects from

attacking the green beans. More than 1000 different chemicals, demonstrating the complexity of green coffee composition, have been reported until this very day in the green beans. These constituents can be classified as soluble and insoluble polysaccharides, aliphatic organic acids, nitrogenous compounds, polyphenols, proteins, lipids, free amino acids. Among these, chlorogenic acids and caffeine are considered vital nutraceuticals promoting beneficial health properties such as antioxidant and weight-loss supplements (Garg 2016).

Various analytical techniques have been used for the identification of the composition of green coffee beans. Combining one-dimensional (1D) and two-dimensional (2D) ^{1}H and ^{13}C NMR spectra of green coffee water extracts, 16 major components were recognized. These compounds were caffeoylquinic acids (chlorogenic acids), organic acids (acetic acid, citric acid, malic acid, and quinic acid), nitrogenous compounds (mainly caffeine and choline and trigonelline), free amino acids (glutamic acid, alanine, and asparagine), myo-inositol, and sucrose which is of the greatest amount calculated by mole (Wei and Tanokura 2015b).

Species and place of origin along with the type of processing applied to the beans affects the amounts of various ingredients to be found in the dry bean and later the aroma formed after roasting (Wei and Tanokura 2015b). Arabica species contains a higher proportion of carbohydrates, lipids, trigonelline, organic acids, and 3-feruoyl-quinic acid. On the other hand, Robusta species is richer in caffeine, proteins, arabinogalactans, chlorogenic acids and metals (Poisson et al. 2017).

According to Table 1, Polysaccharides, lipids, and proteins seem to be the major ingredients. Other minor components, such as nitrogenous compounds (caffeine and trigonelline), polyphenols (chlorogenic acids),

free sugars (largely sucrose), free amino acids, are available. These minor ingredients are nevertheless considered significant as a source of coffee aroma (Poisson et al. 2017). It is also worth mentioning that among simple carbohydrate sucrose is known to be the major one quantitatively while other simple saccharides are of almost negligible amounts. On the amino acids side, Alanine was found to be the free amino acid with the highest amount in green coffee beans (Murkovic and Derler 2006).

Table 1: The main components of green coffee beans. Adopted from Poisson et al. (2017).

Constituent	**Content (% Based on Dry Weight)**	
	Arabica	**Robusta**
Soluble carbohydrates	9-12.5	6-11.5
Monosaccharides	0.2-0.5	0.2-0.5
Oligosaccharides	6-9	3-7
Polysaccharides	3-4	3-4
Insoluble carbohydrates	46-53	34-44
Hemicellulose	5-10	3-4
Cellulose, β (1-4)-mannan	41-43	32-40
Organic acids	2-2.9	1.3-2.2
Chlorogenic acids	6.7-9.2	7.1-12.1
Lignin	1-3	1-3
Lipids	15-18	8-12
Coffee oil	15-17.7	8-11.7
Wax	0.2-0.3	0.2-0.3
N-compounds	11-15	11-15
Free amino acids	0.2-0.8	0.2-0.8
Proteins	8.5-12	8.5-12

Caffeine	0.8-1.4	1.7-4.0
Trigonelline	0.6-1.2	0.3-0.9
Minerals	3-5.4	3-5.4

1.1.2. Roasting.

The production of coffee for consumption requires brown to black beans with the characteristic odour and taste that make coffee beverages widely popular. Roasting is the most important step in the conversion of aroma and flavour of green coffee into these well-defined sensory qualities. This rather simple process is a highly complex chemical and physical transformation that influences colour, flavour and/or aroma of the final product. Roasting is a dry heating process that can be applied through drum roasters where the seeds are directly treated by fire or hot surfaces, or in fluid bed roasters, where hot air or gases are applied to the beans. The latter type of roasters is faster and more controllable (Farah 2012).

Roasting itself is divided to different phases, an endothermic drying phase (up to 100 °C,), followed by an exothermic phase (170-220 °C) resulting in the development of valued characteristic flavour compounds and a subsequent cooling phase (Poisson et al. 2017). The temperature of roasting can even be raised up to 300 °C (Bagdonaite et al. 2008). In the initial phase, limited chemical reactions take place compared to the following higher temperature phase. It is necessary to get to approximately 200 °C in order to trigger the reactions forming the sought colour, aroma, and taste from natural precursors. The roasting is stopped at a certain point using air or water to interrupt the undergoing reactions. The roasting degree and profile vary considerably and should be optimized through mass loss, visual colour inspection or other roaster

specific parameters to preserve the quality of the final product (Farah 2012). Though advanced techniques such as the determination of free amino acids or chlorogenic acids or the continuous on-line monitoring of organic volatiles were also proposed. Manipulating the time and temperature of roasting affects the colour (from light brown to almost black beans), acidity (decrease with further roasting), bitterness (increase by further roasting), soluble solids (higher in fast roasting), and the overall volatile profile (more roasty and buttery at fast roasting) of final coffee beans (Poisson et al. 2017).

Various chemical reactions take place in the second phase of the roasting process e.g. caramelisation, Maillard reaction, dehydration, hydrolysis, enolization, cyclization, cleavage, fragmentation, recombination of fragments, pyrolysis, and polymerization reactions. Also in this step we can notice clearly a size expansion which is due to the increased internal pressure after release of carbon dioxide as well as the migration of lipids to the surface of the bean forming an oily layer that entraps volatiles. The darkening of the beans is attributed to the formation of higher molecular weight melanoidins by the Maillard reaction, caramelisation and polymerization of coffee components (Poisson et al. 2017).

The Maillard reaction or non-enzymatic browning is of special importance as it leads to the development of volatile and non-volatile components. This complex multi-step reaction occurs when amino acids and reducing sugars interact generating a set of intermediates (i.e. Amadori and Heyns products) that degrade to lower molecular weight compounds with diverse aromas, tastes and colours. Another outstanding chemical transformation is the Strecker degradation during which amino acids undergo deamination and oxidative decarboxylation leading to aldehydes contributing to the aroma. The Maillard reaction is favoured

over caramelisation due to the lower activation energy. The degradation of chlorogenic acids and quinic acid is linked to the formation of bitter lactones. Amino acids condense into diketopiperazines exhibiting also a bitter taste. The oxidation of unsaturated fatty acids gives rise to aliphatic aldehydes that can further react during roasting (Poisson et al. 2017).

The resulting coffee beans contain carbohydrates, protein fragments, small aliphatic acids, caffeine, trigonelline, lipids, unknown complex high-weight molecules (melanoidins), and hundreds of volatiles carried mostly in the coffee oil. The compounds that are no longer found in roasted beans (sucrose, free amino acids, and chlorogenic acids) along with compounds that decrease significantly (e.g. trigonelline) are linked to the formation of aroma, taste and colour (Wei and Tanokura 2015a). The availability of the free amino groups and the quick thermal hydrolysis of sucrose is the reason for their high reactivity early in the roasting process; however, the degradation of chlorogenic acids and the debranching of arabinose moieties needs a higher activation energy leading to their somewhat delayed activity after the beginning of the roasting process. Proteins, cellulose and mannans, most lipids, metal ions, and alkaloids are stable during roasting or undergo limited breakdown, e.g. polysaccharides and proteins. This diverse composition comprises antioxidants and chemo-preventatives as well as undesirable components, e.g. acrylamide and furan (Poisson et al. 2017).

1.1.3. Brewing.

Coffee is primarily consumed as beverages. In fact, it is considered to be the second beverage consumed after water (Ding 2014). The chemical composition of this beverage is greatly influenced by the brewing method applied. Hot water is used to brew coffee either by direct boiling or at

temperatures between 90 °C – 95 °C. The cupping ratio of coffee to water varies widely according to cultural and individual preferences, but is usually around 8–20 g coffee/100 mL water. The length of extraction and the fineness of the powder are also considerably changeable from one method to another. The most common brewing methods worldwide are simple filtration, Turkish boiled coffee, electric drip brewer, espresso machine, Italian and French presses.

Medium-ground coffee is used for the filtration method where it is evenly spread in a filter and hot water is poured over the coffee. On the other hand, Turkish coffee uses finely ground coffee in boiled with water in a pan and poured directly into the drinking cup. The electric drip brewer applies automatic pouring of water on fine or medium-ground coffee that is placed on a filter paper. Espresso makers are quite different where a pressure of 9 bar is used to percolate water on a coarse or medium ground coffee.

Italian press uses a pressure valve to drive heated water continuously through the medium coarse coffee, whereas a French press mixes coarse coffee and hot water for a few minutes then the brew is separated through a sieve which is pressed into the mixture.

Instant coffee production involves the extraction of water-soluble compounds with high temperature and pressure. Later, the brew is cooled and concentrated by heating, and finally freeze-dried or spray-dried to remove the moisture forming a powder. Afterwards steam, water and/or oil are used to cover the surface of the powder to form granules in a process called agglomeration. In contrast to usual coffee brews, instant coffee uses a high percentage of Robusta since they naturally hold a higher proportion of soluble solids thus increasing the yield (Farah 2012).

1.1.4. Health effects.

The issue of health effects associated with coffee consumption is largely controversial. Coffee drinking is believed by many to be beneficial for type 2 diabetes, stroke, cancer, and all-cause mortality, while others suggest side effects of coffee consumption can negatively affect coronary heart disease, myocardia infraction, angina, arrhythmias, stroke, Parkinson's disease, osteoporosis, impaired iron and zinc absorption, delay in conception, spontaneous abortions, fetal growth restriction and is attributed to mortality in drinkers under 55 years of age. Nevertheless, an undisputed beneficial effect of coffee on the liver is reported. The use of different types of coffee and brewing methods might be the reason for these contradicting results (Ding 2014).

Phyto biomolecules in green coffee are promising molecules with positive health effects in the prevention of various diseases, such as obesity, diabetes, oxidative stress, cardio-vascular diseases, multiple hepatic disorders, anticancer activities, Parkinson's disease, Alzheimer's disease, antiaging effects, asthma, headache, antibacterial effects, anti-inflammatory effects, immunomodulation and it is said to reduces suicide risk. Chlorogenic acids consumed from green coffee extracts are distributed throughout body fluids. In the colon they can inhibit the proliferation of cancerous cells while their metabolites can interact with colonic microflora exhibiting prebiotic activity. It is worth mentioning that chlorogenic acids have an antioxidant capacity more than that of ascorbic acid or mannitol. On the other hand, constituents of green coffee beans can induce sensitivities, asthma, allergic rhinitis, and allergic conjunctivitis, and contact allergy (Garg 2016).

1.1.5. Chemical classes of coffee volatiles.

Flavour of foods can be classified to volatile flavour that can be nasally perceived and non-volatile flavour which is tasted by the mouth. The aroma of coffee is considered a high value quality parameter that contributes to not more than 0.1% of the total coffee weight. The analysis of this aroma is challenging due to the trace concentration of important moieties (in the ppt to ppm range) and their volatility and instability. A major breakthrough was made by the use of gas chromatography-olfactometry (the implementation of a human nose as a sensitive detector), and the Sensomics approach to estimate the concentration to odour threshold, where mass spectrometry and nuclear magnetic resonance are used to elucidate the structure (Poisson et al. 2017).

The formation of coffee aroma is an extremely complex process. During the roasting process, key chemical reactions take place resulting in a wide range of compounds affecting the colour, flavour as well as the aroma of roasted coffee (Martins et al. 2001). Interactions between diverse reaction mechanisms can be observed where Maillard reaction, caramelization, Strecker degradation, pyrolysis, degradation of sulfur amino acids, hydroxy-amino acids, trigonelline, quinic acid and minor lipids take place (Wei and Tanokura 2015a).

As an outcome, the aroma is composed of a complex mixture of volatile compounds of which more than 900 volatiles were identified (Franca et al. 2009) comprising diverse chemical groups such as furans, furanones, pyrans, pyridines, pyrazines, pyrroles, cyclopentenes, ketones, phenols hydrocarbons, alcohols, aldehydes, acids, anhydrides, esters, lactones, thiophenes, oxazoles, thiazoles, amines, and various sulphur and nitrogen compounds (Moon and Shibamoto 2009, Farah 2012). Not all compounds

of the same chemical class give the same sensory impact (Farah 2012) and only 25-30 compounds were proven to contribute substantially to the final aroma with 2-furfurylthiol being the most relevant. Stable isotope dilution experiments were used to identify the main components responsible for the aroma of coffee brew (Poisson et al. 2017). Some of these compounds are mentioned in Table 2.

Table 2: Key volatiles contributing to the aroma of roasted coffee and their availability in Arabica and Robusta coffee species. Adopted from Poisson et al. (2017).

Aroma compound	**Arabica**	**Robusta**
Methanethiol		*
Dimethyl (mono-/di-/tri-) sulphide	*	*
2-Furfurylmercaptane		*
3-Mercapto 3-methylbutyl formate	*	*
Methional	*	
2-Methylbutanal		*
3-Methylbutanal	*	*
2,3-Butanedione	*	*
2,3-Pentanedione	*	
2-(Ethyl/Ethenyl) 3,5-dimethylpyrazine		*
2,3-Diethyl 5-methylpyrazine		*
2-Methoxy 3-isobutylpyrazine	*	
2-Methoxyphenol		*
4-(Ethyl/Vinyl) 2-methoxyphenol		*
Sotolon	*	*
Furaneol	*	

The availability of precursors in the green beans affects the type of aroma compounds to be formed as seen in the difference between the aroma of Arabica (sweet due to greater formation of diketones, furfurals, and furaneol) and Robusta (spicy and earthy due to greater formation of pyrazines and phenols), whereas the roasting parameters determine the kinetic of the formation of these aroma compounds as seen in the difference between light (fruity aroma), medium and dark (spicy/ashy aroma) roasts (Poisson et al. 2017). Defective seeds, when available in green beans batch, give rise to off flavours. Also, the maturation stage of the coffee fruits when harvested may affect the aroma of their roasted beans. Various carbohydrates can be considered as precursors for furans, aldehydes, ketones, phenols, and volatile acids (formic and acetic acid), while proteins, peptide, and amino acids lead to the generation of ketones, pyrroles, and pyrazines. Lipids generate aldehydes and ketones, whereas chlorogenic acids produce phenolic volatile compounds and trigonelline gives rise to pyrroles, pyridines, and pyrazines (Farah 2012). Generally, low molecular weight precursors lead to diverse chemical groups of heterocycles with phenols and organic acids, while the high molecular weight fraction leads mainly to alkyl pyrazines of earthy or nutty flavour beside 2-furfuylthiol (Poisson et al. 2017).

Furan derivatives are believed to be the main components of coffee aroma, followed by pyrazines, pyrroles, and ketones (Capuano and Fogliano 2011). Petisca et al. (2013) have found them to be the major chemical class of compounds in the volatile part of all ground and espresso coffee samples that they analysed. Of which, furfuryl alcohol (FFA) was found in an extremely high percentage representing more than 50 % of all furans quantified. These derivatives are not naturally present in green beans; rather they are formed during the roasting process. The

level of furan derivatives correlates well with the colour of roasting form light brown to almost black (Anese 2015).

It is worth mentioning that the amounts of aroma moieties have an optimum and afterwards many volatiles will decrease such as diketones, furfurals, or 4-vinylguaiacol leaving a burnt flavour associated with over-roasting. However, other volatiles continue to rise in amounts even at excessive roasting, e.g. 2-furfurylthiol, N-methyl pyrrole, and dimethyl trisulfide (Poisson et al. 2017).

1.2. Hydroxymethyl-substituted furan derivatives:

Furan derivatives are diverse heterocyclic chemicals that share a five-membered aromatic ring containing an oxygen atom. This widely spread class of compounds in foods and beverages is especially important due to its pleasant contributions to the aroma, colour and taste (Hu et al. 2013) at a low threshold (Pérez-Palacios et al. 2012), leading to their current use as in the food and feed industry as flavourings or flavour enhancers (Anese and Suman 2013). This ring can carry one or more of a different variety of substituents affecting its physio-chemical properties such as solubility and volatility (Anese 2015). Examples of substituents are aldehydes, ketones, esters, alcohols, acids, thiols, and sulphide groups. Furan derivatives are mainly produced upon the heat and/or acid treatment of foods containing monosaccharides (Chinnici et al. 2003). Key furan derivatives in roasted coffee are 2-furfurylthiol, 2-methyl-3-furanthiol, 5-hydroxymethyl furaldehyde (HMF), furfural and FFA (Capuano and Fogliano 2011).

Furan derivatives which are substituted with a hydroxymethyl group have undergone numerous research activities and received a lot of attention. For once, they are available in higher quantities compared to other

derivatives. Moreover, they were found in toxicological studies to give rise to negative health effects. Regarding roasted coffee, the two most known examples of this group of substituted furan derivatives are furfuryl alcohol (FFA) (2-hydroxymethyl furan) and 5-hydroxymethyl furaldehyde (HMF) since they are found in great quantities in coffee along their carboxylic acid derivatives 2-furoic acid (2-FA) and 5-hydroxymethyl furoic acid (HMFA), respectively. Fig. 1 illustrates the chemical structure of the hydroxymethyl furan derivatives of interest along with 2-FA.

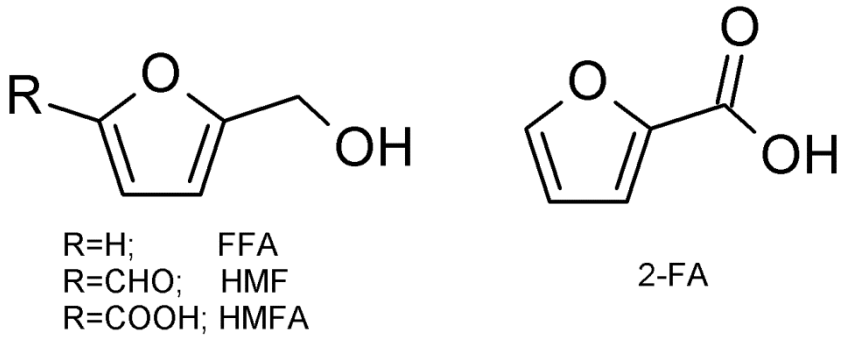


Figure 1: The chemical structure of the most prevalent hydroxymethyl furan derivatives in roasted coffee and the structure of 2-furoic acid. FFA: Furfuryl alcohol. HMF: 5-Hydroxymethyl furaldehyde. HMFA: 5-Hydroxymethyl furoic acid. 2-FA: 2-Furoic acid.

1.2.1. Presence in foods.

Industrial or domestic food processing, e.g. fermentation, pasteurization, thermal treatment, UV treatment, and preservation or aging, leads to a significant alteration of the chemical composition through various chemical reactions. Furan derivatives such as FFA, HMF, and many others are known to ensue upon similar reactions (Hu et al. 2013). Sugary-products provide necessary prerequisites for the formation of

these molecules as in the higher concentration of sugars and organic acids, the acidic pH and the minimal water activity (Silva et al. 2017).

FFA occurs mainly in thermally processed foods like coffee, fruit juices, baked foods, wines, paste fish, and butter. FFA polymers are formed under acidic food conditions contributing to the brown colour (Okaru and Lachenmeier 2017). Coffee is considered the main source of exposure of FFA Since it occurs at comparably low concentrations in other foods. Also, it is highly water soluble and therefore most of it is transferred to the brew (Murkovic & Swasti 2013). FFA alone composes more than 50% of all furans quantified in coffee (Petisca et al. 2013).

HMF, a less volatile furan derivative, with amounts in foods that differentiate widely and can reach more than 1 g/kg like in dried fruits, caramel, roasted coffee, chicory, barley, malt, and balsamic vinegar (Anese and Suman 2013). HMF was the major volatile chemical in light roasted coffee (Moon and Shibamoto 2009). HMF quantities are proportional to the heat applied during carbohydrate-rich food processing thus it can be taken as a quality marker of these products. HMF is also found in honey, bakery products, fruit juices, pasta, milk, and cereals, not to mention pharmaceutical preparations and cigarette smoke. Bread and coffee are considered the main HMF dietary sources in human diet. In recent investigations, the daily intake of HMF can reach up to 23 mg which is several orders of magnitude higher than that of other process contaminants such as acrylamide and furan (Capuano and Fogliano 2011).

Alongside FFA and HMF, we find 2-FA and HMFA in coffee in considerable amounts (Capuano and Fogliano 2011).

1.2.2. Analysis.

Gas and liquid chromatography with UV-Vis, biosensor, fluorescence, or mass spectrometric detection have been reported for the analysis of furan derivatives (Okaru and Lachenmeier 2017). A preceding extraction step is usually implemented to avoid matrix interferences. Nevertheless, these preparation steps may result in a too-diluted sample, insufficient sensitivity, or the consumption of big volumes of solvents as in liquid-liquid or solid phase extraction, however the use of micro-extraction by packed sorbent can help to avoid these disadvantages with minimizing time and money spent (Silva et al. 2017). Solid-phase extraction and micro-extraction are based on the entrapment of analytes to release them at a later step. These techniques have been employed vastly in the isolation, clean-up and concentration of furan derivatives from their samples (Hu et al. 2013). When analysing furan derivatives in coffee, a clean-up step by water extraction and filtration must be applied, along a clarification step by solid-phase extraction (Anese 2015).

At the mean time gas chromatography coupled with headspace or headspace-micro extraction is the common method for the monitoring of furan derivatives given the volatility of some members of this class (Hu et al. 2013). In the headspace technology the analytes are partitioned from the food sample into the headspace gas which is injected directly into the GC system, whereas in the headspace-micro extraction, the analytes in the headspace are adsorbed on a polymeric fibre and the substances are analysed by GC after thermos release in the injector. Different times and temperatures as well as food composition affect the results of the headspace (Anese 2015). Extraction temperature, time, sample volume, and salt concentration need to be optimised in the headspace-micro extraction technology (Pérez-Palacios et al. 2012). But since furan

derivatives form during heat treatment of food samples that contain their precursors, the application of heat on these samples that are being injected into GC systems might lead to the formation of artefacts or can change the actual amounts of these derivatives, such as the documented on-fibre formation of furan from related compounds, e.g. 2-butenal and furfural, during thermal desorption (Hu et al. 2013).

HPLC and UPLC have been reported multiple times for the analysis of furan derivatives in apple cider and wine (Hu et al. 2013), traditional balsamic vinegars (Chinnici et al. 2003), fruit juices (Palmers et al. 2015), sugarcane honey (Silva et al. 2017) and treacle (Edris et al. 2007) while publications concerning coffee focus on the analysis of a single furan derivative (Murkovic and Pichler 2006, Rodrigues and Bragagnolo 2013).

Other colorimetric and spectrophotometric methods have been reported for the analysis of HMF (Anese 2015).

1.2.3. Metabolism.

FFA is gradually oxidized upon absorption to furfural and then to 2-FA. This carboxylic acid forms a thioester with coenzyme A that undergoes further metabolism to a glycine conjugate and excreted in urine. Alternatively, the thioester can condense with acetyl-coenzyme A forming 2-furanacryloyl coenzyme A that is also conjugated with glycine and excreted through urine (JECFA 2001).

The oral administration of HMF results in fast absorption via the gastrointestinal track of rats and mice. HMF is also taken up by cell cultures where the sample composition and concentration affect the total uptake. After absorption HMF is quickly metabolised in the kidney, bladder or to a smaller extent in the liver. Finally, it is excreted via urine.

HMF goes through oxidation to form HMFA that is conjugated with glycine. Later, this glycine derivative is excreted through urine. Higher administration of HMF will result in higher excretion of 2-FA and 2,5-furan dicarboxylic acid in the urine. Other metabolites recovered from urine include 5-carboxylic acid-2-furoyl glycine, 5-carboxylic acid-2-furoyl amino-methane, and 5-hydroxymethylfuranacryloylglycine, while no conjugates of HMF with either glucuronide or sulfate were recovered. HMF is proposed to undergo allylic chlorination in gastric juice because of the availability of plenty of chloride ions giving 5-chloromethylfurfural that is an extremely mutagenic agent in *Salmonella typhimurium*. However, this compound was never retrieved *in vivo*. It is also notable that HMF can be converted by colonic bacteria into FFA (Capuano and Fogliano 2011).

Another pathway of the metabolism of furan derivatives is the ring opening to α-ketoglutaric acid followed by complete oxidation to carbon dioxide through the citric acid cycle. This pathway is rather minor and it was only proven in rodents (JECFA 2001, Capuano and Fogliano 2011).

On the other hand, FFA and HMF can be sulfonated to 2-sulfo-oxymethylfuran and 5-sulfoxymethyfurfural, respectively, in the presence of phosphoadenosine-phosphosulphate. This reaction is catalysed by sulfotransferases (SULT). The activated compound contains sulfate as a good leaving group resulting in unstable intermediates that can react with DNA and other macromolecules (Glatt et al. 2012, Sachse et al. 2016).

1.2.4. Toxicological effects.

FFA and HMF are potentially harmful moieties to living organisms, particularly those producing SULTs either naturally or after genetic engineering. Several animal studies have proven that FFA and HMF can

form DNA adducts through a similar conversion mechanism. Intracellular SULTs are involved in the production of reactive electrophiles from FFA and HMF which in turn react with DNA molecules leading to mutations (Glatt et al. 2012, Sachse et al. 2016).

According to the 76th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2012), FFA is suggested to be converted by intracellular mouse and human SULTs to produce a reactive electrophile, i.e. 2-sulfooxymethylfuran as in Fig. 2 that can interact with DNA forming 2-methylfuran adducts of 2′-deoxyadenosine and 2′-deoxyguanosine (JECFA 2012). Monien et al. (2011) detected DNA adducts in FFA exposed *S. typhimurium* TA100 expressing human SULT 1A1 and in DNA of liver, lung and kidney of orally exposed FVB/N mice which also express SULT 1A1. While Høie et al. (2015) reported that FFA exposure enhanced the adduct levels in colon and liver of wild type mice. It is also mentioned that FFA is able to induce sister chromatid exchange (SCE) in human lymphocytes. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded at the 76th meeting, that newly published *in vitro* and *in vivo* studies raise concerns regarding the potential genotoxicity of FFA and that the previously established ADI will need to be reconsidered at a future meeting (JECFA 2012).

HMF poses a carcinogenic risk due to its structural characteristics such as the furan ring, the α, β-unsaturated carbonyl, and the allylic hydroxyl group (Anese and Suman 2013). The administration of HMF at high concentrations is shown to cause cytotoxicity, and the irritation of eyes, upper respiratory tract, skin and mucous membranes. An LD50 of HMF on rats was calculated to be between 2.5 to 5.0 g/kg of body weight. In rodents, HMF induced colonic pre-neoplastic lesions in a concentration dependent manner, skin papilloma after topical administration,

lipomatous kidney tumours, multiple intestinal neoplasia, hepatocellular adenomas. HMF promoted no or low mutagenicity *in vitro* in bacterial strains. On the other hand, HMF exhibited genotoxicity in *S. typhimurium* TA104 in the presence of rat hepatic SULTs and in *S. typhimurium* TA100 strain expressing human SULT1A1 and in mammalian cells engineered for expression of human SULT1A1. This can be attributed to the fact that HMF is enzymatically transformed *in vivo* to produce 5-sulfooxymethylfurfural (as in Fig. 2), a known compound to induce tumours in mice skin. This metabolite is a reactive electrophile that can interact with DNA-molecules forming adducts in cell-free systems and is directly mutagenic to bacterial and mammalian cells. Topically it is more actively causing mouse skin papilloma than HMF. Recently, this metabolite was identified in the blood of mice after administration of HMF. Also, it was found to be a strong nephrotoxic and liver damaging. Different SULT forms showed different conversion kinetics of HMF, with human 1A1 being the most active form. With this type of enzymes being more expressed in human non-liver tissues than in rodents, implying that humans are more sensitive to HMF than rodents. However, data obtained from literature is controversial regarding a serious health risk relevant to human consumption of HMF although some foods may have concentrations close to the active range in cells. Multiple exposure limits for HMF were set from 132-540 mg/day. EFSA concluded that a sufficient evidence to raise concern about genotoxicity is available (Capuano and Fogliano 2011).

2-FA is not a hydroxymethyl furan derivative, so it does not share the same underlying bio-activation mechanism as for FFA and HMF leading to its toxicity. Nonetheless, 2-FA is believed to be a precursor of a different toxic molecule. The decarboxylation of 2-FA is hypothesised to

lead to furan in food during pasteurisation or sterilization (as seen in Fig. 2) (Varelis and Hucker 2011). Furan is a known toxic and carcinogen to humans after its transformation by cytochrome P450 to cis-2-butene-1,2-diol (Mesias and Morales 2015). It was reported to induce hepatocellular adenomas and carcinomas in rodents and high incidence of cholangiocarcinoma in rats at high doses (Anese 2015). Lately, it was classified by the International Agency for Research on Cancer as a possible carcinogen to humans (group 2B) (Mesias and Morales 2015).

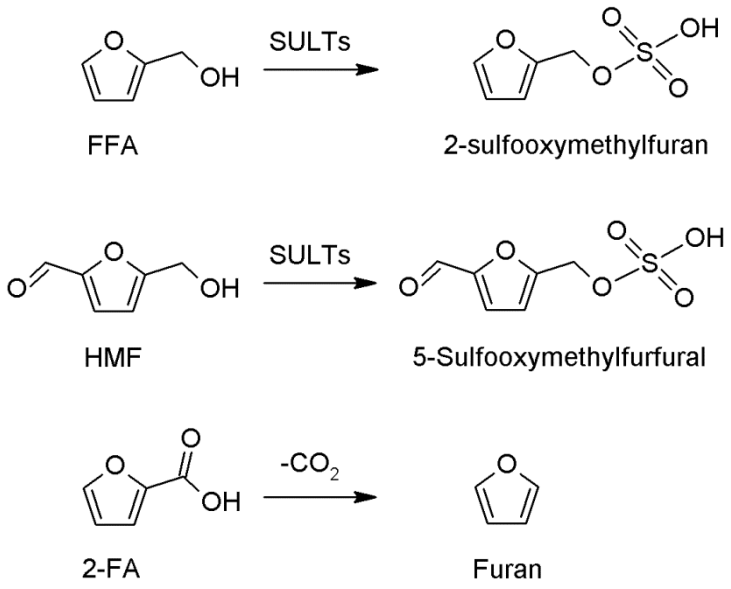


Fig. 2: The conversion of furfuryl alcohol (FFA), 5-hydroxymethylfurfural (HMF) and 2-furoic acid (2-FA) into potentially toxic compounds. SULTs: Sulfotransferases.

1.2.5. Formation mechanism.

Studies indicate clearly that the production of coffee volatiles and flavour compounds follows a massively complex network of reactions of a wide variety of possible precursors that interact and sometimes compete with each other. The elucidation of formation mechanisms is realised through many strategies. Model systems of a limited number of presumed

precursors were roasted mimicking actual conditions usually applied with aim of reducing the complexity of real coffee beans. Thiols, diketones, and pyrazines are examples of studied aroma molecules under dry heat conditions. A model system is mostly a simple mixture of a sugar and an amino acid in the case of Maillard reaction products. Nonetheless, these model systems cannot provide the identical complex chemical and physical atmosphere as in the green coffee bean. The comparison of roasting whole beans to fragments or powdered beans was reported previously. Therefore, model systems may not be helpful in the elucidation of the authentic reactions taking place in the coffee bean. Recently, the intact coffee bean was suggested as a carrier vessel of the precursors to be studied and this approach was called the biomimetic in-bean system. In this system, the beans are extracted with water and then freeze dried to permit the use of the empty shell by spiking precursors in question via soaking the shells in saturated solution of these compounds. The introduction of real food matrix characteristics gives improved and more realistic understanding of the ensuing reactions. This technique was used to study the formation of 2-furfurylthiol, 2- and 3-methylbutanal, α-diketones, guaiacols, alkyl pyrazines, dicarbonyls and coffee melanoidins. Another advancement was achieved using labelled precursors (Poisson et al. 2017).

The widespread of furan derivatives in various food types can be attributed to the fact that these products can be formed via multiple routes and from a wide range of precursors like carbohydrates, amino acids, mixtures of carbohydrates and amino acids, vitamins, polyunsaturated fatty acids and carotenoids (Anese and Suman 2013). Looking at green coffee, furan derivatives are found (if any) in negligible amounts. Thus, such compounds can be termed as process contaminants since they are

mostly formed through the roasting process from precursors found naturally in green coffee.

FFA formation mechanism is still not fully understood as few papers have presented hypotheses concerning its formation in food matrix. Yaylayan and Keyhani (2000) have used ^{13}C labelled model systems of glucose and alanine to study the carbon source of Maillard reaction products under pyrolysis conditions. They observed the formation of FFA from intact C1-C5 or C2-C6 carbon skeleton of glucose. It was hypothesised that an oxidation at C1 and C6 of the glucose takes place to produce both gluconic and glucuronic acid, followed by decarboxylation to give pentitiol and aldopentose, respectively. By further dehydration and cyclisation FFA was formed (Fig. 3b, c). In addition, FFA was also formed from fragments of both glucose and alanine through an unknown mechanism, with glyceraldehyde as potential intermediate (Fig. 3a). In another manuscript Wnorowski and Yaylayan (2000) suggested a different reaction pathway for the formation of FFA, in which they hypothesised that a retro-aldol cleavage takes place at the C1-C2 bond of 1-deoxy-1-amino-3-hexulose that is formed from the Amadori-compound. This reaction leads to an aldo-pentose that can dehydrate and cyclise to FFA (Fig. 3d). Brands and van Boekel (2001) have detected minor amounts of FFA in heated aqueous solutions of monosaccharides and casein model systems, and proposed that it can be formed from an 1,2-enediol by β-elimination and α-dicarbonyl cleavage (Fig. 3e). Another pathway that they presented was the Maillard reaction which forms N-glycosylamine followed by rearrangement to a Schiff base and subsequently to 1,2-enaminol which can be deaminated followed by α-dicarbonyl cleavage leading to FFA. Although they mentioned that this pathway is not significant in ketose model systems (Fig. 3f). Later, Moon

and Shibamoto (2010) indicated FFA as one of the major compounds formed from the roasting of quinic or caffeic acids, and suggested that the formation pathway is possible through a sequence of dehydration, formic acid elimination, homolytic bond cleavage, and radical reactions (Fig. 3g). Also, FFA is reported to originate from the enzymatic or chemical reduction of furfural during the storage of alcoholic beverages (Fig. 3h) (Okaru and Lachenmeier 2017).

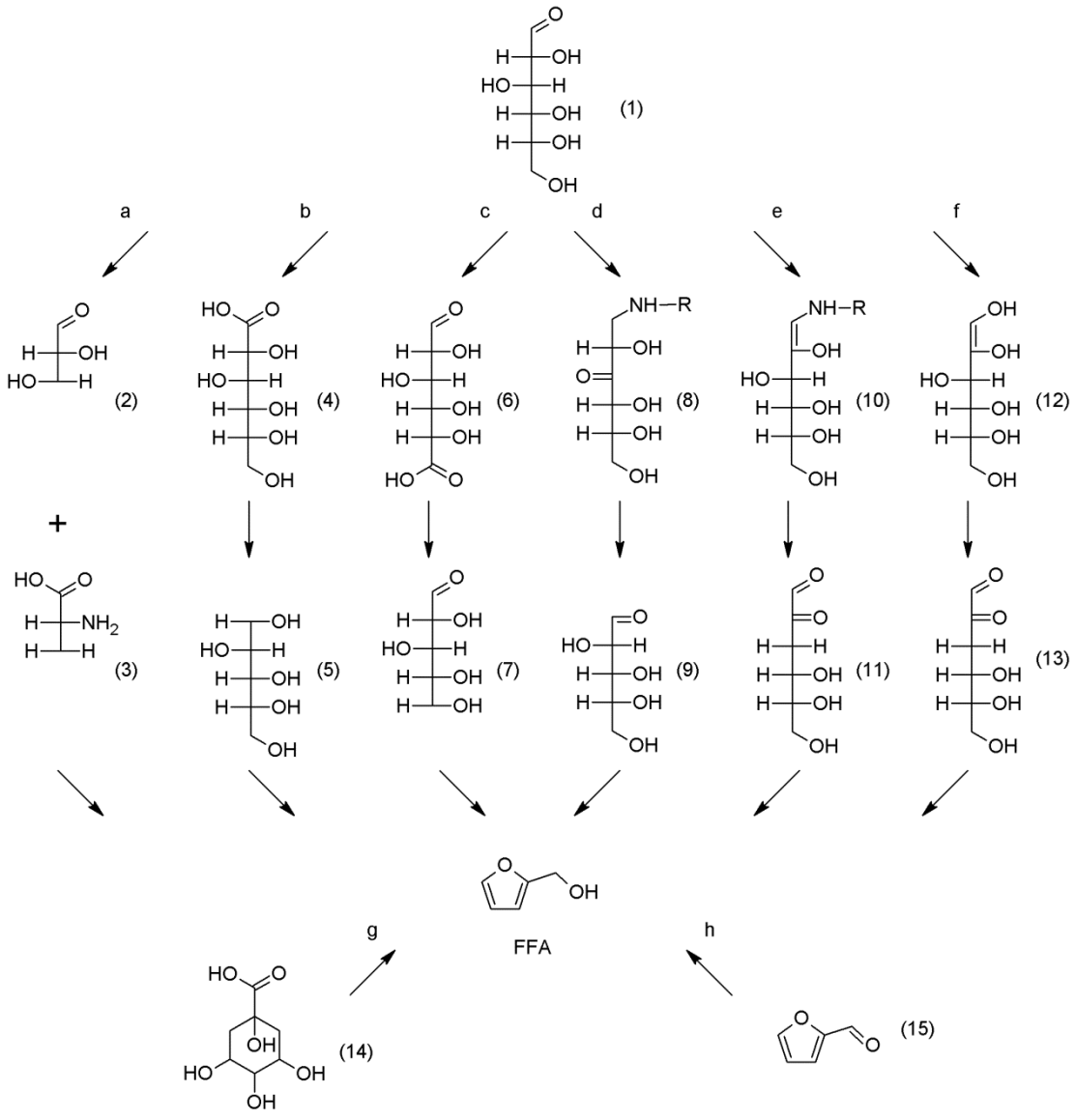


Fig. 3: Different pathways leading to the formation of furfuryl alcohol (FFA) in food matrix. 1: Glucose. 2: Glyceraldehyde. 3: Alanine. 4:

Gluconic acid. 5: Pentitol. 6: Glucuronic acid. 7: Aldopentose. 8: 1-Amino-1-deoxy 3-hexulose. 9: Aldopentose. 10: 1,2-Eneaminol. 11: 3-Deoxyosone. 12: 1,2-Enediol. 13: 3-Deoxyosone. 14: Quinic acid. 15: 2-Furfural.

The kinetics of HMF formation are described as zero-order or an exponential trend (Capuano and Fogliano 2011). Cämmerer et al. (1999) elucidated the formation mechanisms of HMF in caramelisation and Maillard reaction conditions. When no amino acid is present, sugars (e.g. glucose and fructose) in acidic food medium tend to undergo 1,2-enolization followed by dehydration to give a 3-deoxyosone. Fructose is reported to be more actively participating in HMF formation than glucose (Fig 4b) (Anese and Suman 2013). The heating of sugars in the presence of proteins and amino acids leads to the same key intermediate through the Maillard reaction, directly from the Amadori product. Later, 3-deoxyosone produces HMF after dehydration and cyclization (Fig 4a). In addition, the same researchers proposed that methyl glyoxal together with glyceraldehyde through an Aldol pathway lead to the formation of HMF in heated aqueous solutions under caramelisation conditions (Fig 4d). Another prominent theory is the direct formation of HMF from sucrose/fructose by the intact fructofuranosyl cation that converts quickly under dry heat conditions to HMF (Fig 4c) (Perez Lucas and Yaylayan 2008). The low pH value in the dough can increase HMF levels in bakery products. It is even possible for HMF to form in acidic medium even at low temperatures, although higher temperatures result in greatly elevated levels. Other parameters affecting levels of HMF in food are the type of sugar available, pH, water activity, and the amounts of divalent cations (Capuano and Fogliano 2011).

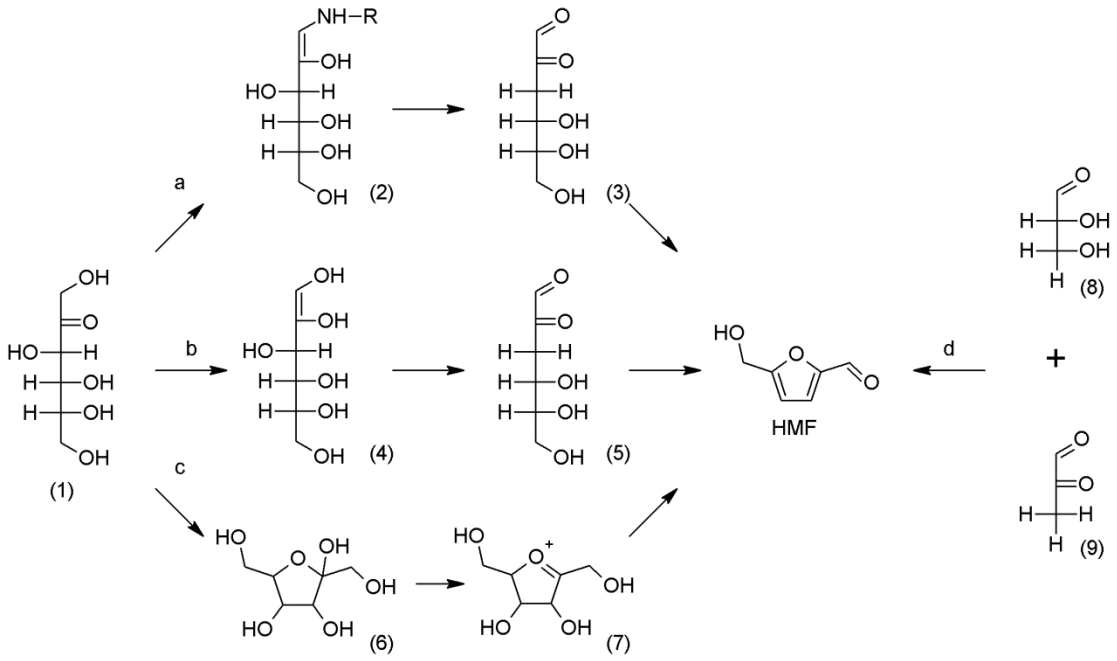


Fig. 4: Different pathways leading to the formation of 5-hydroxymethyl furaldehyde (HMF) in food matrix. 1: Fructose. 2: 1,2-Eneaminol. 3: 3-Deoxyosone. 4: 1,2-Enediol. 5: 3-Deoxyosone. 6: Fructofuranose. 7: Fructofuranosyl cation. 8: Glyceraldehyde. 9: Methyl glyoxal.

2-FA is believed to originate from the caramelisation of sugars, ascorbic acid or dehydroascorbic acid. It was reported that the thermal degradation of aqueous solutions of dehydroascorbic acid results into 2-FA, probably through 2,3-diketo-L-gulonic acid that is quickly decarboxylated into a ketene intermediate (Fig. 5a). However, ascorbic acid produces 2-furfural instead. 2-Furfural, in turn, is proposed to give rise to 2-FA through a disproportionation Cannizzaro reaction, where 2-furfural is hydrated forming 2-furylmethanediol that transfers a hydride to another 2-furfural molecule resulting in a FFA moiety and a 2-FA moiety (Fig. 5b). 2-Furfural can also take part of the Cannizzaro reaction after being formed from the caramelisation of sugars (Fig. 5c) (Varelis and Hucker 2011).

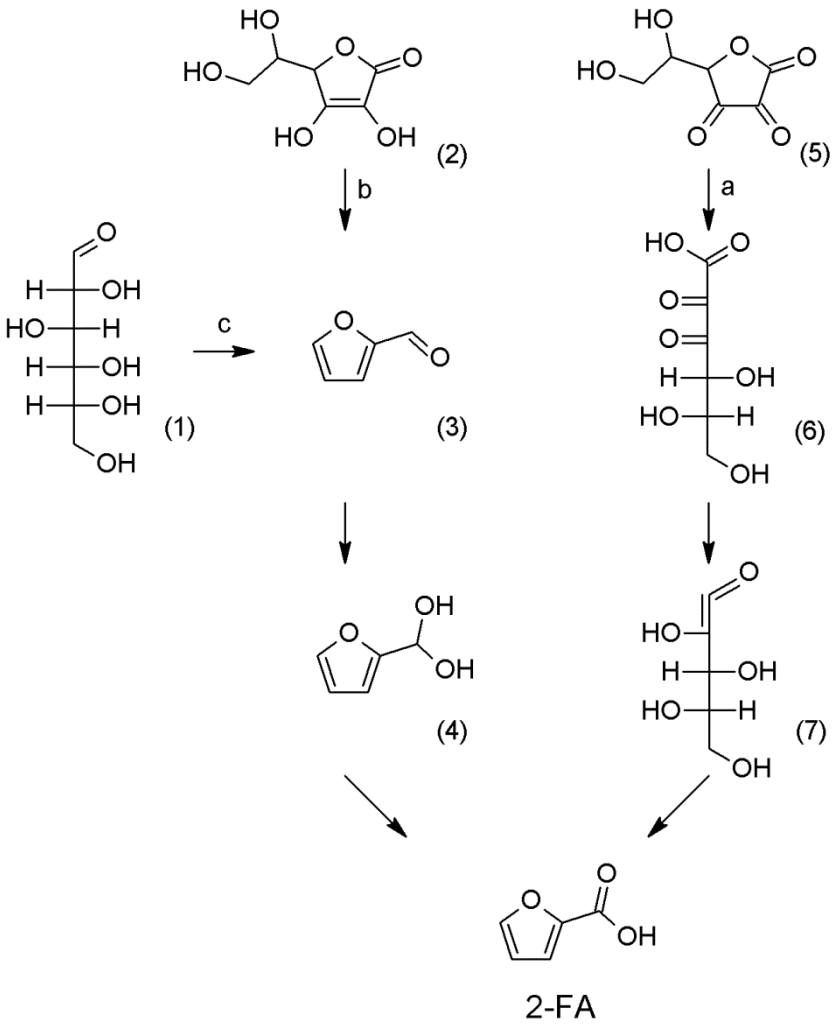


Fig. 5: Different pathways leading to the formation of 2-furoic acid (2-FA) in food matrix. 1: Glucose. 2: Ascorbic acid. 3: 2-Furfural. 4: 2-Furylmethanediol. 5: Dehydroascorbic acid. 6: 2,3-diketo-L-gulonic acid. 7: Ketene intermediate.

In contrast, very limited number of papers discusses the formation mechanisms of HMFA in food matrix. Murkovic and Bornik (2007) suggested that HMFA is formed by an Aldol reaction of glyceraldehyde with pyruvic acid.

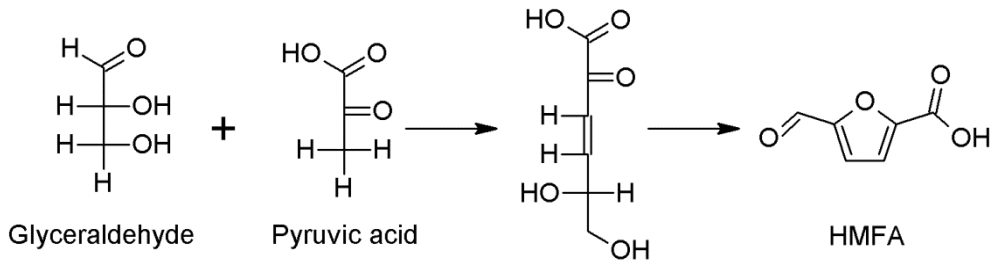


Fig. 6: Formation pathway of 5-hydroxymethyl-furoic acid (HMFA) in food matrix as per Murkovic and Bornik (2007).

1.3. Mitigation experiments:

Process contaminants like acrylamide, furan and HMF are of great concern due to their potential toxicological effects as well as their widespread in foods. This raised awareness prompted industry to keep their as low as practically possible. Possible routes to mitigate food process contaminants were investigated in order to reduce consumer intake. Sometimes it is useful to deduce information about mitigation of process contaminants from research dealing with various compositions or process parameters leading to their formation. Examples are through the manipulation of formulations, process and post-process activities (Anese and Suman 2013).

1.3.1. Approaches.

Several approaches have been suggested for the mitigation of potentially harmful substances formed during processing of foods. Anese and Suman (2013) classified these technological approaches to preventive approaches that aim to lessen the formation of process contaminants during thermal or other processing activities, and eliminative approaches that target the removal of such compounds after being created.

Preventative approaches can rely on changing the reaction conditions to less favourable ones by altering precursors or formation mechanisms. Modifying the heat load (i.e. low thermal input roasting) or the formulation of the product are regarded as preventative methods also. Specific precursors can be removed or substituted with others that do not lead to the formation of relevant process contaminants. Alternatively, new ingredients can be added to the formula to compete with highly reactive precursors of process contaminants. However, from an industrial point of view all these preventative interventions offer no assessment of the sensory characteristics of the re-formulated products thus affecting the possibility of these approaches before a full assessment is done (Anese and Suman 2013).

Furan and HMF are process contaminants that are affected by the temperature of roasting. Thus, low temperature roasting for a prolonged period of time can be an effective preventative approach for the mitigation of these process contaminants (Anese 2015). The manipulation of the time-temperature profile is likely to affect the development of the brown colour of foods (Capuano and Fogliano 2011). Another innovative way to lower the heat load is the use of dielectric heating that is instant and more uniform since it generates heat from inside the product by frictional interactions of polar molecules (water) with the electric field and electromagnetic radiation. Furan and its derivatives were less produced by microwave–hot air roasting (Anese 2015).

Eliminative or post-processing approaches can be done sometimes simply by incorporating food additives after thermal treatment of the product, thus limiting their contribution to the formation of process contaminants although this is industrially unrealistic. Occasionally, the alteration of environmental conditions such as the introduction of nitrogen as a gas can

limit formation of specific process contaminants. Otherwise, the volatility of some process contaminants (e.g. furan) can be exploited to remove them from the finished food product (i.e. vacuum treatment) (Anese and Suman 2013). However, such treatments lower the total volatiles and odour intensity, implying the need to a flavour enrichment step just before packaging to neutralize the negative effects of this treatment. Different food preparation techniques, such as the method of coffee brewing or cooking in an open vessel, can affect the amounts left of process contaminants in the final consumed product. A good example is the lower levels of furan in filter-drip prepared coffee compared to espresso and automated coffee machines. The reason is that the closed environment and direct consumption of these products decrease the losses of process contaminants through normal volatility (Anese 2015). Ionizing radiation or yeast fermentation can be used to decompose some process contaminants (Anese and Suman 2013).

1.3.2. Process contaminants.

Food processing introduces vast changes to the composition of food, some of these changes are important to the edibility of foods (colour, flavour and aroma) while others are of harmful characteristics. Food process contaminants can thus be defined as impurities that are not naturally a part of a food that were introduced during food cooking or processing and are harmful to the quality of the product or the people consuming that product (Curtis et al. 2014).

The mitigation of various process contaminants was carried out previously, such as acrylamide (Constantinou and Koutsidis 2016, Oral et al. 2014), furan and/or its derivatives (Zheng et al. 2015, Navarro and

Morales 2017, Oral et al. 2014, Totlani and Peterson 2007), and pyrazines and pyrroles (Totlani and Peterson 2007).

Acrylamide is a known hazardous compound that forms during thermal treatment (e.g. roasting, baking and frying) of foods (Bagdonaite et al. 2008). It is formed through the Maillard reaction with the level of free asparagine considered the limiting precursor for its production (Curtis et al. 2014). Acrylamide is described as a probable carcinogen to humans by the International Agency for Research of Cancer due to its ability to form adducts to haemoglobin and DNA after being metabolised to an epoxide (Bagdonaite et al. 2008). Acrylamide was found to be related to breast cancer mortality, low birth weight and low head circumference in babies beside other toxic neurological and reproductive effects that were detected in rodents. The Joint Expert Committee of Food Additives of the FAO and WHO considered the presence of acrylamide in food a concern to public health. The European Commission and the American Food and Drug Administration (FDA) are trying to lower the current levels of acrylamide by issuing indicative levels that were agreed upon with the industry. Acrylamide is found in high concentrations in coffee as well as many potato or cereal-based dishes. A 'Toolbox' collecting possible approaches for reducing acrylamide in food industry was issued by Food Drink Europe comprising many approaches such as the manipulation of proceeding parameters, lowering the pH before processing, water pre-soaking, and the addition of various mitigation agents (antioxidants, divalent cations, asparaginase). Reducing free asparagine accumulation in grains of cereals by agronomical or genetic practices is also an investigated approach for limiting acrylamide in bakery products. Such approach requires looking for plant varieties (weather already cultivated or not) and environmental factors with low potential of acrylamide

formation. It is well known that low sulphur supply to cereal plants raises the production of asparagine in the grains, as well as the deficiency in potassium, phosphorus and magnesium. However, elevated nitrogen supply is correlated with increased free asparagine content in grains. Refining of the flour may help in the reduction of acrylamide formed from these products compared to wholegrain products. Examples of environmental factors that affect the accumulation of free asparagine in cereals are cadmium exposure, drought and salt stress, pathogen attack. Genetic intervention was achieved in genetically modified potato by manipulating genes responsible for asparagine synthetase, thus reducing the acrylamide formation potential. Other genetic approaches suggest the induction of glutamine accumulation instead of asparagine; consequently 2-pyrrolidinone will be formed rather than acrylamide (Curtis et al. 2014). The higher roasting times of coffee lead to the degradation of acrylamide that was formed during the first few minutes of the process where around 95% of all acrylamide formed being lost during roasting. But a balance is needed between optimal aroma and the amount of acrylamide left in coffee since both depend on the roasting time and conditions. Different brewing techniques can reduce the amount of acrylamide present in the brew. Although acrylamide is highly water soluble, espresso brewing helps in lowering the final level of acrylamide in the brew compared to filter brewing. Acrylamide is also reported to decrease after different periods of storage (especially in instant coffee) with temperature of storage playing an important role (Bagdonaite et al. 2008).

Furan is another food process contaminant that is known to cause liver cancer in rodents and it is currently classified as a possible carcinogen to humans by the International Agency for Research on Cancer. Furan has

several formation pathways, such as the oxidation of poly unsaturated fatty acids, through Maillard reaction, and from direct degradation of sugars, ascorbic acid or dehydroascorbic acid. Its concentrations in foods (especially in coffee or to a minor extent in cereal foods) raises concern of health risk too. Mitigation of furan can rely on the modification of processing parameters such as temperature, time, phosphate level and pH. Lower free amino acids and reducing sugars content in cereals besides reducing the content of unsaturated fatty acids in plant oils are potential approaches for the mitigation of furan formation in foods (Curtis et al. 2014). Vacuum application is a promising technique for the removal of furan from high moisture foods. Others suggest introducing products with minimal heat processing, e.g. light roasted coffee instead of dark ones, to limit the formation of furan, but analogous approaches might lead to an entirely different aroma and taste of the product thus affecting consumer acceptability. Nevertheless, this approach might lead to an elevation of other process contaminants, e.g. acrylamide, in the final product since prolonged roasting help in the destruction of process contaminants that are produced early in the roasting process (Anese 2015). Furan mitigation experiments do not provide conclusive results due to the large number of precursors and the complexity of their interactions (Anese and Suman 2013).

HMF is a food process contaminant that was the topic of multiple mitigation experiments due to its higher exposure rates and the potential mutagenicity. However, no industrial HMF-specific mitigation techniques are available yet (Capuano and Fogliano 2011), however several formulation-based mitigation strategies are studied at lab scale and need further effort to gain industrial applicability (Anese and Suman 2013). The application of certain temperature, time, and pressure conditions can

help remove furan derivatives (e.g. HMF) through volatility. However, a hydration step of coffee powder is necessary before vacuum application to induce the release of volatiles (Anese 2015). Nevertheless, the mitigation of furan derivatives in foods is very difficult to achieve industrially due to many reasons, e.g. multiple precursors that are healthy food components that cannot be omitted as well as the close relation between reactions leading to the formation of furan derivatives and other desired sensory attributes (colour and flavour) of heated foods (Anese and Suman 2013). For instance, HMF levels are reported to correlate with the brown colour of foods (Capuano and Fogliano 2011). In fact, the mitigation of furan derivatives might compromise food acceptability by consumers. (Anese and Suman 2013).

1.3.3. Mitigation by chemical additives.

Within various mitigation approaches we can find that the addition of different chemical compounds that can either interfere with the building mechanism or destroy the final produced process contaminants is a widely applied strategy. Numerous chemical additives belonging to different chemical classes were applied in these experiments. Mostly antioxidants of polyphenol characteristics were employed since they are known to exhibit dicarbonyl trapping effects. These effects play a pivotal role in capturing important intermediates for the formation of process contaminants, especially those formed through caramelisation or Maillard reaction (Zheng et al. 2015). Different research groups used different matrices to investigate the mitigation effects of various agents. Some papers applied mitigation agents to well defined and simplified model systems while others employed them during the processing of actual food samples (usually biscuits).

The addition of reducing agents was capable of mitigating furan formation from ascorbic acid model systems, whereas the availability of ferric ions promoted its formation from unsaturated fatty acids. (Curtis et al. 2014) Furan production is reported to decrease by 30% by replacing oxygen with nitrogen in model systems, thus an air exhaustion step can limit its formation in canned foods (Anese 2015). Radical scavengers such as tocopherol acetate and butyl-hydroxy anisole are reported to reduce furan formation in a poly unsaturated fatty acid system by various degrees depending on the processing conditions (pressure cooking or dry heating). Asparagine addition to a sugar-malic acid model system reduced HMF formation significantly (Suman and Anese 2013).

The formation and mitigation of process contaminants is studied mostly by using model systems while experiments carried out in foods are rather limited. However, mitigation agents added to these systems can exert positive or negative results depending on the system's composition and the conditioned applied during processing, e.g. furan formation from ascorbic acid gave contradicting results depending on the matrix composition. At the same time, model systems of pure compounds do not give similar levels of process contaminants as in actual foods due to competing reactions that can be only found in real foods. Hence, the results deduced form model systems must be carefully viewed before they can be used to understand reactions and mechanism in foods (Anese and Suman 2013).

During mitigation experiments in cereal based foods, the exchange of reducing sugars with other sugars or polyalcohols affected the overall colour of the product; also, the addition of sucrose resulted in higher HMF levels due to the direct formation of the fructofuranosyl cation (Capuano and Fogliano 2011). α-Tocopherol did not exhibit any furan

mitigation capacity in soybean oil-starch emulsions. Ascorbic acid was added to orange juice in order to lower the formation of furan, whereas its addition to carrot juice or pumpkin puree induced furan production (Anese and Suman 2013).

Coffee is the focus of many mitigation investigations due to its high consumption rate which might lead to potential health risks to humans. (Bagdonaite et al. 2008) Mitigation techniques can be applied at different coffee production steps (roasting, brewing or consumption), although some of these are barely achievable (Anese 2015).

1.4. Aim of study.

Hydroxymethyl substituted furan derivatives exhibit a widespread presence in foods and they were shown to acquire toxic effects on human health. Coffee is considered a food material that is both rich in furan derivatives and at the same time being heavily consumed by the population. FFA, a volatile and reactive hydroxymethyl substituted furan derivative, is the major furan derivative in coffee, nevertheless other major furan derivatives, namely HMF, 2-FA and HMFA, are also considered important due to their potential toxicological properties. The volatile profile of a cup of coffee, including furan derivatives, differs widely due to many parameters, e.g. roasting conditions, coffee species used, residual water content in the green beans, the volatility of certain molecules, storage conditions, the interaction with gastrointestinal fluids upon consumption, brewing method applied. As well, the number of published reports about the mitigation of process contaminants in coffee (including furan derivatives) is extremely limited due to the fact that mitigation agents are hard to incorporate into coffee before being roasted.

For all the fore mentioned reasons, the research was aimed to achieve the following objectives:

- To optimize a simple and reliable analytical method for the simultaneous determination of FFA, 2-FA, HMF and HMFA in roasted ground coffee and brews using an HPLC-DAD system equipped with a C8 column and by implementing different extraction solvents and techniques for this purpose.
- To study the formation kinetics of FFA in roasted coffee and the various factors that may affect its levels, e.g. different species, residual moisture level, and the volatility of FFA.
- To explore the parameters affecting the exposure to FFA, such as the effect of storage conditions on the level of FFA in coffee and the effect of gastrointestinal fluids on FFA after consumption of a coffee beverage.
- To investigate the effects of various brewing techniques on the levels of FFA, 2-FA, HMF and HMFA in coffee brews to determine relative exposure of these components from coffee consumption.
- To estimate the mitigation effect of 14 different agents being applied to the coffee matrix, and compare this to their effect when applied to a simplified model system containing probable precursors of process contaminants in question.

2. Results and Discussion

A detailed description of the methods applied to achieve the objective of this research along with the results obtained and a consequent discussion of these results, are presented here in the form of four research papers. These papers were submitted to scientific journals for peer-review. The four manuscripts are as follows:

- **Abdullatif Albouchi**, & Michael Murkovic. (2018). A simple and selective liquid chromatography method for the determination of furan derivatives in roasted coffee extracts and brews.
- **Abdullatif Albouchi**, & Michael Murkovic. (2018). Formation kinetics of furfuryl alcohol in a coffee model system. *Food Chemistry*. 243: 91–95.
- **Abdullatif Albouchi**, Julia Russ, & Michael Murkovic. (2018). Parameters affecting the exposure to furfuryl alcohol from coffee.
- **Abdullatif Albouchi**, & Michael Murkovic. (2018). Mitigation of furan derivatives in coffee and coffee-related model systems.

2.1. Paper 1:

'A Simple and Selective Liquid Chromatography Method for the Determination of Furan Derivatives in Roasted Coffee Extracts and Brews'

A simple and selective liquid chromatography method for the determination of furan derivatives in roasted coffee extracts and brews

Abdullatif Albouchi, Michael Murkovic

Institute of Biochemistry, Graz University of Technology, Graz, Austria

Petersgasse 10-12/II, 8010, Graz, Austria

Corresponding author:

E-mail: Abdullatifl.albouchi@outlook.de - Tel. +43 6648749438.

Abstract:

The simultaneous separation and quantitation of four major furan derivatives in roasted coffee were performed using a simple and selective HPLC-DAD method. No sample pre-treatment other than centrifugation was needed. The purity of the peaks of furfuryl alcohol, 2-furoic acid, 5-hydroxymethyl furaldehyde and 5-hydroxymethyl furoic acid in coffee extracts and brews was checked by the diode array detector against authentic standards. The validation results affirmed the linearity (in the range of 5-25 µg/mL), accuracy (recovery was ≥ 89.9%) and reproducibility (intra-day relative standard deviation was ≤ 4.2%, inter-day relative standard deviation was ≤ 4.5%) of the developed method. The values for the limit of detection and quantification were in the range of 0.11-0.76 and 0.35-2.55 µg/mL, respectively. Sonication and shaking with or without heating were applied as different extraction techniques for the extraction of the four furan derivatives from ground coffee. No significant difference was observed between the three techniques when water was used as solvent. Water and methanol were compared to a water-methanol mixture in the clean-up of ground coffee samples. Water acted as the best extraction solvent among the ones tested by extracting a minimum of 97.3% of furan derivatives from ground coffee from the first extraction cycle. 50% (v:v) methanol in water showed slightly lower clean-up whereas methanol was the least suitable solvent, especially for the extraction of 2-furoic acid, 5-hydroxymethyl furaldehyde and 5-hydroxymethyl furoic acid.

Keywords: HPLC-DAD; furan derivatives; coffee; extraction techniques; extraction solvents.

Introduction:

Furan derivatives are a wide range of chemicals that share a five-membered ring containing an oxygen atom. This ring can carry one or more substituents affecting its physio-chemical properties such as solubility and volatility (Anese 2015). This class of compounds is receiving a lot of attention due to their pleasant contributions to the aroma, colour and taste of many foods and beverages as well as their potentially harmful effects (Hu et al. 2013).

Coffee is one of these foods with a specific aroma related to furan derivatives. In green coffee, furans are not naturally present. They are formed during the roasting process where the level of these compounds correlates well with the colour of roasting. It was found in several studies that this class of compounds comprises the major components of its aroma (Anese 2015; Petisca et al. 2013). Of special importance are furfuryl alcohol (FFA) and 5-hydroxymethyl furaldehyde (HMF) which are found in great quantities in coffee along with their carboxylic acid derivatives 2-furoic acid (2-FA) and 5-hydroxymethyl furoic acid (HMFA). FFA alone composes more than 50% of all furans quantified (Petisca et al. 2013) while HMF was the major volatile chemical in light roasted coffee (Moon and Shibamoto 2009).

On the other hand, several animal studies have proven that FFA and HMF can form DNA adducts through a similar conversion mechanism. Intracellular sulfotransferases (SULT) are involved in the production of reactive electrophiles from FFA and HMF (2-sulfo-oxymethylfuran and 5-sulphooxymethylfurfural, respectively), which in turn react with the DNA leading to mutations (Glatt et al. 2012; Sachse et al. 2016). 2-FA and HMFA are natural metabolites of FFA and HMF, respectively (Glatt et al. 2012;

JECFA 2012). In addition, HMFA is formed during coffee roasting (Murkovic and Bornik 2007).

This compiled information about the presence of furan derivatives and their toxicological effects gives rise to the importance of monitoring these compounds quantitatively in foods that are both rich in them and also heavily consumed such as coffee. Currently, gas chromatography is the common method for the monitoring of furan derivatives given the volatility of some members of this class. However, furan derivatives form during heat treatment of food samples that contain their precursors, the application of heat on these samples that are being injected into GC systems might lead to the formation of artefacts or can change the actual amounts of these derivatives (Hu et al. 2013). HPLC has another advantage in comparison with GC which is the ability to retrieve the analysed compounds after analysis while this is not possible in GC. HPLC have been reported multiple times for the analysis of furan derivatives in apple cider and wine (Hu et al. 2013), traditional balsamic vinegars (Chinnici et al. 2003), fruit juices (Palmers et al. 2015), sugarcane honey (Silva et al. 2017) and treacle (Edris et al. 2007) while publications concerning coffee focus on the analysis of a single furan derivative (Murkovic and Pichler 2006; Rodrigues and Bragagnolo 2013; Albouchi and Murkovic 2018).

The objective of this manuscript is to describe the implementation and optimization of a simple and reliable analytical method for the extraction and simultaneous determination of FFA, 2-FA, HMF and HMFA in roasted ground coffee and brews using an HPLC-DAD system equipped with a C8 column.

Materials and methods:

Coffee sample:

A commercially roasted and ground coffee was purchased from a local market. The product was labelled as a medium roast (Vienna roast) of 100% Arabica type coffee.

Chemicals:

FFA (≥ 98 %) was obtained from Fluka Chemicals (Basel, Switzerland). 2-FA (98%) and HMF (99%) were obtained from Aldrich Chemistry (St. Louis, MO, USA). HMFA was obtained from Matrix Scientific (Columbia, SC, USA). Methanol (HPLC grade) was obtained from Chem Lab NV (Zedelgem, Belgium). Water for HPLC was obtained from Ultra clear TWF-UV system (Siemens water technologies, Germany). Glacial acetic acid (99-100%) was obtained from Avantor performance material (Deventer, Netherlands).

HPLC analysis of furan derivatives:

2 µL of each sample or standard were injected into an Agilent 1100 series HPLC system (Agilent Technologies, Germany) with a diode array detector and a Zorbax Eclipse (XBD-C8 4.6×150 mm, 5 µm, Agilent Technologies, USA) with a pre-column of the same material. The separation was done using a gradient mobile phase of 0.1% acetic acid in water (A) and methanol (B) at 25 °C. The gradient started with 100% A, at 2.5 minutes B was increased to 16 %, between 10 and 10.5 minutes B was increased to 100% and held until the end of the run (15 minutes) with a flow rate of 0.5 mL/min. Detection was done at a wavelength of 217 nm for FFA. HMF was detected at 284 nm. 2-FA and HMFA were detected at 250 nm.

Method validation:

Standards containing FFA, 2-FA, HMF and HMFA at 5 different concentrations were used for the validation procedure. The five points of the calibration curve were at 5, 10, 15, 20 and 25 µg/mL of each compound.

The linearity of the analytical method was determined by plotting the mean value of the peak area of each standard at each concentration against the corresponding concentration. The correlation coefficient (r^2), regression data (slope and intercept), and the curve standard error were calculated.

The reproducibility of the analytical method was examined by calculating the relative standard deviation of peak areas for each furan derivative at each concentration. For intra-day precision, the respective areas of three replicates performed at the same day were used while for inter-day precision the respective areas of three replicates performed at the three consecutive days were used.

The recovery of the analytical method was conducted by spiking 50 mg of the commercial roasted coffee sample with 10, 20, 30, 40, or 50 µL of furan derivatives stock solution (500 µg/mL of each of the four compounds) and then extracting with 1 mL of water using heat-assisted shaking in the thermomixer (Eppendorf, Germany) for 10 minutes at 60 °C and 1,000 rpm. The samples were then centrifuged (Eppendorf 5804 R centrifuge, Germany) at 5 °C and 14,000 rpm for 15 minutes before the supernatant was taken for HPLC analysis. Duplicates were prepared for each spiking level. The obtained areas were plotted against the expected theoretical concentrations of the furan derivatives after spiking. The final recovery was calculated as the ratio of the slope of the spiked samples regression line to the slope of

the furan derivatives standards regression line.

The limits of detection (LOD) and the limit of quantification (LOQ) of the four furan derivatives were calculated from the signal to noise ratio (S/N). This ratio was calculated with the help of the difference between the maximum and minimum noise responses from a blank water injection in a range equal to five times the width of the standard peak at half height, this range is distributed equally around the retention time of the standard peak. LOD was determined as (3 × S/N) while LOQ was determined as (10 × S/N) (LoBrutto and Patel 2007).

Assessment of different extraction techniques:

50 mg of ground roasted coffee were extracted with 1 ml of distilled water in an Eppendorf reaction tube (2 mL) using sonication for 10 minutes, shaking with the vortex mixer Genie 2 (Scientific Industries Inc., USA) for 10 minutes or heat-assisted shaking in the thermomixer for 10 minutes at 60 °C and 1,000 rpm. The samples were then centrifuged at 5 °C and 14,000 rpm for 15 minutes before the supernatant was taken for HPLC analysis. Three samples were prepared for each method.

Assessment of sample clean up:

50 mg of ground coffee were weighed carefully into an Eppendorf reaction tube (2 mL). 1,000 µL of deionized water were added to the tube and the total weight of the tube and its content was noted (w_1). The tube was heated in the thermomixer for 10 minutes at 60 °C with shaking at 1,000 rpm. The tube was then centrifuged at 5 °C and 14,000 rpm for 15 minutes before the supernatant was taken for analysis by HPLC. The final weight of the tube and its content after removal of the supernatant was also noted (w_2). The amount of each furan derivative remaining in the solvent

adsorbed to the coffee (C_{rem}) could be calculated by Eq. 1 from the concentration in the first extract (C_1) in µg/g of solvent. A second round of extraction was performed and the extracted furan derivatives were also calculated as µg/g of solvent (C_2). The additional amount extracted (C_{extra}) of each furan derivative was calculated by subtraction (Eq. 2). Then a percentage of the additional amount extracted to the total extractable amount was calculated (Eq. 3).

$$C_{rem} = C_1 \times \frac{1000-(w_1-w_2)}{1000} \qquad \text{(Eq. 1)}$$

$$C_{extra} = C_2 - C_{rem} \qquad \text{(Eq. 2)}$$

$$P = \frac{C_{extra}}{C_1+C_{extra}} \times 100 \qquad \text{(Eq. 3)}$$

The same experiment was repeated with 50% (v:v) methanol in water and pure methanol to assess different extraction efficiencies of different solvents. Each experiment was performed in triplicates.

Results and discussion:

Development of the analytical method:

A comprehensive reversed phase liquid chromatography method development was established by manipulating multiple parameters affecting the chromatographic performance. Among the four furan derivatives of interest, it was found in our experiments that the retention time of FFA was independent of the pH of the mobile phase. Therefore we started with optimising conditions for FFA separation since it is harder to control. Different chromatographic columns (C18, C8, PFP, HILIC and ion exchange) were used to examine FFA in coffee extracts. Using isocratic elution it was

observed that the C8 column retained FFA substantially longer with better separation from other peaks and a satisfactory peak purity. A range of flow rates from 0.2 to 1.0 mL/min was tested and the value of 0.5 mL/min was chosen for a column with a diameter of 4.6 mm. Different organic modifiers (methanol, acetonitrile and tetrahydrofuran) with diverse percentages of them at the initial chromatographic conditions were tested. FFA is a hydrophilic molecule that gets eluted faster by stronger organic modifiers or by increasing the percentage of that modifier, thus the initial mobile phase composition was set to 0% of organic modifier. Isocratic and gradient elution with a variety of slopes were also tested to optimise the peak shape and purity and a mild gradient slope using methanol was specifically developed. Finally, the pH of the aqueous part of the mobile phase was manipulated by adding different acidifiers (formic or acetic acid) at different percentages (0.0004-2%) with the aim of modifying the retention time of the other three furan derivatives. Therefore acetic acid 0.1% was added to the aqueous part of the mobile phase for optimal separation of the four furan derivatives as seen in Fig. 1. The temperature of the column compartment was altered in the range of 17.5-35 °C. It was observed that higher temperatures resulted in shorter retention times at temperatures above 30 °C.

The final HPLC method took less than 15 min to analyse the four furan derivatives with good separation and no significant co-elution. The purity of the peaks was tested using the integrated peak purity tool of the software. No co-elution with the matrix was observed at the respective wavelength. This is show in the chromatogram (Fig. 1). The samples were analysed directly after extraction without any further pre-treatment. The method was also applied to the analysis of filter drip and espresso coffee brews prepared according to ISO 6668:2008 (cupping ratio: 7 g of ground coffee to 100 mL of water) immediately after centrifugation only. The chromatograms

of the brews were similar to that of laboratory prepared extracts with no interaction with peaks from the matrix. Fig. 1 is an HPLC chromatogram of a coffee brew showing all four substances measured at the absorption maximum of the respective compound.

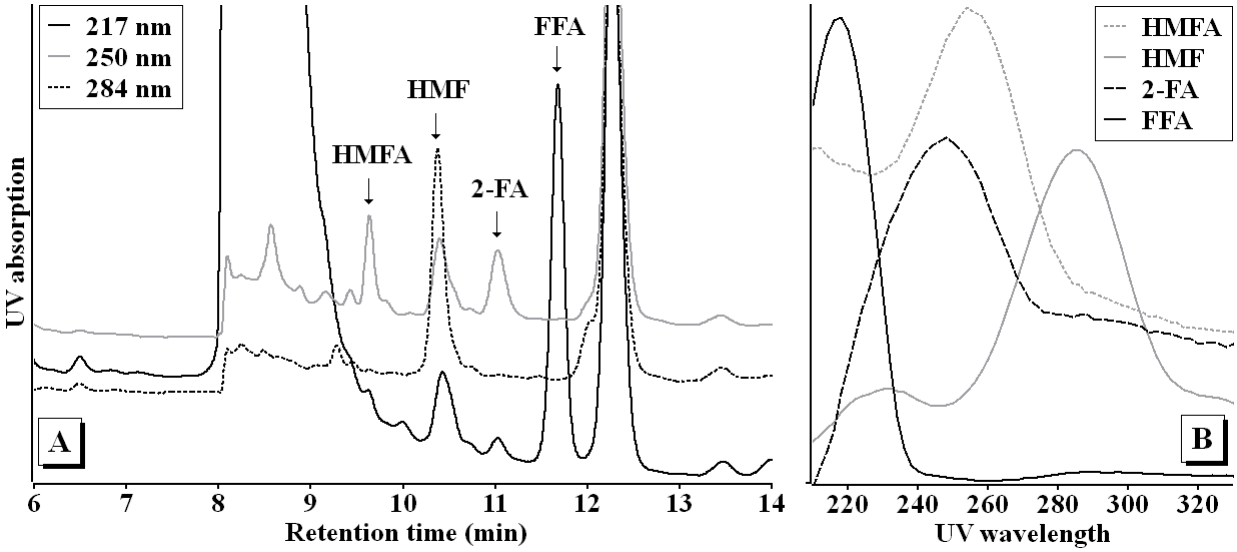


Fig. 1: A: HPLC chromatogram of a coffee brew indicating the peaks of furfuryl alcohol (FFA), 5-hydroxymethyl furaldehyde (HMF), 2-furoic acid (2-FA) and 5-hydroxymethyl-2-furoic acid (HMFA). B: The baseline corrected UV absorption spectrum at the peak maximum of the four furan derivatives.

Validation of the analytical method:

Table 1 shows all validation parameters obtained for the four furan derivatives in question. The acquired values for the r^2 (≥0.998), recovery (≥89.9%) and the reproducibility (intra-day precision ≤ 4.2%, inter-day precision ≤ 4.5%) indicate that the developed method was linear, accurate and precise, respectively. The linearity was also tested by Mandel's test and a linear regression was accepted at a significance level of 99%. The obtained values of LOD and LOQ illustrate the sensitivity of the method. Although the detection wavelength for FFA (217 nm) is below the cut-off of the acetic acid the FFA could be detected sufficiently sensitive due to the low acetic

acid content (0.1%).

Assessment of different extraction techniques:

Three different extraction techniques were applied to ground coffee to extract FFA, 2-FA, HMF and HMFA. The same extraction solvent and ground coffee were used for the three techniques to allow a rational explanation of results. Fig. 2 shows the results of the three extraction techniques. No significant differences were observed in the amounts of the four furan derivatives extracted by the three extraction techniques. This implies that the four furan derivatives of interest are highly water soluble and are easily extracted with water, independent of the extraction method used.

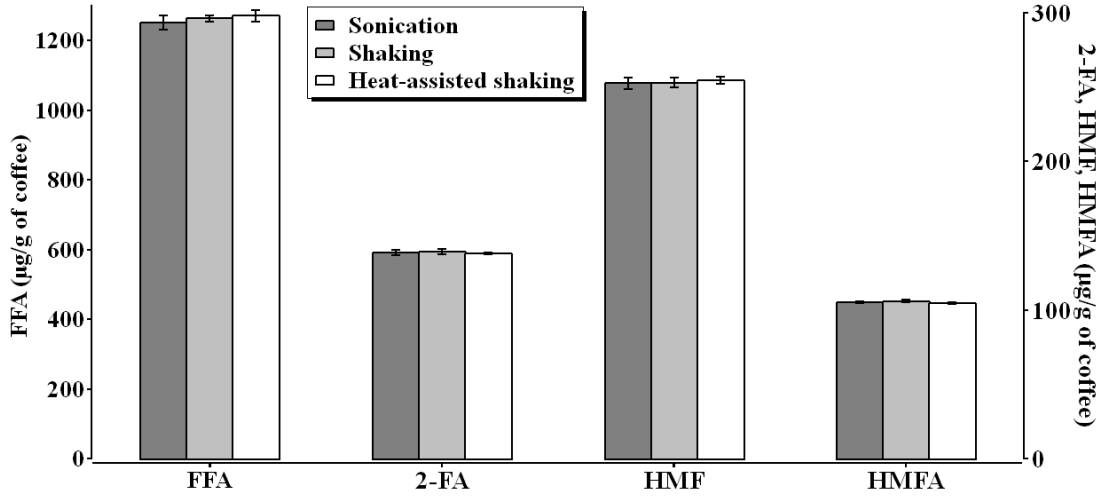


Fig. 2: Amounts of FFA, 2-FA, HMF and HMFA in µg/g of coffee extracted by three different extraction techniques.

Table 1: Result values for the validation of the analytical method. The values of the slope and Y intercept are given ± standard deviation. RSD: relative standard deviation (n=3).

	r^2	Slope	Y intercept	Curve standard error (n=15)	Intra-day precision (RSD%)	Inter-day precision (RSD%)	LOD (µg/mL)	LOQ (µg/mL)	Recovery (%)
FFA	0.9982	11.07 (± 0.34)	18.44 (± 5.35)	3.70	0.68 – 4.21	0.71 – 3.33	0.76	2.55	91.00
2-FA	0.9999	14.83 (± 0.01)	-2.43 (± 0.57)	1.24	0.14 – 1.11	0.28 – 4.50	0.12	0.41	97.57
HMF	0.9997	16.67 (± 0.13)	-5.11 (± 1.80)	2.19	0.12 – 0.97	0.81 – 3.30	0.11	0.35	89.94
HMFA	0.9998	11.64 (± 0.08)	0.18 (± 1.24)	1.09	0.43 – 1.04	0.22 – 3.54	0.23	0.78	99.57

Table 2: Results of two rounds of extraction applied to 50 mg of ground coffee. C_1: amount extracted by first extraction round (µg/g of solvent). C_{extra}: amount extracted by second extraction round (µg/g of solvent). P: percentage of the amount extracted by the second round to the total amount extracted. Values are given ± standard deviation.

	Water			**50% (v:v) methanol in water**			**Methanol**		
	C_1	C_{extra}	P (%)	C_1	C_{extra}	P (%)	C_1	C_{extra}	P (%)
FFA	64.0 (±0.71)	0.13 (±0.22)	0.20 (±0.35)	70.1 (±1.65)	0 (±0.0)	0 (±0.00)	80.3 (±5.10)	0.49 (±0.64)	0.62 (±0.80)
2-FA	6.95 (±0.04)	0.05 (±0.02)	0.68 (±0.22)	7.68 (±0.27)	0.16 (±0.07)	2.09 (±0.92)	7.78 (±0.46)	1.22 (±0.21)	13.5 (±1.28)
HMF	12.8 (±0.12)	0.28 (±0.06)	2.17 (±0.45)	14.3 (±0.04)	0.26 (±0.07)	1.79 (±0.46)	13.3 (±0.80)	1.21 (±0.18)	8.30 (±0.69)
HMFA	5.26 (±0.02)	0.10 (±0.03)	1.81 (±0.58)	5.96 (±0.02)	0.16 (±0.01)	2.56 (±0.17)	5.07 (±0.28)	0.80 (±0.21)	13.5 (±2.35)

Assessment of the clean-up of ground coffee:

Three different extraction solvents were used to examine the clean-up of FFA, 2-FA, HMF and HMFA from ground coffee while extracting with heat-assisted shaking for a fixed time, temperature and shaking conditions. Table 2 reveals that water and a mixture with 50% (v:v) methanol were similar in the extraction efficiency (except for 2-FA where water showed better results) from roasted coffee samples. Methanol presented similar results to the other two extraction solvents regarding the clean-up of FFA from coffee with a lower extraction efficiency of 2-FA, HMF, and HMFA. Less than 2.8% of the total amounts extracted of the four furan derivatives were extracted by the second extraction round with water or 50% (v:v) methanol in water. In the case of methanol, the amounts extracted by the second extraction round reached 16.2% of the total amounts extracted of HMFA. These results also point out that FFA is the furan derivative which is extracted most efficiently using any of the tested methods while 2-FA and HMF were more difficult to extract.

Conclusions:

It is shown here that the separation and simultaneous quantitation of four major furan derivatives in roasted coffee were carried out successfully by an improved HPLC-DAD method using a c* column. The chromatographic run took 15 minutes to completely separate FFA, 2-FA, HMF and HMFA with no disturbing co-elution, neither from ground coffee nor the brews. The developed HPLC method was fast and appropriate for routine analysis of these four furan derivatives in roasted coffee samples. The method performed well in the analysis of real coffee drip filter and espresso brews. The diode array detector was used to demonstrate the purity of the four peaks of interest compared to the spectrum of authentic standards. The validation

results proved the linearity, accuracy, precision (intra and inter-day), and sensitivity of the new method. The different extraction techniques applied to ground coffee yielded similar extraction results for all furan derivatives tested using water as an extraction solvent. The comparison of water, methanol, and water-methanol mixtures as extraction solvents revealed that unmodified water was optimal. On the other hand, methanol was not suitable to completely extract 2-FA, HMF, and HMFA from roasted coffee grounds.

Acknowledgements: The authors would like to thank Erasmus Mundus Avempace plus program for their financial support.

References:

Albouchi A, Murkovic M. 2018. Formation kinetics of furfuryl alcohol in a coffee model system. Food Chem. 243:91-95.

Anese M. 2015. Furan and other furanic compounds in coffee: Occurrence, mitigation strategies, and importance of processing. Preedy V. (editor), *Processing and Impact on Active Components in Food*. Cambridge (MA) United States: Academic Press; p. 541-547.

Chinnici F, Masino F, Antonelli A. 2003. Determination of furanic compounds in traditional balsamic vinegars by ion-exclusion liquid chromatography and diode-array detection. *J Chromatogr Sci*. 41:305-310.

Edris AE, Murkovic M, Siegmund B. 2007. Application of headspace-solid-phase microextraction and HPLC for the analysis of the aroma volatile components of treacle and determination of its content of 5-hydroxymethylfurfural (HMF). *Food Chem*. 104:1310-1314.

Glatt H, Schneider H, Murkovic M, Monien, Meinl W. 2012. Hydroxymethyl-substituted furans: Mutagenicity in *Salmonella typhimurium* strains engineered for expression of various human and rodent sulphotransferases. *Mutagenesis*. 27:41-48.

Hu G, Hernandez M, Zhu H, Shao S. 2013. An efficient method for the determination of furan derivatives in apple cider and wine by solid phase extraction and high performance liquid chromatography diode array detector. *J Chromatogr A*. 1284:100-106.

[ISO] International Organization for Standardization, Technical Committee ISO/TC 34, Food products, Subcommittee SC 15, Coffee. 2008. ISO 6668:2008: Green coffee-Preparation of samples for use in sensory analysis.

[JECFA] Joint FAO/WHO Expert Committee on Food Additives. 2012. 76th meeting: Safety evaluation of certain food additives. *WHO Food Addit. Ser*. 67.

LoBrutto R, Patel T. 2007. Method Validation, Kazakevich Y and LoBrutto R (editors), *HPLC for Pharmaceutical Scientists.* Wiley: New Jersey (US); p. 455-502.

Moon JK, Shibamoto T. 2009. Role of roasting conditions in the profile of volatile flavor chemicals formed from coffee beans. *J Agri Food Chem*. 57:5823-5831.

Murkovic M, Bornik MA. 2007. Formation of 5-hydroxymethyl-2-furfural (HMF) and 5-hydroxymethyl-2-furoic acid during roasting of coffee. *Mol Nutr Food Res.* 51:390-394.

Murkovic M, Pichler N. 2006. Analysis of 5-hydroxymethylfurfual in coffee, dried fruits and urine. *Mol Nutr Food Res*. 50:842-846.

Palmers S, Grauwet T, Celus M, Wibowo S, Kebede BT, Hendrickx ME, Van Loey A. 2015. A kinetic study of furan formation during storage of shelf-stable fruit juices. *J Food Eng.* 165:74-81.

Petisca C, Pérez-Palacios T, Farah A, Pinho O, Ferreira IMPLVO. 2013. Furans and other volatile compounds in ground roasted and espresso coffee using headspace solid-phase microextraction: Effect of roasting speed. *Food Bioprod Process*. 91:233-241.

Rodrigues NP, Bragagnolo N. 2013. Identification and quantification of bioactive compounds in coffee brews by HPLC–DAD–MS^n. *J Food Compos Anal*. 32:105-115.

Sachse B, Meinl W, Sommer Y, Glatt H, Seidel A, Monien BH. 2016. Bioactivation of food genotoxicants 5-hydroxymethylfurfural and furfuryl alcohol by

sulfotransferases from human, mouse and rat: A comparative study. *Arch Toxicol*. 90:137-148.

Silva P, Silva CL, Perestrelo R, Nunes FM, Câmara JS. 2017. A useful strategy based on chromatographic data combined with quality-by-design approach for food analysis applications: The case study of furanic derivatives in sugarcane honey. *J Chromatogr A*. 1520:117-126.

2.2. Paper 2:

'Formation Kinetics of Furfuryl Alcohol in a Coffee Model System'

Food Chemistry 243 (2018) 91–95

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Formation kinetics of furfuryl alcohol in a coffee model system

Abdullatif Albouchi*, Michael Murkovic

Institute of Biochemistry, Graz University of Technology, Petersgasse 10-12/II, 8010 Graz, Austria

ARTICLE INFO

Chemical compounds studied in this article:
Furfuryl alcohol (PubChem CID: 7361).

Keywords:
Furfuryl alcohol
Coffee roasting
Formation kinetics
Process contaminants

ABSTRACT

The production of furfuryl alcohol from green coffee during roasting and the effect of multiple parameters on its formation were studied employing HPLC-DAD. Results show that coffee produces furfuryl alcohol in larger quantities (418 μg/g) compared to other beans or seeds (up to 132 μg/g) roasted under the same conditions. The kinetics of furfuryl alcohol production resemble those of other process contaminants (e.g., HMF, acrylamide) produced in coffee roasting, with temperature and time of roasting playing significant roles in quantities formed. Different coffee species yielded different amounts of furfuryl alcohol. The data point out that the amounts of furfuryl alcohol found in roasted coffee do not reflect the total amounts produced during roasting because great amounts of furfuryl alcohol (up to 57%) are evaporating and released to the atmosphere during roasting. Finally the effect of the moisture content on furfuryl alcohol formation was found to be of little impact.

1. Introduction

Coffee is one of the most consumed beverages around the world. In fact it is considered to be the second beverage consumed after water (Ding, 2014). Due to its importance coffee has been investigated intensively, from production till consumption and associated health issues.

The production of coffee for consumption requires the roasting of green coffee to produce brown to black beans with the characteristic odour and taste that make coffee beverages widely popular. Roasting is done at high temperatures up to 240 °C (Farah, 2012). During roasting a diverse range of chemical reactions occurs, which includes the Maillard reaction, Strecker degradation, caramelization of sugars, and the breakdown of many simple and polymeric compounds originally found in green coffee beans (Simões, Maricato, Nunes, Domingues, & Coimbra, 2014; Wei & Tanokura, 2015). These reactions influence colour, flavour and/or aroma of the final product.

Nearly 900 volatile compounds resulting from coffee roasting have been identified (Franca, Oliveira, Oliveira, Agresti, & Augusti, 2009). Moon and Shibamoto (2009) identified 52 different compounds in the volatile part of roasted coffee belonging to different chemical groups, such as pyridines, pyrazines, pyrroles, furanones, furans, cyclopentenes, phenols, and other miscellaneous compounds. Petisca, Pérez-Palacios, Farah, Pinho, and Ferreira (2013) reported that furans were the major class of compounds found in the volatile part of all ground and espresso coffee samples that they analysed. Of which, furfuryl alcohol was found in an extremely high percentage representing more than 50% of all furans quantified.

Furfuryl alcohol is a volatile compound of roasted coffee which is not present in green coffee but formed during roasting. A few papers have presented hypotheses concerning the formation of furfuryl alcohol in a food matrix. Yaylayan and Keyhani (2000) used ^{13}C-labelled model systems of glucose and alanine to study the carbon source of Maillard reaction products under pyrolysis conditions. They observed the formation of furfuryl alcohol from the intact C1–C5 or C2–C6 carbon skeleton of glucose. It was hypothesised that an oxidation at C1 and C6 of the glucose takes place to produce both gluconic and glucuronic acid, followed by decarboxylation, to give pentitol and aldopentose, respectively. By further dehydration and cyclisation furfuryl alcohol was formed (Fig. 1). In addition, furfuryl alcohol was also formed from fragments of both glucose and alanine through an unknown mechanism, with glyceraldehyde as a potential intermediate. In another manuscript Wnorowski and Yaylayan (2000) suggested a different reaction pathway for the formation of furfuryl alcohol, in which they hypothesised that a retro-aldol cleavage takes place at the C1–C2 bond of 1-deoxy-1-amino-3-hexulose that is formed from the Amadori-compound. This reaction leads to an aldopentose that can dehydrate and cyclise to furfuryl alcohol. Brands and Van Boekel (2001) detected minor amounts of furfuryl alcohol in heated solutions of monosaccharides and casein model systems, and proposed that it can be formed from a 1,2-enediol by β-elimination and α-dicarbonyl cleavage. Another pathway that they presented was the Maillard reaction, which forms *N*-glycosylamine followed by rearrangement to a Schiff base and subsequently to 1,2-enaminol, which can be deaminated followed by α-

* Corresponding author.
E-mail addresses: abdullatif.albouchi@student.tugraz.at (A. Albouchi), michael.murkovic@tugraz.at (M. Murkovic).

http://dx.doi.org/10.1016/j.foodchem.2017.09.112
Received 26 June 2017; Received in revised form 19 September 2017; Accepted 21 September 2017
Available online 22 September 2017
0308-8146/

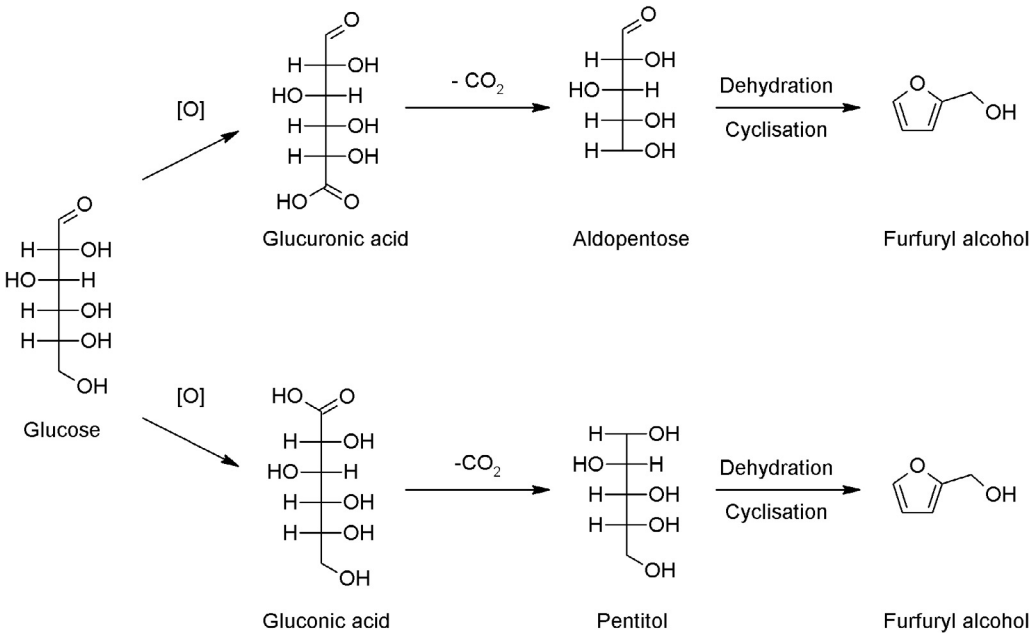


Fig. 1. Proposed hypothesis for furfuryl alcohol formation from glucose under pyrolysis conditions adapted from Yaylayan and Keyhani (2000). ([O]: oxidation, $-CO_2$: decarboxylation.)

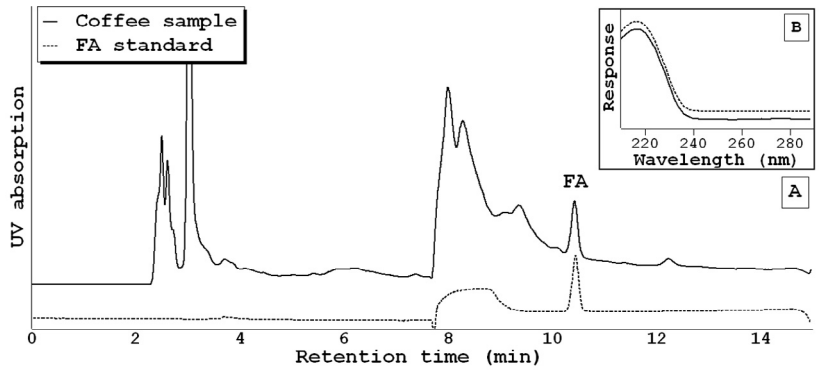


Fig. 2. A: HPLC chromatogram indicating the peak of furfuryl alcohol. B: UV spectrum at apex of furfuryl alcohol peak.

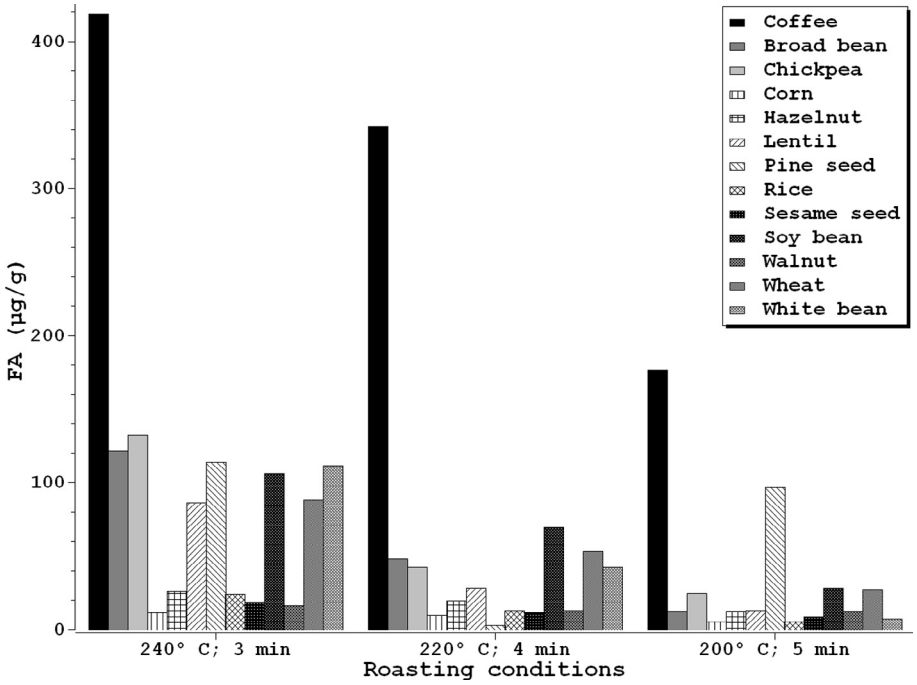


Fig. 3. Furfuryl alcohol in roasted foods (ground model roasting at 50-mg scale) under different roasting conditions.

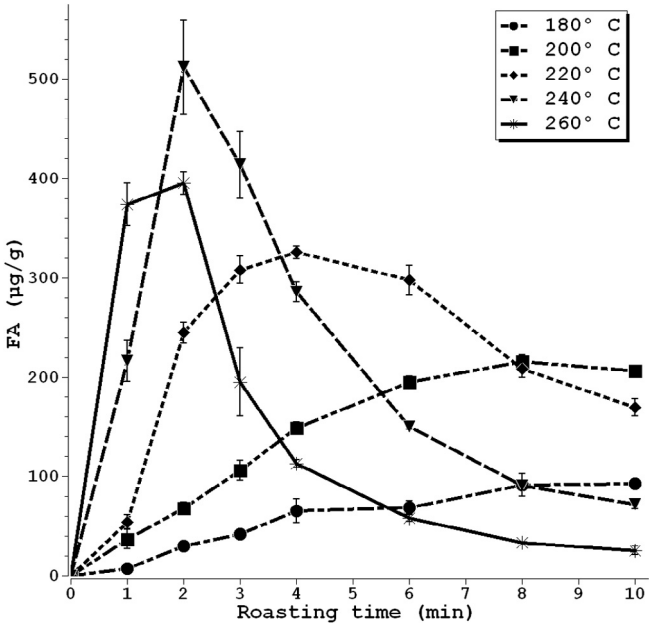


Fig. 4. Kinetics of furfuryl alcohol formation in ground green coffee (model roasting at 50-mg scale) roasted at different temperatures for different roasting times.

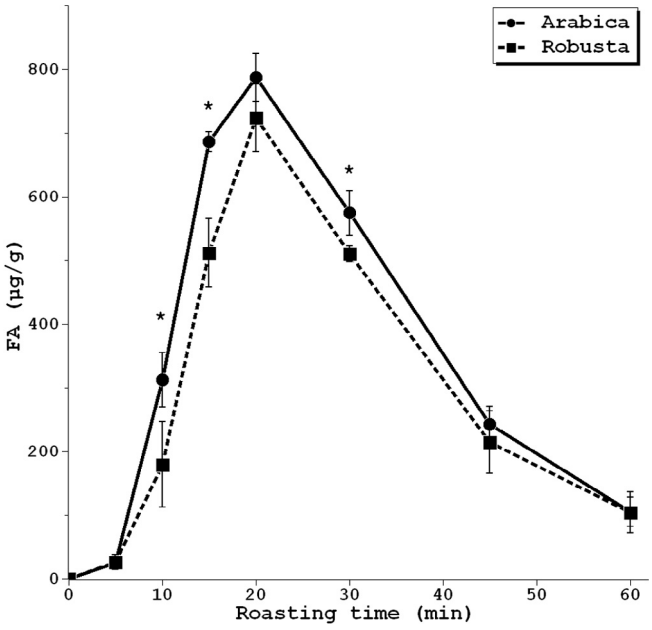


Fig. 5. Comparison of the formation of FA in Arabica and Robusta coffee (whole beans model roasting at 3-g scale) roasted at 220 °C. (*: Time points with significant difference).

dicarbonyl cleavage, leading to furfuryl alcohol; the authors mentioned that this pathway is not significant in ketose model systems. Later, Moon and Shibamoto (2010) indicated furfuryl alcohol as one of the major compounds formed from the roasting of quinic or caffeic acids, and suggested that the formation pathway is possible through dehydration, formic acid elimination, homolytic bond cleavage, and radical reactions.

What makes furfuryl alcohol noteworthy compared to other coffee components is the fact that it is highly water soluble and therefore most of it is transferred to the brew. Since furfuryl alcohol occurs at comparably low concentrations in other foods, coffee is the main source of exposure (Murkovic & Swasti, 2013). It was proven in several animal models that furfuryl alcohol can form DNA adducts, which might lead to mutations. Monien, Herrmann, Florian, and Glatt (2011) detected

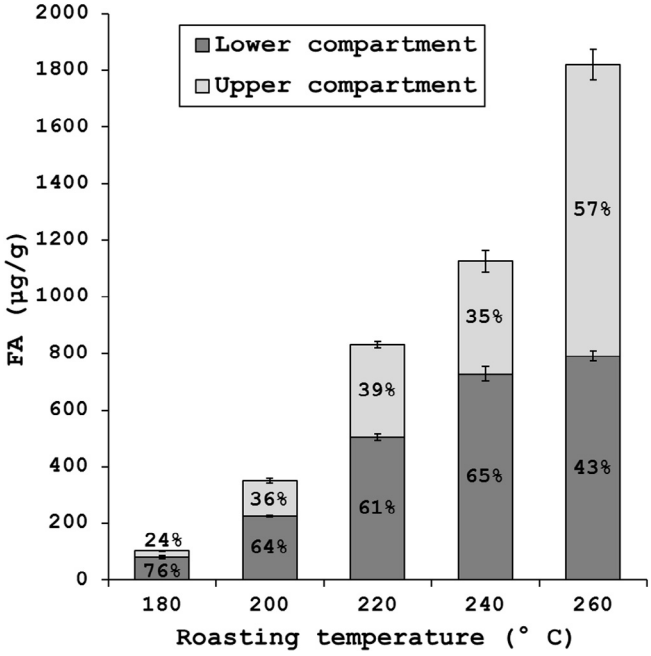


Fig. 6. Amounts of furfuryl alcohol produced from roasting ground green coffee (model roasting at 50-mg scale) under different roasting conditions in a closed system, divided between upper and lower compartments. Numbers represent relative percentage detected in each compartment of total amount measured at each roasting condition.

DNA adducts in furfuryl alcohol exposed to *S. typhimurium* TA100 expressing human sulfotransferase (SULT) 1A1 and in DNA of liver, lung and kidney of FVB/N mice that also express SULT 1A1. In another paper Høie et al. (2015) reported that furfuryl alcohol exposure enhanced the adduct levels in colon and liver of wild type mice. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded at the 76th meeting, that newly published *in vitro* and *in vivo* studies raise concerns regarding the potential genotoxicity of furfuryl alcohol and that the previously established acceptable daily intake will need to be reconsidered at a future meeting (JECFA, 2012).

These findings as well as the fact that the volatile profile of roasted coffee is affected by the roasting conditions and other parameters (Baggenstoss, Poisson, Luethi, Perren, & Escher, 2007; Franca et al., 2009; Kučera, Papoušek, Kurka, Barták, & Bednár, 2016; Moon & Shibamoto, 2009; Petisca et al., 2013), have prompted us to shed more light on the formation kinetics of furfuryl alcohol in roasted coffee, the major source of human exposure.

2. Materials and methods

2.1. Coffee samples

Three different types of green coffee were used: an Arabica type was used for ground coffee bean experiments, while a Robusta type (Uganda) and an Arabica type (Peru) were used for experiments using whole beans. For the small-scale experiments 10 g of green coffee were ground in a mill (A11 basic mill; IKA, Staufen, Germany) and sieved through a 500-µm mesh sieve.

2.2. Other food samples

Rice, wheat, corn, chickpea, broad bean, pine seed, white bean, soy bean, lentil, hazelnut, walnut, and sesame seed were bought from a local market as whole dry samples, and were subsequently ground with an A11 basic mill (IKA).

2.3. Chemicals

Furfuryl alcohol (≥98%) was obtained from Fluka Chemicals (Basel, Switzerland). Methanol (HPLC grade) was obtained from Chem Lab NV (Zedelgem, Belgium). Water for HPLC was obtained from an Ultra Clear TWF-UV system (Evoqua Water Technologies, Barsbuettel, Germany).

2.4. Roasting of food samples

Fifty milligrams of each ground food sample were weighed into a 2-mL glass vial. The ground food sample was roasted in the vial in a metal block heated by a heating plate (Heidolph, Schwabach, Germany). Roasting was achieved at 240 °C, 220 °C, and 200 °C for 3, 4, and 5 min, respectively. The samples were left to stand at room temperature for 1 min and then put on ice for cooling until extraction. These experiments were carried out singly, since the results were only used for comparison with coffee and not for exposure quantification.

2.5. Roasting of ground coffee for determination of kinetics

Fifty milligrams of ground green coffee were weighed into a 2-mL glass vial. The content was roasted in the heated metal block at 260 °C, 240 °C, 220 °C, 200 °C, and 180 °C for 1, 2, 3, 4, 6, 8, or 10 min. The samples were left to stand at room temperature for 1 min and then kept on ice until extraction. Every roasting experiment was carried out three times. This small scale was selected to improve homogeneity, by eliminating the uneven heat transfer in whole beans and larger-scale roasting. No inhomogeneity of the samples during roasting was observable.

2.6. Roasting of whole coffee beans for determination of kinetics

Whole green coffee beans (3 g) were put on a glass Petri dish and roasted at 220 °C for 5, 10, 15, 20, 30, 45, or 60 min in an oven. The longer roasting times were chosen to see the chemical fate of furfuryl alcohol at prolonged roasting. Then the samples were left to stand at room temperature for 5 min and then put on ice for cooling. Afterwards, the beans were ground and 50 mg were sampled and extracted.

2.7. Roasting in closed system

Ground green coffee beans (50 mg) were weighed in a 2-mL glass vial. The vial opening was connected to a bigger vial and sealed. The lower vial (containing the ground coffee) was roasted in a heated metal block keeping the top vial cold. The roasting was achieved at 260 °C, 240 °C, 220 °C, 200 °C, and 180 °C for 2, 2, 4, 8 and 10 min, respectively. The samples were left to stand at room temperature for 1 min and then put on ice for cooling until extraction. Each compartment was extracted individually. It was expected that if furfuryl alcohol evaporates after formation it will be trapped in the cold (upper) vial.

2.8. Moisture removal from ground green coffee

Ground green coffee beans (50 mg) were weighed into a 2-mL glass vial. The coffee in the vials was either freeze dried overnight in Modulyo freeze drier (Savant instruments Inc., USA) or dried in a drying oven at 105 °C for 5 h. Each vial was weighed before and after treatment. No detectable amounts of furfuryl alcohol were found after the drying treatment. The green coffee in the vials was then roasted in the heated metal block. The roasting was achieved at 240 °C for 3 min. The samples were left to stand at room temperature for 1 min and then put on ice for cooling until extraction. Each experiment was carried out three times.

2.9. Extraction procedure

One millilitre of deionised water was added to each sample. Shaking was applied at medium speed using a Vortex-Genie 2 (Scientific industries Inc., Bohemia, NY) for 10 min. The samples were then centrifuged (Eppendorf AG, Hamburg, Germany) for 15 min at 5 °C and 14,000 rpm.

2.10. HPLC analysis

A 2-μL aliquot of the extract or furfuryl alcohol standard was injected into an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) with diode array detector and using a Zorbax Eclipse column (XBD-C8, 4.6 × 150 mm, 5 μm; Agilent Technologies, Santa Clara, CA) with pre-column. The separation was done using a gradient mobile phase of water (A) and methanol (B) at 25 °C. The gradient started with 100% **A**, at 2.5 min **B** was increased to 16%, between 10 and 10.5 min **B** was increased to 100% and held until the end of the run (15 min) with a flow rate of 0.5 mL/min. Detection was done at a wavelength of 217 nm (Fig. 2).

3. Results and discussion

3.1. Furfuryl alcohol production from food samples

Fig. 3 shows the amount of furfuryl alcohol produced from dry ground food samples, including beans, cereals and nuts, which were roasted under the same conditions as coffee. We noticed that the amount of furfuryl alcohol produced by coffee exceeded to a large extent the amount produced by the other foods. We noted also that the amount of furfuryl alcohol produced increases with higher roasting temperatures. In Fig. 3 the results of different roasting conditions are shown. The longer roasting times were chosen to obtain comparable colours in the product. Although foods (except for coffee) are normally not roasted using such high temperatures, these results indicate that coffee is probably the prominent source of furfuryl alcohol in the human diet. This is consistent with data suggesting that roasted coffee is the major source of furfuryl alcohol exposure to humans (Murkovic & Swasti, 2013).

3.2. Furfuryl alcohol formation kinetics

To reduce the influence of heat transfer and the 3-dimensional structure of the coffee beans the green coffee was ground to a fine powder and minute amounts were roasted. In addition, the variation between the experiments was reduced significantly. The trend of furfuryl alcohol formation in ground green coffee (Fig. 4) resembles that of whole beans (Fig. 5). In Fig. 4, the kinetics of furfuryl alcohol formation at all roasting temperatures (except at 180 °C) can be described as a curved line reaching a maximum of furfuryl alcohol that declines with further roasting. The maximum concentration of furfuryl alcohol is reached faster with higher roasting temperatures. It has to be pointed out that the highest amount of furfuryl alcohol observed was at 240 °C (512 μg/g) and that the amount of furfuryl alcohol produced at 180 °C was the lowest in the experiments described here (92 μg/g). The trend of furfuryl alcohol formation kinetics is very similar to that of other furan derivatives produced in coffee, such as hydroxymethylfurfural and hydroxymethylfuroic acid (Murkovic & Bornik, 2007), as well as acrylamide (Bagdonaite, Derler, & Murkovic, 2008). Furfuryl alcohol has been found to polymerise during the roasting process forming dimers and higher oligomers (Swasti & Murkovic, 2012), and this (in addition to evaporation and other possible conversions of furfuryl alcohol) could be the reason for furfuryl alcohol decline observed at later stages of roasting.

3.3. Furfuryl alcohol formation from different Coffea species

Although many species of coffee are available, only two of them are of commercial importance, *Coffea arabica* and *Coffea canephora* (better known as Robusta) (Farah, 2012). Many chemical, biological and agricultural differences can be observed between these two species. Regarding the formation of process contaminants such as furfuryl alcohol, differences in chemical composition of green beans are of great importance, since different amounts of precursors will certainly affect the amount of these compounds formed. As seen in Fig. 5, the amount of furfuryl alcohol produced in Arabica is higher than in Robusta after 10, 15, and 30 min. This can be attributed to the higher content of sucrose in Arabica (on average 7.5% based on dry matter) as compared to Robusta (on average 3.25%, based on dry matter). Sucrose is the major sugar in coffee, and is considered an important precursor of aroma and volatile compounds in coffee (Murkovic & Derler, 2006; Wei & Tanokura, 2015).

3.4. Evaporation of furfuryl alcohol during roasting

Furfuryl alcohol has a boiling point of 171 °C, which is well below the roasting temperatures of coffee. To assess the amount of furfuryl alcohol released into the atmosphere during roasting, ground green coffee was roasted in a tightly closed 2-compartment system in which one compartment was kept at room temperature. The amount of furfuryl alcohol produced from roasting in the lower compartment, alongside that condensed on the cold surface of the upper compartment was measured. In Fig. 6, by comparing the amount of furfuryl alcohol in both system compartments, it was observed that with higher roasting temperature, the amount of evaporating furfuryl alcohol increased and reached more than 50% of the total amount of furfuryl alcohol produced (in the case of roasting at 260 °C). Furfuryl alcohol evaporation can explain why the amount of furfuryl alcohol detected in roasted coffee in Section 3.2, increases with increasing roasting temperature up to 240 °C, and does not further increase at higher roasting temperatures (e.g. 260 °C).

3.5. Effect of moisture on furfuryl alcohol formation

To study the effect of moisture content of green coffee on furfuryl alcohol formation, two moisture removal treatments were applied to ground green coffee, with the aim of removing natural moisture in green coffee. Normally green coffee is dried in agricultural processing to a final water content of around 12% (Kleinwächter, Bytof, & Selmar, 2015). We were able to remove 6.2% and 9.1% of green coffee weight with freeze drying and oven drying, respectively. No significant difference in furfuryl alcohol produced from either treated groups was observed compared to the amount determined from untreated (water-containing) green coffee. This means that the removal of most of the water from green coffee before roasting has not interfered with furfuryl alcohol production from these samples, and that water (alongside other volatile components of green coffee that might have been removed during treatments) does not play a major role in the formation mechanism of furfuryl alcohol.

4. Conclusion

Analysis of diverse roasted food samples (conditions similar to coffee roasting), showed that coffee contains comparably high amounts of furfuryl alcohol. The profile of furfuryl alcohol formation kinetics is similar to a great extent to other process contaminants, with a peak produced quickly after onset of roasting and then concentrations decrease with ongoing roasting, which can be attributed to evaporation, polymerisation, and probably other conversions of furfuryl alcohol that may occur. The amount of furfuryl alcohol formed from different *Coffea* species differs, as seen between *Coffea arabica* and *Coffea canephora*, and this can be attributed to the different composition of these two species. The study of furfuryl alcohol emission to the atmosphere during roasting, revealed that a high percentage (up to 57%) of furfuryl alcohol produced during roasting is evaporating, especially at higher roasting temperatures, leading to a lower amount of furfuryl alcohol determined in samples roasted under these conditions. The reduction of the original water content did not affect the production of furfuryl alcohol from these samples, compared to the control with normal moisture content. All these experiments bring into focus the importance of different parameters, such as temperature of roasting, and coffee species used, on the production of furfuryl alcohol.

Acknowledgements

Abdullatif Albouchi would like to thank Erasmus Mundus Avempace plus program for their financial support.

Reference

Bagdonaite, K., Derler, K., & Murkovic, M. (2008). Determination of acrylamide during roasting of coffee. *Journal of Agricultural and Food Chemistry, 56*, 6081–6086.
Baggenstoss, J., Poisson, L., Luethi, R., Perren, R., & Escher, F. (2007). Influence of water quench cooling on degassing and aroma stability of roasted coffee. *Journal of Agricultural and Food Chemistry, 55*, 6685–6691.
Brands, C. M. J., & Van Boekel, M. A. J. S. (2001). Reactions of monosaccharides during heating of sugar-casein systems: Building of a reaction network model. *Journal of Agricultural and Food Chemistry, 49*, 4667–4675.
Ding, W. X. (2014). Drinking coffee burns hepatic fat by inducing lipophagy coupled with mitochondrial β-oxidation. *Hepatology, 59*(4), 1235–1238.
Farah, A. (2012). Coffee constituents. In Y. F. Chu (Ed.). *Coffee: Emerging health effects and disease prevention* (pp. 21–58). John Wiley & Sons Inc.
Franca, A. S., Oliveira, L. S., Oliveira, R. C. S., Agresti, P. C. M., & Augusti, R. (2009). A preliminary evaluation of the effect of processing temperature on coffee roasting degree assessment. *Journal of Food Engineering, 92*, 345–352.
Høie, A. H., Monien, B. H., Sakhi, A. K., Glatt, H., Hjertholm, H., & Husøy, T. (2015). Formation of DNA adducts in wild-type and transgenic mice expressing human sulfotransferases 1A1 and 1A2 after oral exposure to furfuryl alcohol. *Mutagenesis, 30*, 643–649.
Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2012). Safety evaluation of certain food additives. In WHO food additives series; 67. World Health Organization.
Kleinwächter, M., Bytof, G., & Selmar, D. (2015). Coffee beans and processing. In V. R. Preedy (Ed.). *Coffee in health and disease prevention* (pp. 73–81). Academic Press, Elsevier Inc.
Kučera, L., Papoušek, R., Kurka, O., Barták, P., & Bednár, P. (2016). Study of composition of espresso coffee prepared from various roast degrees of *Coffea arabica* L. coffee beans. *Food Chemistry, 199*, 727–735.
Monien, B. H., Herrmann, K., Florian, S., & Glatt, H. (2011). Metabolic activation of furfuryl alcohol: Formation of 2-methylfuranyl DNA adducts in *Salmonella typhimurium* strains expressing human sulfotransferase 1A1 and in FVB/N mice. *Carcinogenesis, 32*, 1533–1539.
Moon, J. K., & Shibamoto, T. (2009). Role of roasting conditions in the profile of volatile flavor chemicals formed from coffee beans. *Journal of Agricultural and Food Chemistry, 57*(13), 5823–5831.
Moon, J. K., & Shibamoto, T. (2010). Formation of volatile chemicals from thermal degradation of less volatile coffee components: Quinic acid, caffeic acid, and chlorogenic Acid. *Journal of Agricultural and Food Chemistry, 58*, 5465–5470.
Murkovic, M., & Bornik, M. A. (2007). Formation of 5-hydroxymethyl-2-furfural (HMF) and 5-hydroxymethyl-2-furoic acid during roasting of coffee. *Molecular Nutrition & Food Research, 51*, 390–394.
Murkovic, M., & Derler, K. (2006). Analysis of amino acids and carbohydrates in green coffee. *Journal of Biochemical and Biophysical Methods, 69*, 25–32.
Murkovic, M., & Swasti, Y. R. (2013). 5-Hydroxymethyl-furfural and furfuryl alcohol: Occurrence, exposure and detection. In F. P. Plasencia, & Z. Ciesarova (Eds.). *Chemical food safety and health* (pp. 43–55). New York: Nova Science Publishers.
Petisca, C., Pérez-Palacios, T., Farah, A., Pinho, O., & Ferreira, I. M. P. L. V. O. (2013). Furans and other volatile compounds in ground roasted and espresso coffee using headspace solid-phase microextraction: Effect of roasting speed. *Food and Bioproducts Processing, 91*, 233–241.
Simões, J., Maricato, É., Nunes, F. M., Domingues, M. R., & Coimbra, M. A. (2014). Thermal stability of spent coffee ground polysaccharides: Galactomannans and arabinogalactans. *Carbohydrate Polymers, 101*, 256–264.
Swasti, Y. R., & Murkovic, M. (2012). Characterization of the polymerization of furfuryl alcohol during roasting of coffee. *Food & Function, 3*(9), 965–969.
Wei, F., & Tanokura, M. (2015). Organic compounds in green coffee beans. In V. R. Preedy (Ed.). *Coffee in health and disease prevention* (pp. 83–91). Academic Press, Elsevier Inc.
Wnorowski, A., & Yaylayan, V. A. (2000). Influence of pyrolytic and aqueous-phase reactions on the mechanism of formation of maillard products. *Journal of Agricultural and Food Chemistry, 48*, 3549–3554.
Yaylayan, V. A., & Keyhani, A. (2000). Origin of carbohydrate degradation products in L-alanine/D-[13C]glucose model systems. *Journal of Agricultural and Food Chemistry, 48*, 2415–2419.

2.3. Paper 3:

‘Parameters affecting the exposure to furfuryl alcohol from coffee’

Parameters affecting the exposure to furfuryl alcohol from coffee

Abdullatif Albouchi[a], Julia Russ[b], Michael Murkovic[c]

[a,b,c] *Institute of Biochemistry, Graz University of Technology, Graz, Austria*

Petersgasse 10-12/II, 8010, Graz, Austria

[a]Corresponding author:

E-mail: Abdullatif.albouchi@student.tugraz.at - Tel. +43 6648749438.

Highlights

- Furfuryl alcohol is stable in dry coffee matrix for at least 8 weeks.
- Furfuryl alcohol shows different extraction efficiencies with different coffee brewing methods.
- The concentration of furfuryl alcohol in coffee brews is relatively higher compared to other furan derivatives.
- The levels of furan derivatives vary widely among brews prepared from different coffee grounds.
- Furfuryl alcohol's decrease in simulated gastric fluid could not be observed in coffee brews at normal ingestion ratios.

Abstract:

Recently, furfuryl alcohol (FFA) was labelled a human potential carcinogenic (group 2B) by the International Agency for Research on Cancer. Its alimentary exposure is mostly from coffee since other foods comprise insignificant concentrations. The various storage conditions of roasted coffee, the different brewing techniques applied and the bio-accessibility after ingestion are potential parameters that might alter the exposure to FFA from coffee. An 8 weeks stability study at varying temperatures showed that FFA is stable in the ground coffee matrix. Moreover, different brewing techniques yielded differences in the extraction efficiency and the final concentration of FFA. The evaluation of relative exposure to four furans (FFA, 5-hydroxymethyl-furaldehyde, 2-furoic acid and 5-hydroxymethyl-2-furoic acid) revealed that FFA amounts were at least 2-fold the amounts of other studied furans in the same brew. A variation of up to 22-fold could be observed in the concentration of the four furans in brews prepared using different coffee grounds and brewing techniques. 90% of the four furans were extracted by the first 25-30% fraction of the drip filter brew. A significant decrease of FFA is observed after stressing with simulated gastric fluid. However, this decrease could not be reproduced when mimicking a regular coffee ingestion situation.

Keywords: furfuryl alcohol; furan derivatives; exposure level; coffee brewing techniques; stability; bio-accessibility.

1. Introduction:

Coffee, as one of the most important commodities of modern life, is investigated intensively from production till consumption. Roasting of coffee is a crucial step for coffee production, in which high temperatures reaching 270 °C are required to produce the special taste and aroma of coffee (Farah 2012). During this roasting process, key chemical reactions such as the Maillard reaction take place. With its related network of reactions, it results in a wide range of compounds affecting the colour, flavour as well as the aroma of roasted coffee (Martins et al. 2001). As an outcome, the aroma of coffee is composed of almost 900 different volatile compounds comprising diverse chemical groups (Franca et al. 2009) such as furans, furanones, pyridines, pyrazines, pyrroles, cyclopentenes and phenols (Moon and Shibamoto 2009). It was shown that furans were the major chemical class of compounds found in coffee samples with furfuryl alcohol (FFA) accounting for more than 50 % of all furans quantified (Petisca et al. 2013).

FFA is an increasingly interesting furan derivative found in coffee which is hypothesized to originate from the rearrangement of sugar molecules or from interaction of sugar and amino acids in green coffee (Brands and van Boekel 2001). Another hypothesis speaks of chlorogenic acids involvement in the formation of FFA (Moon and Shibamoto 2010). What draws the interest to FFA is that coffee is considered the major source for human exposure to it (Murkovic and Swasti 2013), whilst FFA was classified as a possible carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC).

Adenoma, carcinoma, or squamous cell carcinoma have been observed in the renal tubule and the nasal respiratory epithelium of rodents exposed to FFA through inhalation (Grosse et al. 2017). Several animal studies have proven that FFA can form

DNA adducts which might lead to mutations (Monien et al. 2011; Høie et al. 2015); for example, FFA-specific DNA adducts were discovered in non-tumour tissue of lung cancer patients, in mice, and in bacteria expressing human sulfotransferases (Grosse et al. 2017). Also, it can induce sister chromatid exchange (SCE) in human lymphocytes. According to the 76th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2012), FFA is suggested to be converted by intracellular mouse and human sulfotransferases (SULT) to produce a reactive electrophile (2-sulfo-oxymethylfuran) that can interact with DNA forming 2-methylfuran adducts of 2′-deoxyadenosine and 2′-deoxyguanosine. The presence of these adducts was confirmed in mice orally exposed to FFA.

The assessment of actual exposure levels to a volatile and reactive compound such as FFA (Flament and Bessière-Thomas 2002), requires taking multiple parameters into consideration as they might affect its levels. Different storage conditions and periods of roasted coffee along with several brewing techniques were tested to investigate its effects on FFA's levels. Additionally, the bio-accessibility of FFA was examined by the imitation of an ingestion scenario in the presence of simulated human gastrointestinal fluids.

As FFA is not the only furan derivative to be found in coffee brews, three chemically-related furan derivatives that are usually found in coffee brews in considerable quantities, namely 5-hydroxymethyl furaldehyde (HMF), 2-furoic acid (2-FA) and 5-hydroxymethyl-2-furoic acid (HMFA) were additional quantified in brews from 11 different coffee grounds brewed using two different brewing methods. The goal was to present a wider outlook on the sample to sample variation in the final cup and give a perspective on the relative exposure of these compounds compared to

that of FFA. The stepwise extraction of these four derivatives from the coffee ground during filter drip brewing was also examined.

The aim of this manuscript is to evaluate the effect of important parameters on the alimentary exposure to substances which can be activated by sulfotransferases. Practically all hydroxymethyl furan derivatives can be activated with these liver enzymes forming highly reactive intermediates resulting in potential DNA reactive compounds. By knowing the alimentary uptake of these compounds, a risk estimation is possible.

2. Materials and methods:

2.1.Coffee for stability study:

10 g of whole green Robusta type (Uganda) coffee beans were put on a glass petri-dish and roasted at 220 °C for 20 minutes in an oven. Then the sample was left to stand at room temperature for 5 minutes and then put on ice for cooling. Afterwards, the beans were ground with an A11-basic mill (IKA, Germany) and the powder was distributed evenly in 3 glass tubes and tightly closed using screw-caps. One tube was stored at room temperature (RT), the second at +4 °C, while the third was stored at -20 °C.

2.2.Coffee for comparison of different brewing procedures:

A commercial ground coffee product was purchased from a local market. The product was labelled as a medium roast (Vienna roast) of 100% Arabica type coffee.

2.3.Coffee for the analysis of furan derivatives:

80 g of coffee beans were roasted in-house using a Probat PRE1Z laboratory scale coffee roaster (Probat-Werke, Emmerich am Rhein, Germany) at 150 ± 10 °C. A medium roast (S1) or a dark roast (S2) of Arabica type (Peru) coffee beans and a dark

roast of Robusta type (Uganda) coffee beans (S3) were prepared. Another 3 commercially roasted whole coffee beans (no coffee type mentioned on the package) labelled as a light roast (S4), a medium roast (S5) and a dark roast (S6) were purchased. Whole bean samples were ground using a house hold coffee grinder.

The remaining 5 samples were commercially roasted ground coffee labelled as 100% Arabica type (S7), a mixture of Arabica and Robusta (S8) and two unlabelled samples regarding the type of coffee used (S9, S10). The last sample was an old open package of 100% Arabica type coffee ground, it was meant to be used before the end of 2013 (S11).

2.4.Chemicals:

FFA (≥ 98 %) and sodium chloride (p.A.) were obtained from Fluka Chemicals (Basel, Switzerland). 2-FA (98%) and HMF (99%) were obtained from Aldrich Chemistry (St. Louis, MO, USA). HMFA was obtained from Matrix Scientific (Columbia, SC, USA). Sodium hydroxide (≥ 99 %) and fuming hydrochloric acid (37 %) were obtained from Carl Roth GmbH (Karlsruhe, Germany). Potassium dihydrogen phosphate (p.A.) was obtained from E. Merck (Darmstadt, Germany). Methanol and acetonitrile (HPLC grade) was obtained from Chem Lab NV (Zedelgem, Belgium). Water for HPLC was obtained from Ultra clear TWF-UV system (Siemens water technologies, Germany). Glacial acetic acid (99-100%) was obtained from Avantor performance material (Deventer, Netherlands).

2.5.Extraction procedure of stability samples:

50 mg of ground coffee were taken weekly from each stability glass tube. 1 mL of deionized water was added to each sample. Shaking was applied at medium speed using Vortex genie 2 (Scientific Industries Inc., USA) for 10 minutes. The samples

were then centrifuged (Eppendorf, Germany) for 15 minutes at 5 °C at 14,000 rpm. The supernatants were analysed by HPLC.

2.6.Brewing methods for the comparison of brewing techniques:

For the filter drip brewing: 5.5 g of commercially roasted ground coffee were put in a paper filter and then inserted into a commercial filtered coffee maker (Severin, Germany). 100 mL of deionized water were added into the water tank of the machine. The coffee maker was started and the first brew was collected into the coffee pot. A second brew was collected from the same spent coffee using another 100 mL of deionized water into a second pot.

For the espresso brewing: 5.5 g of the commercial ground coffee were put in the metal filter of the espresso maker and then inserted into a household espresso coffee maker (Junior express, Italy). 100 mL of deionized water were added into the water tank of the machine. The coffee maker was put on a hot plate for 5 minutes and the first brew was collected into the coffee pot after resting the coffee maker for another 5 minutes. A second brew was collected from the same spent coffee using another 100 mL of deionized water into a second pot.

For the boiling brewing: 100 mL of deionized water were added into a stainless-steel pot and the pot was put on a heating plate until the water starts to boil. 5.5 g of the commercial ground coffee were added to the metal pot and boiled with continuous stirring for 5 minutes and the first brew was collected into a glass beaker after resting the coffee mixture for another 5 minutes. A second brew was collected from the same spent coffee using another 100 mL of deionized water into a second glass beaker.

1 mL of each brew was put into a reaction tube and put on ice for cooling. The

samples were then centrifuged for 15 minutes at 5 °C at 14,000 rpm. The supernatants were analysed by HPLC.

2.7.Brewing methods for the quantification of furan derivatives:

For the filter drip brewing: 35 g of each coffee sample (S1-11) were put in a paper filter and then inserted into a commercial filtered coffee maker. 500 mL of deionized water were added into the water tank of the machine. The coffee maker was started and the brew was collected into the coffee pot.

For the espresso brewing: 14 g of each coffee sample (S1-11) were put in the metal filter of the espresso maker and then inserted into a household espresso coffee maker. 200 mL of deionized water were added into the water tank of the machine. The coffee was brewed until all the water has moved into the upper chamber of the machine.

Two samples (1 mL each) were taken from each brew into a reaction tube and centrifuged for 20 minutes at 15 °C at 14,000 rpm. The supernatants were analysed by HPLC.

2.8.Brewing method for the progressive extraction experiment:

70 g of commercially roasted ground coffee (S11) were put in a paper filter and then inserted into a commercial filtered coffee maker. 1000 mL of deionized water were added into the water tank of the machine. The coffee maker was started and 50 mL fractions of the brew were collected into separate vessels. Two samples (1 mL each) were taken from each fraction of the brew into a reaction tube and centrifuged for 20 minutes at 15 °C at 14,000 rpm. The supernatants were analysed by HPLC.

2.9.Preparation of simulated gastrointestinal fluids according to USPC (2008):

For preparation of simulated gastric fluid (SGF), 0.2 g of sodium chloride was dissolved in a few millilitres of water and 0.7 mL of fuming hydrochloric acid was added carefully. Then the mixture was diluted with water up to 100 mL. No pepsin was added during SGF preparation.

For preparation of simulated intestinal fluid (SIF), 0.68 g of potassium dihydrogen phosphate was dissolved in 25 mL of water. 7.7 mL of 0.2 N sodium hydroxide solution was added and another 50 mL of deionized water. The pH was adjusted to 6.8 and the volume was completed to 100 mL with deionized water. No pancreatin was added during preparation of SIF.

2.10. Stressing FFA with gastrointestinal fluids:

150 µL of a FFA standard solution in water (500 µg/mL) were put into a reaction tube. Then 850 µL of SGF, SIF or deionized water (control) were added. All tubes were incubated in a thermomixer (Eppendorf, Germany) at 37 °C and mixing speed of 500 rpm for 2 hours. 100 µL from each tube were Sampled at the following time points: 0, 10, 20, 30, 45, 60, 90, 120 minutes. Then 500 µL of SIF were added to neutralize the pH in SGF tubes. Similarly, 500 µL of water were added to SIF and control tubes. The tube content was analysed by HPLC.

2.11. Ingestion scenario with gastrointestinal fluids:

For the FFA standard: 500 µL of FFA standard solution in water (75 µg/mL) were put into a reaction tube. 200 µL of SGF, SIF or deionized water (control) were added. All tubes were incubated in a thermomixer at 37 °C and mixing speed of 500 rpm for 2 hours. Then 1000 µL of SIF were added to neutralize the pH in SGF tubes. Similarly,

1000 µL of water were added to SIF and control tubes. The tube content was analysed by HPLC.

For the coffee brew: 500 µL of filter brewed coffee were put into a reaction tube. 200 µL of SGF, SIF or deionized water (control) were added. All tubes were incubated in a thermomixer at 37 °C and mixing speed of 500 rpm for 2 hours. Then 1000 µL of SIF were added to neutralize the pH in SGF tubes. Similarly, 1000 µL of water were added to SIF and control tubes. All tubes were then centrifuged for 15 minutes at 5 °C at 14,000 rpm. The supernatants were analysed by HPLC.

For the coffee mixture with milk: 500 µL of filter brewed coffee were put into a reaction tube. 500 µL of pasteurised milk (3.5% fat) were added. Then 200 µL of SGF, SIF or deionized water (control) were added. All tubes were incubated in a thermomixer at 37 °C and mixing speed of 500 rpm for 2 hours. Then 500 µL of SIF were added to neutralize the pH in SGF tubes. Similarly, 500 µL of water were added to SIF and control tubes. 500 µL of the final mixture were taken into a new reaction tube and 1000 µL of acetonitrile were added and the tubes were shaken vigorously for 1 minute to promote protein precipitation. All tubes were then centrifuged for 15 minutes at 5 °C at 14,000 rpm. The supernatants were analysed by HPLC.

2.12. HPLC analysis of furan derivatives:

2 µL of each sample or FFA standard were injected into an Agilent 1100 series HPLC system (Agilent Technologies, Germany) with a diode array detector and a Zorbax Eclipse (XBD-C8 4.6×150 mm, 5 µm, Agilent Technologies, USA) with a pre-column. The separation was done using a gradient mobile phase of 0.1% acetic acid in water (A) and methanol (B) at 25 °C. The gradient started with 100% A, at 2.5 minutes B was increased to 16 %, between 10 and 10.5 minutes B was increased to

100% and held until the end of the run (15 minutes) with a flow rate of 0.5 mL/min. Detection was done at a wavelength of 217 nm for FFA. HMF was detected at 284 nm. 2-FA and HMFA were detected at 250 nm. The validation of the analytical method revealed that the developed method was linear in the range of 5-25 µg/mL. The recovery was around 90% whereas inter and intra-day relative standard deviation was ≤ 5%. The values for the limit of detection and quantification ranged within 0.1-0.8 and 0.4-2.6 µg/mL, respectively. Fig. 1 is an HPLC chromatogram of a coffee brew showing all four peaks of different furan derivatives of interest.

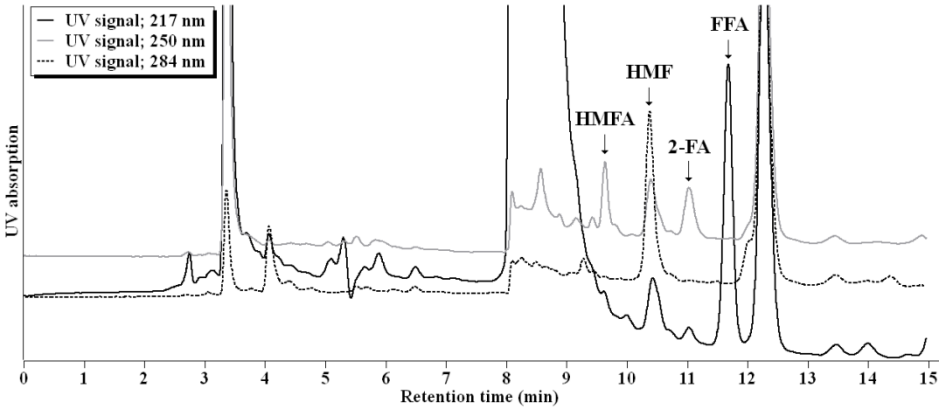


Fig. 1: HPLC chromatogram of a coffee brew indicating the peaks of furfuryl alcohol (FFA), 5-hydroxymethyl furaldehyde (HMF), 2-furoic acid (2-FA) and 5-hydroxymethyl-2-furoic acid (HMFA).

3. Results and discussion:

3.1.Stability of FFA in coffee matrix:

Fig. 2 shows the amount of FFA in an in-house roasted and ground Robusta coffee that was stored at three different storage conditions for 8 weeks. Since there were no observed significant differences in FFA amounts after 8 weeks of storage even at room temperature, we can assume the relative stability of FFA in the ground coffee. Meaning that, roasting conditions are more significant than storage conditions

regarding the exposure of FFA from coffee. This highlights the prominent role that roasting parameters play on the amount of FFA to be formed as previously published (Albouchi and Murkovic 2018). This would also imply that FFA is relatively unreactive towards other coffee constituents during dry storage.

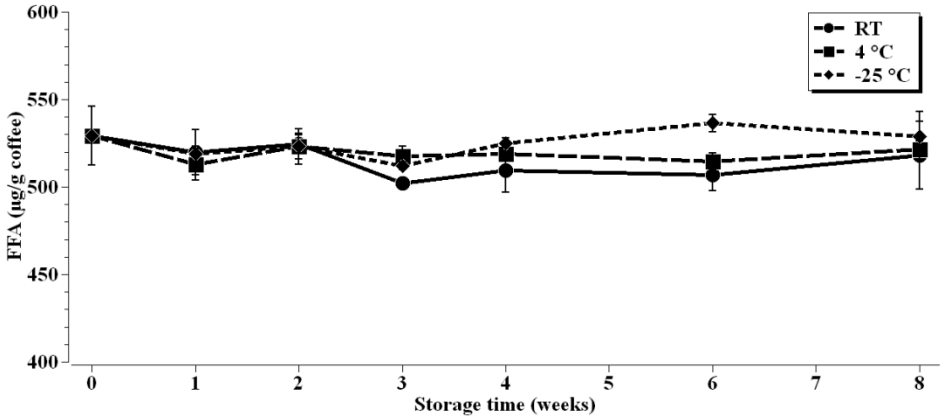


Fig. 2: Amount of furfuryl alcohol (FFA) in ground roasted Robusta coffee during 8 weeks of storage at different temperatures. RT: room temperature.

3.2.The comparison of FFA extraction using different brewing techniques:

Many different brewing techniques and recipes are available for the preparation of a cup of coffee from coffee grounds. The choice of a specific brewing technique or recipe depends on personal preferences, cultural trends and the availability of the desired coffee maker (Guenther et al. 2010). For the purpose of this experiment we decided to use a standardized cupping ratio for the comparison of all brewing techniques. The Specialty Coffee Association of America (SCAA 2012) prepared a standard for cupping ratio that can be adjusted to the brewing vessel size. This standard states that 0.055 g coffee per 1 mL of water shall be used for cupping. The same commercial ground coffee sample was used in the testing to allow rational comparison.

As seen in Fig. 3, a significant difference could be observed when using the boiling technique compared to the other two techniques. FFA amounts in the first boiling brew were shown to be statistically lower than that of filter drip or espresso brews. This might be due to the high volatility of FFA during boiling. The resulting concentrations of FFA in the final cup were 66.7 ± 1.0 µg/mL of boil brewed coffee, 72.7 ± 1.5 µg/mL of espresso brewed coffee, and 75.0 ± 4.1 µg/mL of drip filter brewed coffee.

A second brew using the same spent coffee was prepared to measure the efficiency of the brewing technique to extract all FFA from the ground coffee. Results indicate that espresso brewing technique is more efficient in extracting FFA from ground coffee, extracting 99% of the total sum of FFA from first and second brews. While filter drip and boiling techniques were less efficient, extracting 90% and 92% of FFA, respectively. Apparently, the pressure aided brewing in espresso coffee makers help fully extracting FFA from the ground coffee.

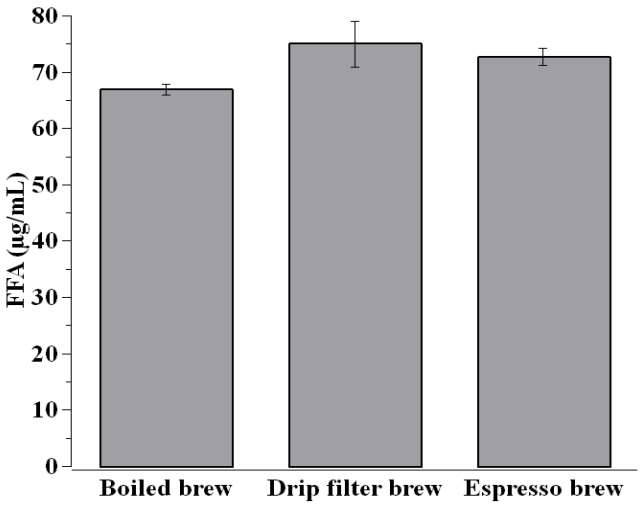


Fig. 3: Comparison of furfuryl alcohol (FFA) concentration in coffee brews prepared by 3 different brewing techniques.

3.3.Relative exposure to furan derivatives from coffee:

FFA is not the only furan derivative with potential negative health effects that can be found in coffee brews. Other furan derivatives such as HMF, HMFA and 2-FA can also be detected. HMF can be converted by SULTs in a similar manner to FFA resulting into an analogue reactive intermediate, which is 5-sulphooxymethylfurfural. This compound has likewise exhibited mutagenic activity on *S. typhimurium* cell cultures engineered to express human SULT enzymes (Glatt et al. 2012). 2-FA and HMFA are natural metabolites of FFA and HMF, respectively (Glatt et al 2012; JECFA 2012), thus their alimentary uptake will add up to their final exposure levels.

A simultaneous quantification of the four furan derivatives in brews of 11 coffee grounds using two different brewing techniques was conducted to allow for the estimation of the relative exposure to these compounds upon coffee consumption and present an overview of fluctuations in their concentrations. For this experiment a higher cupping ratio was used in the preparation of the brews as in the ISO 6668 where 7 g of ground coffee is used per 100 mL of water for the purpose of sensory analysis of coffee brews (ISO 2008). Also in this experiment higher volumes were brewed compared to those in section 3.2.

In a survey conducted by the governmental Food Standards of Australia and New Zealand (FSANZ 2011), a takeaway coffee cup can range in size from 270-600 mL. By using the average values from Table 1 to extrapolate the daily exposure to FFA from one dose of coffee, we can reach an exposure of 33.2 ± 8.4 mg of FFA for 600 mL of filter drip brewed coffee or 41.0 ± 8.8 4 mg of FFA for 600 mL of espresso brewed coffee. These amounts exceed the acceptable daily intake of 0-0.5 mg/kg bodyweight that was established by JECFA at its 55th meeting (JECFA 2012) for a 70 Kg-weighed person. On the other hand, the consumption of similar quantities of filter

drip coffee results in an exposure of 6 mg of HMF, 4.6 mg of 2-FA and 2.7 mg of HMFA. Whereas espresso coffee leads to an exposure of 6.4 mg of HMF, 5.4 mg of 2-FA and 2.8 mg of HMFA. These numbers are far below those for FFA.

Table 1 clearly points out the variability of the four furan derivatives in different brews from different coffee grounds and brewing techniques. FFA has shown the highest concentration of furan derivatives in all brews, and this is consistent with published data (Petisca et al. 2013). The concentration of FFA ranges from 32.9 ± 2.2 up to 69.1 ± 2.4 µg/mL of the drip filter brew. Espresso brews contains noticeably higher concentrations up to 88.7 ± 1.7 µg/mL, with an increase of FFA up to 93% against corresponding filter drip brews. HMF was second to FFA, with Drip filter brews containing between 1.3 ± 0.5 up to 28.5 ± 1.1 µg/mL. Espresso brews had slight differences in the amounts of HMF compared to drip filter brews. HMF concentrations were only 2 to 50% of FFA concentrations in the same brew.

For 2-FA the concentrations range from 5.9 ± 0.8 up to 9.0 ± 0.4 µg/mL of the drip filter brew. Espresso brews showed higher concentrations up to 11.1 ± 0.2 µg/mL, with an increase of 2-FA up to 88% against corresponding filter drip brews. The concentrations of 2-FA were between 10 to 23% of FFA concentrations in the same brew. HMFA was the furan derivative with the lowest concentrations observed from the four compounds of interest. Drip filter brews showed concentrations between 0.7 ± 0.2 up to 7.3 ± 0.5 µg/mL. Espresso brews had quite similar amounts of HMFA compared to drip filter brews. HMFA concentrations were as low as 1 to 22% of FFA concentrations in the same brew.

Coffee sample	FFA (µg/mL brew)		HMF (µg/mL brew)		2-FA (µg/mL brew)		HMFA (µg/mL brew)	
	Filter drip brew	Espresso brew	Filter drip brew	Espresso brew	Filter drip brew	Espresso brew	Filter drip brew	Espresso brew
S1	61.8 ± 2.3	66.5 ± 0.1	14.3 ± 0.3	15.1 ± 0.0	8.3 ± 0.1	8.2 ± 0.0	5.3 ± 0.1	5.5 ± 0.0
S2	36.3 ± 1.1	70.2 ± 1.5	1.9 ± 0.2	1.4 ± 0.1	5.9 ± 0.8	11.1 ± 0.2	0.8 ± 0.1	1.2 ± 0.1
S3	32.9 ± 2.2	54.5 ± 1.1	1.3 ± 0.5	1.0 ± 0.0	6.0 ± 0.6	9.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.1
S4	67.8 ± 1.9	73.6 ± 1.9	3.6 ± 0.0	3.9 ± 0.2	9.0 ± 0.3	9.7 ± 0.7	4.0 ± 0.1	3.8 ± 0.1
S5	69.1 ± 2.4	75.3 ± 1.7	3.4 ± 0.1	3.4 ± 0.1	9.0 ± 0.4	9.1 ± 0.2	3.9 ± 0.2	3.7 ± 0.1
S6	64.1 ± 8.1	75.2 ± 1.9	3.4 ± 0.3	3.7 ± 0.3	8.7 ± 0.9	9.1 ± 0.4	3.9 ± 0.5	4.2 ± 0.2
S7	66.6 ± 2.6	71.7 ± 3.6	28.5 ± 1.1	29.2 ± 1.4	8.0 ± 0.4	8.4 ± 0.3	7.3 ± 0.4	7.4 ± 0.4
S8	57.9 ± 4.5	88.7 ± 1.7	9.4 ± 0.5	13.3 ± 0.3	7.0 ± 0.6	10.2 ± 0.1	5.3 ± 0.4	7.3 ± 0.5
S9	57.4 ± 1.5	79.8 ± 0.7	11.5 ± 0.1	13.4 ± 1.0	6.8 ± 0.2	8.5 ± 0.1	5.2 ± 0.1	5.7 ± 0.6
S10	60.5 ± 3.9	63.6 ± 3.7	16.1 ± 0.7	17.4 ± 0.3	7.3 ± 0.5	7.6 ± 0.4	5.8 ± 0.5	4.7 ± 1.2
S11	33.5 ± 1.6	32.8 ± 0.8	16.7 ± 0.5	14.8 ± 0.4	7.7 ± 0.4	7.5 ± 0.2	7.3 ± 0.5	6.8 ± 0.2
Average	55.3 ± 14.0	68.3 ± 14.7	10.0 ± 8.4	10.6 ± 8.8	7.6 ± 1.1	9.0 ± 1.1	4.5 ± 2.2	4.6 ± 2.2

Table 1: The concentrations of the four furan derivatives in coffee brews from 11 different ground coffee samples using filter drip or espresso brewing techniques. Results are shown as average value ± standard deviation (n=3). FFA: furfuryl alcohol, HMF: 5-hydroxymethyl furaldehyde, 2-FA: 2-furoic acid and HMFA: 5-hydroxymethyl-2-furoic acid.

3.4.Progressive extraction of furan derivatives during drip filter brewing of coffee:

In filter drip brewing technique hot water is poured slowly over coffee grounds and the filtrates pass through a paper filter to settle in the coffee pot. The gradual extraction of furan derivatives from coffee grounds can affect their final amounts to be extracted to the brew. It is shown in Fig. 4 that the concentrations of furan derivatives change drastically in the brew from the beginning to the end of the brewing process. The concentration of FFA in the brew decrease from 321 ± 48 µg/mL with the increase of the volume until it reaches 68 ± 4 µg/mL with the first 300 mL fraction containing up to 90% of the final amount of FFA. Similarly, HMF concentration decreased from 125 ± 25 to 28 ± 1 µg/mL during brewing, also with 90% of the final amount to be extracted by the first 300 mL of the hot water. 2-FA and HMFA showed similar extraction concentrations and profiles with concentrations starting from 40 ± 11 µg/mL that decrease to 8 ± 1 µg/mL, and the first 250 mL fraction extracting 90% of their final amounts.

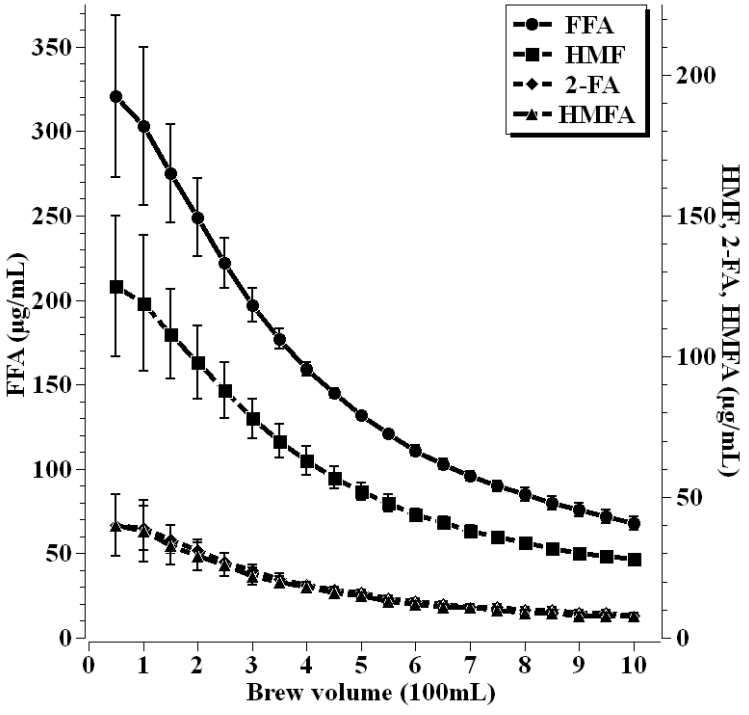


Fig. 4: Progressive extraction of furfuryl alcohol (FFA), 5-hydroxymethyl furaldehyde (HMF), 2-furoic acid (2-FA) and 5-hydroxymethyl-2-furoic acid (HMFA) during filter drip brewing of coffee brews.

3.5.Stressing FFA with gastrointestinal track fluids:

After brewing, coffee is drunk either black or with milk and with or without sweeteners added. During ingestion, the coffee brew has relatively little interaction time in the buccal cavity, so we can assume that the saliva has no or very little effects on the brew. On the other hand, coffee brews spend a considerable amount of time in the stomach and the small intestine, during which it gets in contact with gastric and later intestinal fluids. Gastric fluid is a strong acidic medium while intestinal fluid is a neutral to basic medium. These different fluids with their different pH values can affect FFA in various ways. In the pharmaceutical industry, simulated gastric (pH=1.2) or intestinal (pH=6.8) fluids are used regularly to test the disintegration and dissolution of pharmaceutical dosage forms, thus mimicking the chemical environment in the stomach or intestine (USPC 2008). These fluids were used to stress FFA standards by preparing the 75 µg/mL FFA standards directly in them.

Fig. 5 shows the result of stressing FFA with simulated gastric or intestinal fluid in comparison to a water control for 2 hours at 37 °C with mild shaking. Obviously, the amount of FFA tends to decrease constantly in the SGF while it remained almost constant in the SIF and the water control. After 120 minutes a statistically significant decrease of 16% of FFA was observed in SGF compared to its concentration at the start. This result is in line with previously published data suggesting FFA polymerization in strongly acidic conditions forming oligomers through methylene linkages (Swasti and Murkovic 2012).

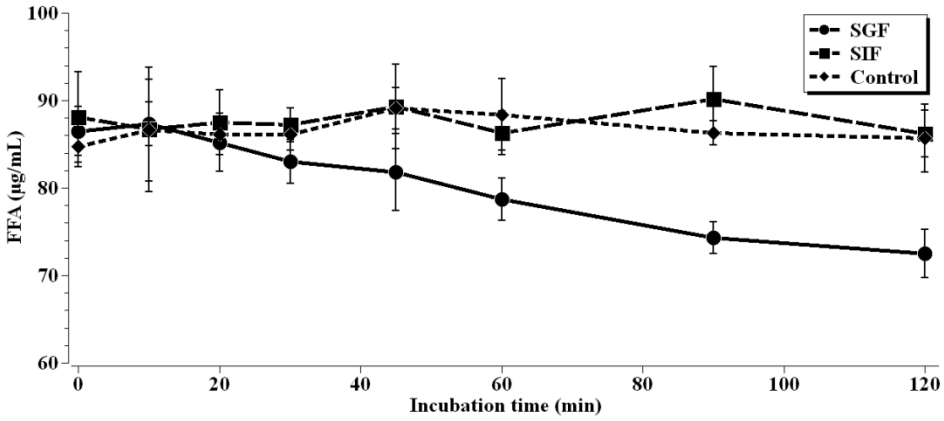


Fig. 5: Effect of stressing furfuryl alcohol (FFA) by simulated gastric fluid (SGF) or simulated intestinal fluid (SIF) in comparison to a water control.

3.6.Effect of gastrointestinal track fluids on FFA in coffee:

Recently, magnetic resonance imaging technology was applied to measure the volume of gastrointestinal fluids in fasting healthy humans (Mudie et al. 2014). This technology showed a volume of 35 ± 7 mL in the fasted stomach and 43 ± 14 mL in the small bowel. These numbers were used in the design of our experiments. Where a 100 mL of FFA standard or a drip filter brew or a (1:1, v:v) mixture of brew and full fat milk were incubated with 40 mL of simulated gastric or intestinal fluid and compared to samples incubated with water as controls. The ratios used mimic coffee drinking at a fasted state.

After 2 hours of incubation at 37 °C with mild shaking, no statistically significant differences could be observed between simulated gastro or intestinal fluid with the water control experiments in all tested scenarios (FFA standard, coffee brew alone or coffee brew with milk 1:1 v:v) as can be noted from Fig. 6. In these results, the expected decrease of FFA during incubation with SGF was not observed. This can be attributed to the dilution effect of the high intake volume on the SGF, thus increasing the final pH to a point where no or very limited polymerisation of FFA

could take place. Another explanation can be the masking effect of the brew constituents on the acid, preventing it from polymerizing FFA. One more remark that is not shown in Fig. 6 in the coffee-milk mixture scenario is that milk did not interfere with FFA and the entire amount of FFA was recover from the mixture after the precipitation of milk proteins using acetonitrile.

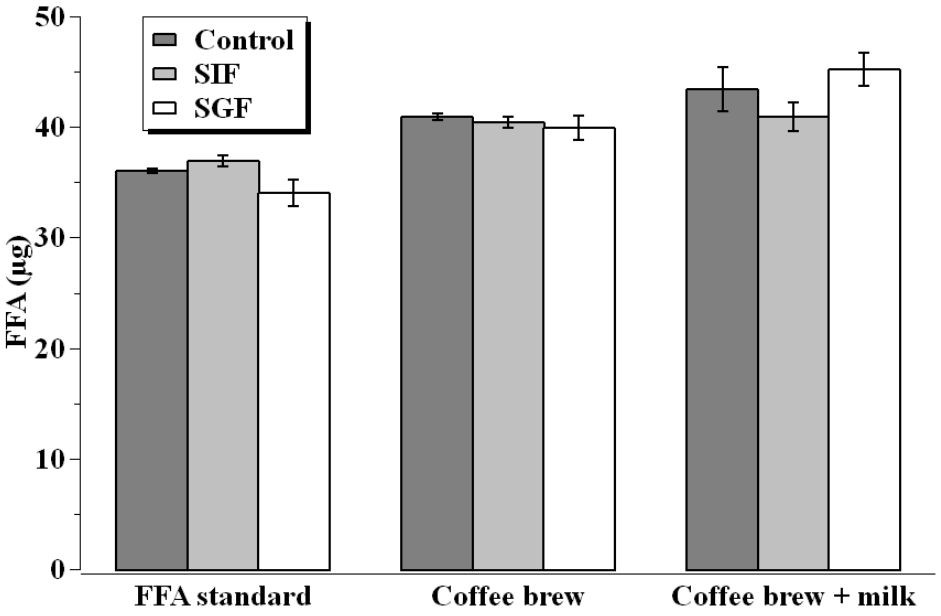


Fig. 6: A simulation of the effect of simulated gastric fluid (SGF), simulated intestinal fluid (SIF) or a water control on the amount of furfuryl alcohol (FFA) in a standard, coffee brew or coffee-milk (1:1, v:v) mixture after incubation in ratios mimicking an actual fasted state coffee drinking scenario.

4. Conclusions:

The various storage conditions applied to ground roasted coffee for 8 weeks revealed that FFA is less volatile and reactive than expected in the dry form of coffee especially at pH conditions relevant in coffee. Different brewing techniques influenced the concentrations of the furan derivatives resulting in different exposure

levels. The assessment of the content of four furan derivatives in 11 different coffee brews points out that FFA exposure was considerably higher compared to HMF, 2-FA or HMFA with a wide sample to sample inconsistency (up to 22-fold) in their concentrations. The consumption of a single dose of 600 mL of filter drip or espresso brewed coffee (equivalent to ca. 6 cups) would result in an exposure greater than the maximum acceptable daily intake of 0.5 mg/kg bodyweight which is suggested by JECFA. This means that high coffee intake raises significant concern of exceeding recommended limits of the daily exposure to FFA. Although FFA tends to polymerize in acidic conditions which are present in the stomach, more work is needed to investigate the influence of this degradation on its bio-accessibility from coffee consumption. This investigation indicates the importance of these different parameters in altering the actual daily exposure levels of potentially carcinogenic furan derivatives that are encountered when consuming coffee drinks.

Acknowledgements: Abdullatif Albouchi would like to thank Erasmus Mundus Avempace plus program for their financial support.

References:

Albouchi A, Murkovic M. 2018. Formation kinetics of furfuryl alcohol in a coffee model system. Food Chem. 243:91-95.

Brands CMJ, van Boekel MAJS. 2001. Reactions of monosaccharides during heating of sugar-casein systems: Building of a reaction network model. J Agri Food Chem. 49:4667-4675.

Farah A. 2012. Coffee constituents. In: Chu YF, editor. Coffee: Emerging health effects and disease prevention. 1st ed. Ames (IA): Wiley; p. 21-58.

Flament I, Bessière-Thomas Y. 2002. Coffee Flavor Chemistry. Chichester: Wiley. p. 64.

Franca AS, Oliveira LS, Oliveira RCS, Agresti PCM, Augusti R. 2009. A preliminary evaluation of the effect of processing temperature on coffee roasting degree assessment. J Food Eng. 92:345-352.

[FSANZ] Food Standards Australia New Zealand. 2011. [accessed 2017 Aug 11] http://www.foodstandards.gov.au/science/monitoringnutrients/ausnut/foodmeasures/Pages/Takeaway-beverage-measures-program---data-table.aspx.

Glatt H, Schneider H, Murkovic M, Monien BH, Meinl W. 2012. Hydroxymethyl-substituted furans: mutagenicity in Salmonella typhimurium strains engineered for expression of various human and rodent sulphotransferases. Mutagenesis. 27(1):41–48.

Grosse Y, Loomis D, Guyton KZ, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Mattock H, Straif K. 2017. Some chemicals that cause tumours of the urinary tract in rodents. The Lancet Oncology. 18(8):1003-1004.

Guenther H, Hoenicke K, Biesterveld S, Gerhard-Rieben E, Lantz I. 2010. Furan in coffee: pilot studies on formation during roasting and losses during production steps and consumer handling. Food Addit Contam. 27(3):283-290.

Høie AH, Monien BH, Sakhi AK, Glatt H, Hjertholm H, Husøy T. 2015. Formation of DNA adducts in wild-type and transgenic mice expressing human sulfotransferases 1A1 and 1A2 after oral exposure to furfuryl alcohol. Mutagenesis. 30:643-649.

[ISO] International Organization for Standardization, Technical Committee ISO/TC 34, Food products, Subcommittee SC 15, Coffee. 2008. ISO 6668:2008 Green coffee-Preparation of samples for use in sensory analysis.

[JECFA] Joint FAO/WHO Expert Committee on Food Additives, 76th meeting. 2012. Safety evaluation of certain food additives. WHO Food Addit Ser. 67.

Martins SIFS, Jongen WMF and van Boekel MAJS. 2001. A review of Maillard reaction in food and implications to kinetic modelling. Trends Food Sci Technol. 11:364–373.

Monien BH, Herrmann K, Florian S, Glatt H. 2011. Metabolic activation of furfuryl alcohol: Formation of 2-methylfuranyl DNA adducts in Salmonella typhimurium strains expressing human sulfotransferase 1A1 and in FVB/N mice. Carcinogenesis. 32:1533-1539.

Moon JK, Shibamoto T. 2009. Role of roasting conditions in the profile of volatile flavor chemicals formed from coffee beans. J Agri Food Chem. 57(13):5823-5831.

Moon JK, Shibamoto T. 2010. Formation of volatile chemicals from thermal degradation of less volatile coffee components: Quinic Acid, caffeic Acid, and chlorogenic Acid. J Agri Food Chem. 58:5465–5470.

Mudie DM, Murray K, Hoad CL, Pritchard SE, Garnett MC, Amidon GL, Gowland PA, Spiller RC, Amidon GE, Marciani L. 2014. Quantification of gastrointestinal liquid volumes and distribution following a 240 mL dose of water in the fasted state. Mol Pharm. 11:3039−3047.

Murkovic M, Swasti YR. 2013. 5-Hydroxymethyl-furfural and furfuryl alcohol: Occurrence, exposure and detection. In: Plasencia FP, Ciesarova Z, editors. Chemical Food Safety and Health. New York (NY): Nova Science Publishers. p. 43-55.

Petisca C, Pérez-Palacios T, Farah A, Pinho O, Ferreira IMPLVO. 2013. Furans and other volatile compounds in ground roasted and espresso coffee using

headspace solid-phase microextraction: Effect of roasting speed. Food Bioprod Process. 91:233-241.

[SCAA] Specialty Coffee Association of America. 2012. SCAA Standard for Cupping: Coffee to Water Ratio for Cupping. v.06_20_2012.

Swasti YR, Murkovic M. 2012. Characterization of the polymerization of furfuryl alcohol during roasting of coffee. Food Funct. 3(9):965-969.

[USPC] The United States Pharmacopeial Convention. 2008. The United States Pharmacopeia 32 – National Formulary 27. Rockville (MD): The United States Pharmacopeial Convention.

2.4. Paper 4:

‘Mitigation of furan derivatives in coffee and coffee-related model systems’

Mitigation of furan derivatives in coffee and coffee-related model systems

Abdullatif Albouchi [a*], Michael Murkovic [b]

[a,b] *Institute of Biochemistry, Graz University of Technology, Graz, Austria*

Petersgasse 10-12/II, 8010, Graz, Austria

Contact information:

[a] E-mail address: Abdullatifl.albouchi@outlook.de - Tel.: +43 6648749438.

[b] E-mail address: michael.murkovic@tugraz.at

Highlights:

1) The mitigation of hydroxymethyl substituted furan derivatives was performed.
2) The mitigation was investigated in two dry model systems and a coffee system.
3) 14 different phenolic and non-phenolic chemical additives were tested.
4) Performance of polyphenols depended on the number of available phenolic groups.
5) Mitigation trend was comparable between sucrose-alanine model systems and coffee.

Abstract:

The mitigation of furfuryl alcohol, 2-furoic acid, 5-hydroxymethyl furaldehyde, and 5-hydroxymethyl 2-furoic acid was conducted in two dry model systems mimicking coffee and an actual coffee system by incorporating 14 chemicals, which can be categorized as hydroxy cinnamates and their esters, hydroxy benzoates, flavonoids, as well as other non-phenolic agents afore a controlled roasting process. High mitigation capacity in the dry model systems was reached after the application of polyphenols (hydroxy cinnamates or benzoates derivatives), quinic acid or EDTA. The mitigation capacity of polyphenols depended on the number and availability of phenolic groups. Certain agents exhibited a furan derivative-specific reducing capacity while most of them showed a generalized effect. The trend of the mitigation in the coffee system was comparable to that in the dry model systems with taurine and sodium sulfite exerting the highest capacity. The mitigation efficacy decreased gradually with the increasing chemical complexity of the tested systems.

Keywords:

Mitigation; furan derivatives; coffee; model system; polyphenols; non-phenolics.

Chemical compounds studied in this article:

Furfuryl alcohol (PubChem CID: 7361); 2-furoic acid (PubChem CID: 6919); 5-hydroxymethyl furaldehyde (PubChem CID: 237332); 5-hydroxymethyl furoic acid (PubChem CID: 80642); chlorogenic acid (PubChem CID: 1794427); caffeic acid (PubChem CID: 689043); ferulic acid (PubChem CID: 445858); (-)-quinic acid (PubChem CID: 6508); protocatechuic acid (PubChem CID: 72); gallic acid (PubChem CID: 370); (+)-catechin hydrate (PubChem CID: 107957); rutin hydrate (PubChem CID: 45479757); naringin (PubChem CID: 442428); L-(+)-ascorbic acid (PubChem CID: 54670067); β-carotene (PubChem CID: 5280489); taurine (PubChem CID: 1123); ethylene diamine tetra acetic acid (PubChem CID: 6049); anhydrous sodium sulfite (PubChem CID: 24437); sucrose (PubChem CID: 5988); DL-alanine (PubChem CID: 602); microcrystalline cellulose (PubChem CID: 14055602).

1. Introduction:

Furan derivatives are a class of heterocyclic five-membered-rings organic molecules that are considered to be of extreme importance among organoleptic compounds of roasted coffee. This can be attributed to furan derivatives being the major group of structurally related compounds found in coffee samples (Petisca, Pérez-Palacios, Farah, Pinho & Ferreira, 2013) as well as their known positive contribution to the sensory attributes of heat-processed foods (Anese & Suman, 2013). Furan derivatives which are substituted with a hydroxymethyl group have undergone numerous research activities and received a lot of attention. For once, they

are available in higher quantities compared to other furans. Moreover they were found in toxicological studies to give rise to negative health effects.

The two most known examples of this group of substituted furan derivatives are furfuryl alcohol (FFA) (2-hydroxymethyl furan) and 5-hydroxymethyl furaldehyde (HMF). In one study, FFA was found to comprise more than 50 % of all furans quantified from coffee samples (Petisca et al., 2013). HMF is obtained from coffee in noteworthy quantities especially as it is considered the major volatile detected in light-roasted coffee (Moon & Shibamoto, 2009). Alongside FFA and HMF, we encounter 2-furoic acid (2-FA) and 5-hydroxymethyl furoic acid (HMFA) in coffee in considerable amounts. 2-FA and HMFA are the carboxylic acid derivatives of FFA and HMF, respectively. Also, it is known that they are natural metabolites of FFA and HMF, respectively, so they are found in biological fluids after consumption of the latter two furan derivatives (Glatt, Schneider, Murkovic, Monien, & Meinl, 2012; JECFA, 2012).

On the other hand, FFA and HMF are associated with toxicological effects on living organisms, particularly those producing sulfotransferases (SULT) either naturally or after genetic engineering. Both of them share a common enzymatic transformation in vivo, where they produce 2-sulfo-oxymethylfuran and 5-sulphooxymethylfurfural (from FFA and HMF, respectively). These final metabolism products are reactive electrophiles that can interact with DNA-molecules forming adducts. These adducts have been related to mutations in the genomic structure (Glatt et al., 2012; Sachse, Meinl, Sommer, Glatt, Seidel, & Monien, 2016).

Looking at green coffee, furan derivatives are found (if any) in negligible amounts. Thus such compounds can be termed as process contaminants since they are mostly formed through the roasting process from precursors found naturally in green

coffee. FFA formation mechanism is still not fully understood. Yaylayan and Keyhani (2000) using ^{13}C-labelled glucose and alanine model systems and after application of pyrolysis conditions, were able to determine that FFA was formed either from intact C1–C5 or C2–C6 glucose fragments or from a mixture of glucose and alanine fragments. This leads researchers to propose oxidation at C1 and C6 of the glucose as the initiation step of this mechanism. Furthermore it was hypothesised that FFA can be formed after a retro-aldol cleavage taking place at C1-C2 of 1-deoxy-1-amino-3-hexulose which is an isomer of the Amadori product that originates in turn from Maillard reaction (Wnorowski & Yaylayan, 2000). Studies performed in aqueous model systems of monosaccharides and casein resulted in minor amounts of FFA to be found. Therefore, it was suggested that α-dicarbonyl cleavage of 1,2-enediol or 1,2-enaminol is the major step in FFA production. The 1,2-enediol forms readily in aqueous solutions of monosaccharides where 1,2-enaminol requires Maillard reaction to be formed, although authors mentioned that formation of the latter in aqueous solutions of ketoses is limited (Brands & Van Boekel, 2001). Another formation mechanism of FFA was introduced involving a multi-step rearrangement of chlorogenic acids (Moon & Shibamoto, 2010).

Cämmerer, Wedzicha and Kroh (1999) elucidated the formation mechanisms of HMF in caramelisation and Maillard reaction conditions. When no amino acid is present, sugars in acidic medium tend to undergo 1,2-enolization followed by dehydration to give 3-deoxyosone. The same key intermediate can also be formed through Maillard reaction from the Amadori reaction product. Later, 3-deoxyosone produces HMF after dehydration and cyclization. In addition, the same researchers proposed that methyl glyoxal together with glyceraldehyde through an aldol pathway lead to the formation of HMF in heated aqueous solutions under caramelisation

conditions. Another prominent theory is the direct formation of HMF from sucrose/fructose by the intact fructofuranosyl cation that converts quickly under dry heat conditions to HMF (Perez Lucas & Yaylayan, 2008). In contrast, very limited number of papers discusses the formation mechanisms of 2-FA or HMFA. It was reported that the degradation of aqueous solutions of dehydroascorbic acid probably into a ketene intermediate results in 2-FA. 2-FA is proposed also to originate from 2-furfural by a Cannizzaro reaction, and the latter is produced from heating of sugars or ascorbic acid (Varelis & Hucker, 2011). Whereas HMFA is suggested to form by an aldol reaction of glyceraldehyde with pyruvic acid (Murkovic & Bornik, 2007).

Several approaches have been suggested for the mitigation of such potentially harmful substances. Anese et al. (2013) classified these technological approaches as preventive that aim in lessening the formation of process contaminants, and eliminative that target the removal of such compounds after being created. Within these approaches we can find that the addition of different chemical compounds that can either interfere with the building mechanism or destroy the final produced process contaminants is a widely applied strategy. This has been previously applied for the mitigation of acrylamide (Constantinou & Koutsidis, 2016; Oral, Dogan, & Sarioglu, 2014), furan and/or its derivatives (Zheng, Chung, & Kim, 2015; Navarro & Morales, 2017; Oral et al., 2014; Totlani & Peterson, 2007), and pyrazines and pyrroles (Totlani et al., 2007). Numerous chemical additives belonging to different chemical classes were applied in these experiments. Mostly antioxidants of polyphenol characteristics were employed since they are known to exhibit dicarbonyl trapping effects. These effects play a pivotal role in capturing important intermediates for the formation of process contaminants, especially those formed through caramelisation or Maillard reaction (Zheng et al., 2015). Different research groups used different

matrices to investigate the mitigation effects of various agents. In some manuscripts the application of mitigation agents to well defined and simplified model systems was described while others described the application during processing of actual food samples (usually biscuits).

The number of published reports about the mitigation of process contaminants in coffee is extremely limited due to the fact that mitigation agents are hard to incorporate into coffee before being roasted. The objective of this study was to investigate the mitigation effect of 14 different agents being applied to the coffee matrix, and compare this to their effect when applied to a simplified model system containing probable precursors of the process contaminants in question. For this purpose, the amounts produced of FFA, HMF along with their carboxylic acid derivatives 2-FA and HMFA, respectively, were quantified. These process contaminants were carefully chosen since they are produced during the roasting of coffee in major amounts and at the same time they are presumed to exert negative effects that can adversely affect human health.

2. Materials and methods:

2.1.Chemicals:

FFA (≥ 98 %) was obtained from Fluka Chemicals (Basel, Switzerland). 2-FA (98 %) and HMF (99 %) were obtained from Aldrich Chemistry (St. Louis, MO, USA). HMFA was obtained from Matrix Scientific (Columbia, SC, USA).

Sucrose and DL-alanine were obtained from E. Merck (Darmstadt, Germany).

Microcrystalline cellulose (ca. 0.038) mm was purchased from Serva Feinbiochemica (Heidelberg, Germany).

L-(+)-ascorbic acid (≥ 99.7 %), (-)-quinic acid (> 98 %), and anhydrous sodium sulfite (pure) were obtained from E. Merck (Darmstadt, Germany). β-Carotene (≥ 97 %) and gallic acid (≥ 98 %) were purchased from Fluka Chemicals (Buchs, Switzerland). Chlorogenic acid (≥ 95 %), ferulic acid, protocatechuic acid (97 %), rutin hydrate (≥ 95 %), naringin (≥ 95 %, water content 1.5 mol/mol), (+)-catechin hydrate (≥ 98 %), ethylene diamine tetra acetic acid (EDTA, 99.5 %), and taurine were obtained from Sigma Aldrich co. (St. Louis, MO, USA). Caffeic acid was obtained from Carl Roth GmbH. (Karlsruhe, Germany).

Methanol (HPLC grade) was obtained from Chem Lab NV (Zedelgem, Belgium). Water for HPLC was obtained from Ultra clear TWF-UV system (Siemens water technologies, Germany). Glacial acetic acid (99-100 %) was obtained from Avantor performance material (Deventer, Netherlands).

2.2.Preparation of the simple and complex model systems base powder:

For the simple model system: A sucrose and alanine mixture was prepared in the molar ratio of 1:1 using simply a mortar and pestle. After several minutes of homogenizing, three samples of the mixture were taken from different positions of the powder and were analysed by HPLC to check the uniformity of the final mixture. The mixture was then kept frozen until its use.

For the complex model system: A defined amount of the simple model system mixture was taken and mixed with an equal amount of microcrystalline cellulose. The mixture was homogenized for several minutes using a mortar and pestle. Later, 3 samples of the mixture were taken from different positions of the powder and were

analysed by HPLC to check the uniformity of the final mixture. The mixture was then kept frozen until its use.

2.3.Incorporation of mitigation agents into the base powder of simple and complex model systems:

50 mg of the simple model system base powder or 100 mg of the complex model system base powder were weighed into a glass vial. The mitigation agent was added in a molar ratio of 3:1 sucrose:mitigation agent. The content of the glass vial was shaken carefully and thoroughly.

2.4.Preparation of the coffee model systems base powder:

6 g of green coffee beans (Robusta, Uganda) were crushed for 1 minute using a homogenizer (A11 basic mill; IKA, Staufen, Germany). Afterwards, 400 mg of coffee crushed powder were put into a ball-mill capsule together with a metal ball. The capsule was attached to the Retsch MM2 ball-mill (Haan, Germany) and the instrument was started at highest possible speed for 15 minutes. The fine powder was scrapped of the walls of the capsule and kept in the fridge until the next step.

2.5.Incorporation of mitigation agents into the base powder of the coffee model system:

200 mg of the coffee model system base powder were weighed into a glass vial. The mitigation agent was added in a molar ratio of 3:1 sucrose:mitigation agent. To the content of the glass vial 500 µL of water were added and the content was mixed with a metal rod. The glass vial was closed and frozen overnight. The content of the glass vial was freeze-dried overnight in Modulyo freeze drier (Savant instruments Inc., USA). Later, the freeze-dried was homogenized again with a metal rod carefully and thoroughly.

2.6.Roasting and extraction of various model systems:

The various control and test samples were roasted in a closed system as previously published in order to capture the entire amounts produced of the volatile furan derivatives, especially the highly volatile FFA (Albouchi & Murkovic, 2018). Briefly, the closed system is a tightly closed 2-compartemt system composed of a smaller vial that contains the sample to be roasted and a bigger vial kept at room temperature to receive the vapour produced from sample roasting. The chosen roasting conditions were 220 °C for 4 minutes. The entire system was then left to rest at room temperature for 1 minute and then quickly put onto ice until extraction.

Each compartment was extracted individually with a 1 mL of water. The upper chamber was shaken at high speed using a Vortex-Genie 2 (Scientific industries Inc., Bohemia, NY) for 1 minute. For the lower chamber, shaking was applied at high speed for 1 minute and then at medium speed for 9 minutes and afterwards centrifuged (Eppendorf AG, Hamburg, Germany) for 15 min at 5 °C and 14,000 rpm.

2.7.HPLC analysis of sucrose in green coffee:

100 mg of the coffee model system base powder were weighed into a reaction tube and extracted twice with a 1 mL of water and a third time with 0.5 mL of water. The extraction was performed by shaking (Vortex-Genie 2, Scientific industries Inc., Bohemia, NY) at high speed for 1 minute and subsequent sonication by Transsonic 460 (Elma, Germany) for 15 minutes. After centrifugation, the extraction aliquots were combined into a 5 mL volumetric flask and the volume was completed with water. The same extraction procedure was performed for three 100 mg coffee samples.

25 µL of each sample or sucrose standard were injected into an Agilent 1100 series

HPLC system (Agilent Technologies, Germany) with a refractive index detector and an Aminex carbohydrate analysis column (HPX-87P, 7.8×300 mm, Bio-rad Laboratories Inc., USA). The separation was done using an isocratic flow of water until the end of the run (15 minutes) with a flow rate of 0.6 mL/min. The column compartment was set at 40 °C during the entire analysis. Detection was carried out with cell temperature of 40 °C.

2.8.HPLC analysis of furan derivatives:

2 µL of each sample or furan derivatives standard were injected into an Agilent 1100 series HPLC system (Agilent Technologies, Germany) with a diode array detector and a Zorbax Eclipse (XBD-C8 4.6×150 mm, 5 µm, Agilent Technologies, USA) with a pre-column. The separation was done using a gradient mobile phase of 0.1 % acetic acid in water (A) and methanol (B) at 25 °C. The gradient started with 100 % A, at 2.5 minutes B was increased to 16 %, between 10 and 10.5 minutes B was increased to 100 % and held until the end of the run (15 minutes) with a flow rate of 0.5 mL/min. Detection was done at a wavelength of 217 nm for FFA. HMF was detected at 284 nm. 2-FA and HMFA were detected at 250 nm.

3. Results and discussion:

3.1.Simple model system:

14 different agents were tested for the mitigation of four furan derivatives in a simple model system composed only of sucrose and alanine in the molar ratio 1:1. The choice of the constituents of the model system was set after numerous trials of model systems mimicking coffee natural constituents (Data not shown). Sucrose is known to be the major simple carbohydrate in green coffee while other simple saccharides are to be found in almost negligible amounts. Alanine was found to be the free amino acid

with the highest amount in green coffee (Murkovic & Derler, 2006). The molar ratio of 1:1 was selected since results will be compared eventually with those from mitigation of furan derivatives in Robusta model system that is reported to have similar molar ratios between simple carbohydrates and free amino acids (Kleinwächter, Bytof, & Selmar, 2015). Simple carbohydrate and free amino acids are considered of major importance as precursors for the formation of a wide range of coffee volatiles through caramelisation, Maillard reaction, aldolisation, Strecker degradation and/or other mechanisms either as whole molecules or as fragments (Wei & Tanokura, 2015).

Fig. 1 illustrates the different mitigation capacity of each mitigation agent on the final amounts of FFA, 2-FA, HMF, and HMFA when added to the model system before roasting in a molar ratio of 3:1 of sucrose:mitigation agent. The results of FFA indicate that chlorogenic acid, quinic acid and EDTA have the highest mitigation capacity (up to 91.2 % of FFA was reduced in the case of quinic acid). On the other hand, ferulic acid and sodium sulfite showed no mitigation capacity for FFA. Also, 2-FA showed similar results. In the case of HMF we can see that quinic acid, EDTA, gallic acid and protocatechuic acid were the most efficient in HMF mitigation (up to 92.7 % was reduced in the case of gallic acid). However taurine had no effect on the determined amounts of HMF. For HMFA, quinic acid and protocatechuic acid had the greatest mitigation capacity (up to 90.3 % in the case of quinic acid). On the contrary, sodium sulfite caused an increase in HMFA of about 17 %.

We can speculate the possible mechanisms that resulted in the mitigation effects of the applied agents as the interaction with precursors, intermediates or final result products. To simplify our interpretation, agents were categorized into groups depending on their chemical structure and natural distribution. Hydroxy cinnamates

are a group of polyphenols that are found naturally in green coffee such as caffeic and ferulic acid and mainly as their esters with quinic acid that is called chlorogenic acids. Chlorogenic and caffeic acids along with quinic acid have shown a high mitigation capacity for all furan derivatives (in the range of 46-92 %). This can be attributed to the interaction with Maillard reaction products through their carbonyl trapping properties (Constantinou et al., 2016), scavenging free radicals (Zheng et al., 2015), or by reducing the final furan derivative since they are strong antioxidants (Oral et al., 2014). Quinic acid is not a hydroxy cinnamate derivative but it is found usually esterified to them, and while it has no phenol groups it appears to interfere strongly with the formation of furan derivatives in our model system. The mode of action of quinic acid is unknown, but it could be theorised that it reacts with amino acids leading to a different Maillard reaction products thus exhausting the amount of amino acids available to react with sugars. On the contrary, ferulic acid has exhibited no mitigating effect on all furan derivatives tested. This is in line with previous published results that refers to the hydroxyl group on the aromatic ring being converted into a methoxy as the reason for the poor free radical and carbonyl scavenging capacity compared to caffeic acid with its free hydroxyl groups (Constantinou et al., 2016; Zheng et al., 2015).

Another group of polyphenols are hydroxyl benzoate derivatives like protocatechuic acid and gallic acid with two and three phenolic groups, respectively. Although it was reported by Navarro et al. (2017) that gallic acid exerted no effects on HMF concentrations in a biscuit model system, we have observed a good mitigation effect of furan derivatives quantitated using both benzoic acid derivatives (in the range of 46-93 %) with gallic acid having somewhat better results (especially on FFA).

However, the same authors stated that gallic acid can prevent the oxidation of the Amadori compound that is formed as an intermediate in Maillard reaction.

The flavonoids are a widely spread polyphenolic antioxidants in plants. In our current study catechin (non-glycoside) as well as rutin and naringin (glycosides of quercetin and naringenin, respectively) were employed for the mitigation of furan derivatives. Catechin and rutin mitigated the four furan derivatives by 29.1-87.8 % whereas naringin resulted in considerably higher concentrations, especially in FFA and 2-FA where it had almost no mitigation capacity. By comparing the structure of the three aglycones we can note that both catechin and quercetin possess two hydroxyl groups on the ring B while naringenin contains only one. Totlani et al. (2007) reported that epicatechin is capable of forming adducts with 3-deoxyosones or subsequent fragments by electrophilic aromatic substitution reactions. These moieties play a prominent role in the formation of furan derivatives as mentioned earlier. Later, it was mentioned that catechin react with aldehydes through alkyl aryl bridges creating adducts, therefore preventing the advancement of the reaction (Constantinou et al., 2016). Quercetin is stated to have the same mechanism as gallic acid where they prevent the oxidation of the Amadori compound (Navarro et al., 2017).

Ascorbic acid showed a good mitigation capacity (in the range of 48-81 %). Adams and De Kimpe (2009) demonstrated that ascorbic acid itself can when caramelised form furan derivatives like FFA, but in the presence of amino acids the reaction mechanisms are in favour of formation of pyrazines rather than furan derivatives. In other words, we can say that ascorbic acid tends to fix nitrogen atoms of amino acids, hence a lower amount of them are available to react with sugar molecules to form furan derivatives through the mechanisms and precursors previously discussed. Also, they reported that the reducing characteristic of ascorbic acid affects the type of

pyrazine formed. Presumably, this applies too to furan derivatives that can be easily reduced such as HMF. In fact from Fig. 1, ascorbic acid had its highest mitigation capacity on this derivative. Very little information is available on the effect of β-carotene on sugar-amino acid model systems. Previously published reports have stated that it is a precursor for furan and in certain concentration under set conditions would promote oxidative activity (Zheng et al., 2015). Our results indicate a moderate mitigation capacity of β-carotene via an unknown pathway. This might be referred to its inherent capabilities of singlet oxygen quenching and peroxyl radical scavenging.

Taurine a non-protein amino acid has shown potential in the mitigation of acrylamide in a potato model system by competing with asparagine a known precursor of acrylamide (Shin, Kim, Lee, Choi, Na, & Lee, 2010). In our experiment, taurine exhibited a low mitigation capacity on FFA and HMFA only 39.6 and 21.7 %, respectively. We believe that this can be attributed to the competition with alanine in the model system leading to different end products. EDTA is a metal complexing agent that is added to stabilise foods and prevent the oxidation of sensitive ingredients such as colourants and flavourings (Kemmei, Kodama, Yamamoto, Inoue, & Hayakawa, 2013). When added to the model system, EDTA had a strong mitigating capacity of 74.6 to 91.3 % on furan derivatives produced. The mechanism in which EDTA exert this effect is not understood, nevertheless the assumption that it may work as a scavenger of reactive intermediates or that it prevents any oxidation steps in the reaction pathways is plausible. We noted that sodium sulfite had a diverse set of actions depending on the furan derivative in question. It had a strong mitigating effect on HMF of 87.7 % while it practiced no effect on FFA or 2-FA and even stimulated the production of HMFA. Zheng et al. (2015) mentioned that sulfites in food systems act by scavenging radicals as well as via nucleophilic attack on reducing sugars

during the Maillard reaction. Since HMF is an aldehyde that is very susceptible to reduction (unlike FFA, 2-FA or HMFA), we can hypothesise that the reducing properties of sodium sulfite is responsible for its ability to mitigate HMF to this great extent.

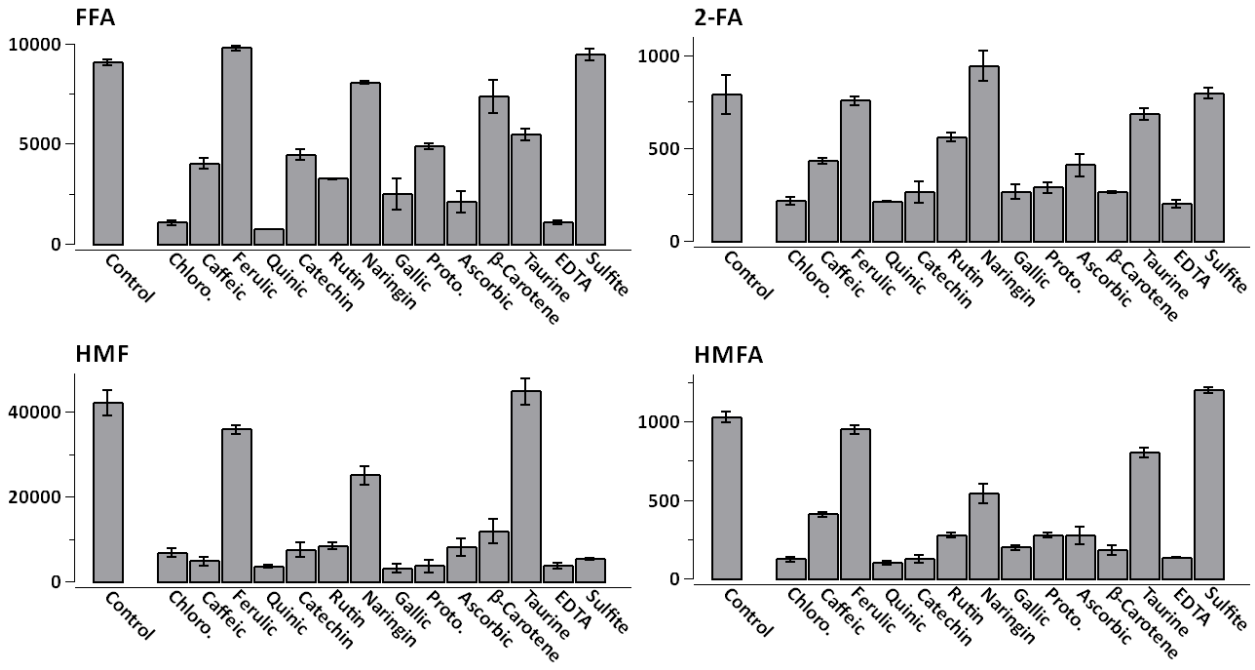


Fig. 1: The mitigation of FFA, 2-FA, HMF, and HMFA in a simple model system of sucrose and alanine in the molar ratio 1:1 that was roasted for 4 minutes at 220 °C in a closed system after incorporation of the mitigation agent in a molar ratio of 3:1 sucrose:mitigation agent. All numbers are given in µmole/mole of sucrose. Chloro: Chlorogenic acid. Proto: Protocatechuic acid.

3.2.Complex model system:

In the effort to come up with a model system that simulates real coffee matrix, microcrystalline cellulose was added to the simple model system in a 1:1 weight ratio. Therefore the final roasted amount of the complex model system was double that of the simple model system in order to keep the amounts of important precursors

(sucrose and alanine) stable. Microcrystalline cellulose was selected because it did not introduce any significant changes in the amounts of the four furan derivatives compared to the simple model system as can be seen by comparing Fig. 1 and Fig. 2. This means that it does neither affect the chemical reactions of sucrose and alanine leading to the formation of these compounds nor act as a precursor of these compounds on its own.

From the comparison between both figures, we can clearly notice that all of the mitigation agents have lost part or all of their mitigation capacity for all four furan derivatives quantified in the presence of microcrystalline cellulose. That is especially true for caffeic acid and naringin. It is even observable that naringin promoted the formation of 2-FA by 51 %. This can be attributed to microcrystalline cellulose forming a physical barrier between the mitigation agents and the reactive moieties. Another explanation may be that microcrystalline cellulose can interact with the mitigation agents reducing its availability for mitigation. Nevertheless, we still can correlate the mitigation capacity in the simple model system with that of the complex model system. Examples of agents that exhibit the greatest reduction in the complex model system are: Chlorogenic acid and quinic acid for FFA, quinic acid and β-carotene for 2-FA, quinic acid and gallic acid and sodium sulfite for HMF, quinic acid for HMFA.

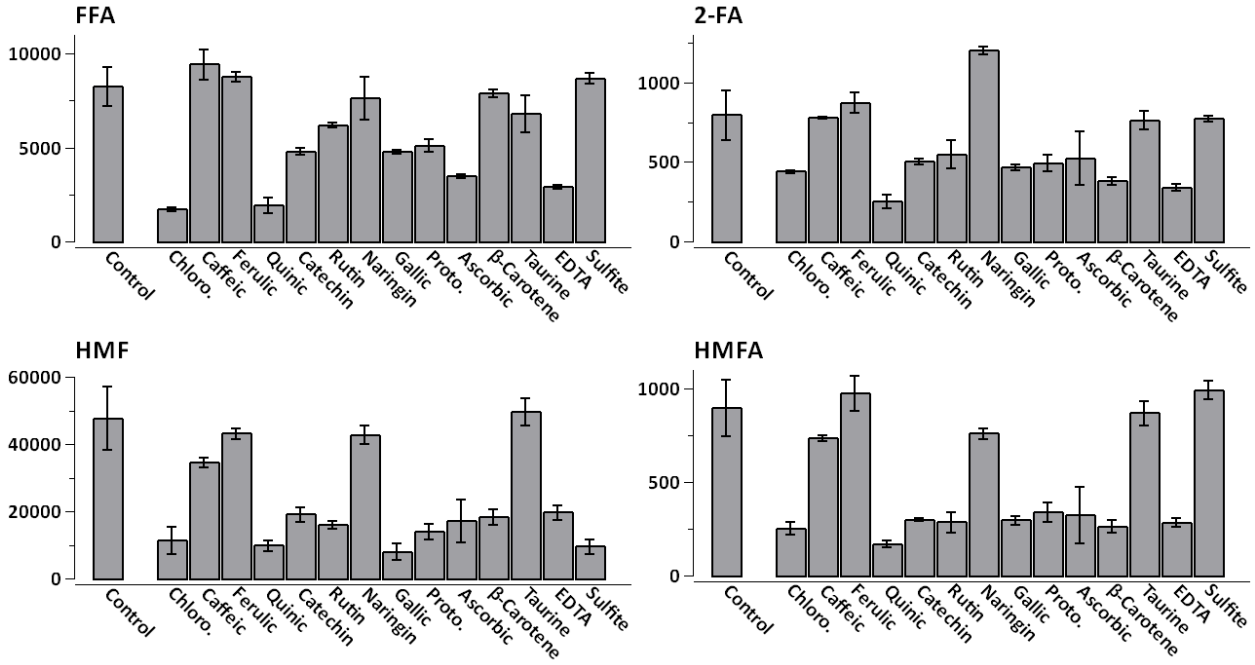


Fig. 2: The mitigation of FFA, 2-FA, HMF, and HMFA in a complex model system of sucrose and alanine in the molar ratio 1:1 plus 50 % (by weight) of microcrystalline cellulose that was roasted for 4 minutes at 220 °C in a closed system after incorporation of the mitigation agent in a molar ratio of 3:1 sucrose:mitigation agent. All numbers are given in µmole/mole of sucrose. Chloro: Chlorogenic acid. Proto: Protocatechuic acid.

3.3.Coffee model system:

The aim of this study was to mitigate furan derivatives in an actual coffee matrix, therefore Robusta type coffee beans were used for this purpose. As discussed earlier, simple carbohydrates and free amino acids are widely recognized as the natural constituents of green coffee beans that take a major part in the formation of various volatile and aroma compounds during the roasting process. Of these reactive precursors, sucrose is the only single compound of major quantity in green coffee beans. Sucrose was determined in the Robusta green coffee by HPLC-RI to be 44.1 ± 0.4 mg/g. Based on this result, the number of moles of sucrose to be present in a 200

mg sample was calculated. Mitigation agents were added in a molar ratio of 3:1 (sucrose:mitigation agent) as in previously prepared model systems. Later, water was added and the mixture was mixed thoroughly to allow the mitigation agents to penetrate the coffee structure to reach the precursors and at the same time to distribute the mitigation agents homogenously throughout the sample. Freeze drying was then applied to remove the water that was added at the last step. By comparing the controls of Fig. 1 or 2 with that of Fig. 3, it is noteworthy that FFA has become the major furan derivative in coffee model system with its amount increasing more than 440 % while HMF suffered from a decrease of about 66 %. The amounts of 2-FA and HMFA in coffee model system were elevated by ca. 350 %. Apparently the reaction atmosphere in coffee favours the formation of FFA, 2-FA, and HMFA on the expense of HMF, particularly as the sum of moles of all furan derivatives formed per mole of sucrose did not differ largely between the three model systems (less than 10 %).

Fig. 3 presents the results of the mitigation experiment in the coffee model system. Most of the mitigation agents have lost all their mitigation capacities; this is especially true for 2-FA and HMF. The complex composition of coffee with various chemical groups of compounds along with the lower percentage of the active precursors to the total amount of the system present a big chance of side reactions that may deplete mitigation agents or prevent them from performing the same actions at equal efficiency as in the simple and complex model systems. The chromatographic purity for the four peaks of interest was checked in all mitigation experiments. Only in the peak of 2-FA in taurine experiment and the peak of HMFA in sodium sulfite experiment a chromatographic interference with unknown moieties was observed. Thus no results were shown for the mitigation of 2-FA by taurine or for HMFA by sodium sulfite. The cause for this can be compounds that are formed from a specific

interaction between coffee constituents other than simple carbohydrates, free amino acids and polysaccharides, with taurine and sodium sulfite that lead to the formation of substances not formed previously in the simple or complex model systems.

For FFA, quinic acid, catechin and EDTA exhibited minor mitigation effect of 3.3-10.8 % while taurine exhibited a 33.1 % decrease which is similar to taurine's effect at the simple model system. Ascorbic acid gave a small increase in FFA of 4.4 %. In the case of 2-FA, only ferulic acid and sodium sulfite exerted a mitigating capacity of 5 % and 22.1 %, respectively. This is against the results obtained from the simple and complex model systems. The effect of sodium sulfite may be referred to the reduction of 2-furfural, which is a precursor of 2-FA, thus preventing the production of the latter. On the other hand, most agents added to coffee model system lead to a small increase of 2-FA in the range of 3-31 %. Ascorbic acid caused an outstanding increase of 2-FA by 170 % and it is attributed to the previously mentioned reports of ascorbic acid being a precursor of 2-FA. Only gallic acid and catechin maintained a rather weak mitigation capacity for HMF by 8.8 % and 11.1 %, respectively, while sodium sulfite demonstrated a moderate mitigation capacity of 38.3 % in the coffee model system. These results are consistent with what we observed in the simple and complex model systems. On the contrary to the results of simple and complex model systems, chlorogenic acid, quinic acid and EDTA brought about stimulated HMF formation by 15.4 %, 29.3 % and 63.9 %, respectively. Looking at HMFA, chlorogenic acid, rutin, ascorbic acid, β-carotene, taurine and EDTA reduced the final amount quantified in a range between 4-18 %.

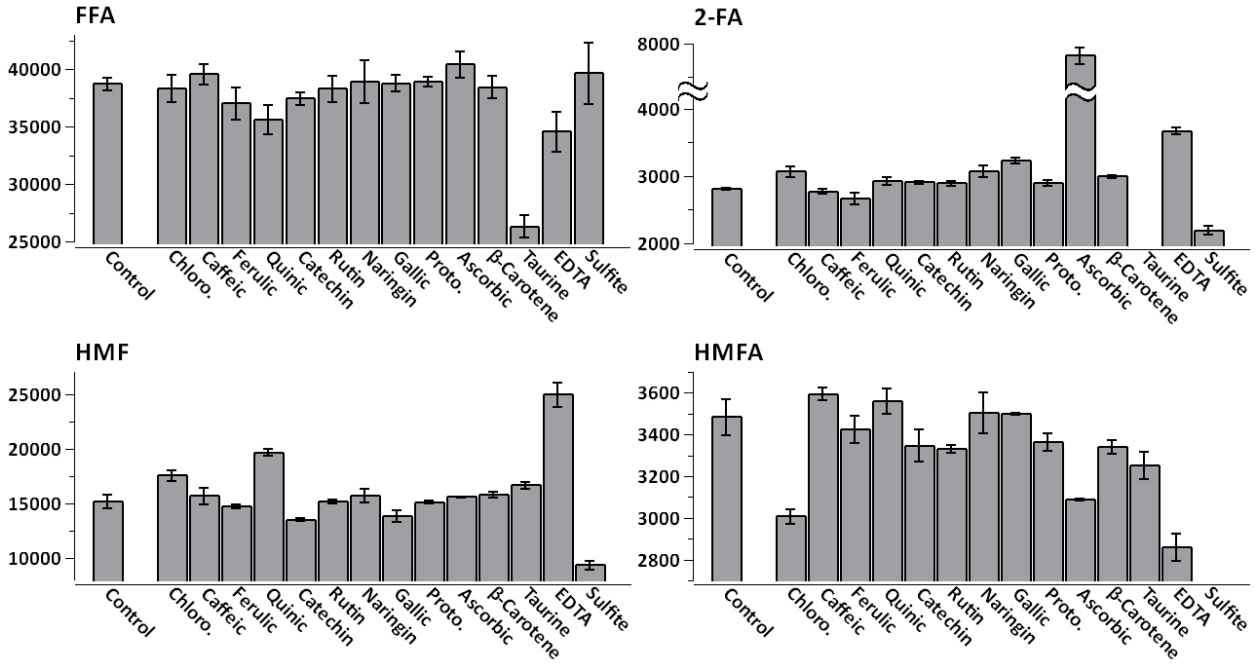


Fig. 3: The mitigation of FFA, 2-FA, HMF, and HMFA in a Robusta coffee model system that was roasted for 4 minutes at 220 °C in a closed system after incorporation of the mitigation agent in a molar ratio of 3:1 sucrose:mitigation agent. All numbers are given in µmole/mole of sucrose. Chloro: Chlorogenic acid. Proto: Protocatechuic acid.

4. Conclusions:

Multiple chemical, physical or microbiological strategies have been proposed for the mitigation of many toxic and probably carcinogenic substances, emerging from numerous processes applied to foods. Of which the addition of different chemical compounds has been previously used for the mitigation of furan, HMF and/or acrylamide.

In this study, the mitigation of four furan derivatives, namely FFA, 2-FA, HMF, and HMFA was performed in two model systems that were carefully chosen to simulate the most suspected precursors of furan derivatives from green coffee's natural components. Later, the mitigation activity was verified in actual coffee matrix.

The 14 different chemical additives were tested by adding them to the model systems in a certain molar ratio depending on the amount of the pre-quantitated precursors prior to a well-defined roasting process.

The agents responsible for the greatest capacity of mitigation in simple and complex model systems were polyphenols (e.g. derivatives of hydroxy cinnamates and hydroxy benzoates) as well as other non-phenolic antioxidants (e.g. quinic acid and EDTA). Flavonoids and sodium sulfite presented a moderate mitigation capability. The efficiency of polyphenolic compounds in reducing furan derivatives relied on the number of phenolic groups available for reaction. Some agents exerted their effect on all furan derivatives quantified while others affected only certain derivatives depending on the hypothesised mitigation pathway and the characteristics of the furan derivative. Microcrystalline cellulose in the complex model system had a negative effect on the mitigation amplitude of all tested agents. Our results are mostly consistent with earlier described model system studies.

The reduction of furan derivatives in the coffee model system followed a similar trend to that observed in the simple and complex model systems (except for 2-FA). Taurine (in the case of FFA) and sodium sulfite (in the case of 2-FA and HMF) seem to be the most efficient mitigation agents in the coffee model system whereas other mitigation agents showed a minor capacity for mitigation of furan derivatives, e.g. catechin and EDTA. As a general rule, the mitigation capacities of tested agents were considerably lower in the coffee model system compared to the simple and complex model systems. Matrix interferences and the fact that presumed precursors form a smaller proportion of the entire coffee system are maybe to blame for this reduction in the mitigation capacity.

The chemical reactions that take place during coffee roasting leading to the production of process contaminants (such as furan derivatives) are the same ones producing important aroma and taste molecules that define the widely popular drink. It is generally accepted that tampering with these reactions and the natural constituents of green coffee is not desirable. That is the main reason why this study aims to provide a closer look at the formation mechanism of furan derivatives and how other chemicals can interfere with the intermediates during this process. From our results, some mitigation experiments in actual coffee matrix validated the results obtained from a model system of sucrose and free amino acids. This fact shed more light on the importance of these two classes of constituents as confirmed precursors for the formation of furan derivatives. Different mitigation agents demonstrated a furan derivative-specific mitigation capacity pointing out the different intermediates or production pathways for formation of the four furan derivatives. The increasing complexity of the model systems (from simple to coffee model systems) indicates how components presumed to be non-precursors can adversely interfere with the work of mitigation agents in the model systems.

Acknowledgements: Abdullatif Albouchi would like to thank Erasmus Mundus Avempace plus program for their financial support.

References:

Adams, A., & De Kimpe, N. (2009). Formation of Pyrazines from Ascorbic Acid and Amino Acids Under Dry-Roasting Conditions. *Food Chemistry,* 115, 1417–1423.

Albouchi, A,. & Murkovic, M. (2018). Formation Kinetics of Furfuryl Alcohol in a Coffee Model System. *Food Chemistry*, 243, 91–95.

Anese, M., & Suman, M. (2013). Mitigation Strategies of Furan and 5-Hydroxymethylfurfural in Food. *Food Research International*, 51, 257–264.

Brands, C. M. J., & Van Boekel, M. A. J. S. (2001). Reactions of Monosaccharides During Heating of Sugar-Casein Systems: Building of a Reaction Network Model. *Journal of Agricultural and Food Chemistry*, 49, 4667–4675.

Cämmerer, B., Wedzicha B. L., & Kroh, L. W. (1999). Nonenzymatic Browning Reactions of Retro-Aldol Degradation Products of Carbohydrates. *European Food Research and Technology*, 209, 261–265.

Constantinou, C., & Koutsidis, G. (2016). Investigations on the Effect of Antioxidant Type and Concentration and Model System Matrix on Acrylamide Formation in Model Maillard Reaction Systems. *Food Chemistry*, 197, 769–775.

Glatt, H., Schneider, H., Murkovic, M., Monien, B. H., & Meinl, W. (2012). Hydroxymethyl-Substituted Furans: Mutagenicity in Salmonella Typhimurium Strains Engineered for Expression of Various Human and Rodent Sulphotransferases. *Mutagenesis*, 27(1), 41–48.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2012). Safety Evaluation of Certain Food Additives. In *WHO Food Additives Series;* 67. World Health Organization.

Kemmei, T., Kodama, S., Yamamoto, A., Inoue, Y., & Hayakawa, K. (2013). Determination of Ethylenediaminetetraacetic Acid in Foods by Reversed-Phase High-Performance Liquid Chromatography. *Food Chemistry*, 138, 866–869.

Kleinwächter, M., Bytof, G., & Selmar, D. (2015). Coffee Beans and Processing. In V. R. Preedy, *Coffee in Health and Disease Prevention* (pp. 73–81). Academic Press, Elsevier Inc.

Moon, J. K., & Shibamoto, T. (2009). Role of Roasting Conditions in the Profile of Volatile Flavor Chemicals Formed from Coffee Beans. *Journal of Agricultural and Food Chemistry*, 57(13), 5823–5831.

Moon, J. K., & Shibamoto, T. (2010). Formation of Volatile Chemicals From Thermal Degradation of Less Volatile Coffee Components: Quinic Acid, Caffeic Acid, and Chlorogenic Acid. *Journal of Agricultural and Food Chemistry*, 58, 5465–5470.

Murkovic, M., & Derler, K. (2006). Analysis of Amino Acids and Carbohydrates in Green Coffee. *Journal of Biochemical and Biophysical Methods*, 69, 25–32.

Murkovic, M., & Bornik, M. A. (2007). Formation of 5-Hydroxymethyl-2-furfural (HMF) and 5-Hydroxymethyl-2-furoic Acid During Roasting of Coffee. *Molecular Nutrition & Food Research*, 51, 390–394.

Navarro, M., & Morales, F. J. (2017). Effect of Hydroxytyrosol and Olive Leaf Extract on 1,2-Dicarbonyl Compounds, Hydroxymethylfurfural and Advanced Glycation Endproducts in a Biscuit Model. *Food Chemistry*, 217, 602–609.

Oral, R. A., Dogan, M., & Sarioglu, K. (2014). Effects of Certain Polyphenols and Extracts on Furans and Acrylamide Formation in Model System, and Total Furans During Storage. *Food Chemistry*, 142, 423–429.

Perez Locas, C., & Yaylayan, V. A. (2008). 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.

Petisca, C., Pérez-Palacios, T., Farah, A., Pinho, O., & Ferreira, I. M. P. L. V. O. (2013). Furans and Other Volatile Compounds in Ground Roasted and Espresso Coffee Using Headspace Solid-Phase Microextraction: Effect of Roasting Speed. *Food and Bioproducts Processing*, 91, 233–241.

Sachse, B., Meinl, W., Sommer, Y., Glatt, H., Seidel, A., & Monien B. H. (2016). Bioactivation of Food Genotoxicants 5-Hydroxymethylfurfural and Furfuryl Alcohol by Sulfotransferases from Human, Mouse and Rat: A Comparative Study. *Archives of Toxicology*, 90, 137–148.

Shin, D. C., Kim C. T., Lee, Y. C., Choi, W. J., Na, Y. J., & Lee, K. W. (2010). Reduction of Acrylamide by Taurine in Aqueous and Potato Chip Model Systems. *Food Research International*, 43, 1356–1360.

Totlani V. M., & Peterson, D. G. (2007). Influence of Epicatechin Reactions on the Mechanisms of Maillard Product Formation in Low Moisture Model Systems. *Journal of Agricultural and Food Chemistry*, 55, 414–420.

Varelis, P., & Hucker, B. (2011). Thermal Decarboxylation of 2-Furoic Acid and Its Implication for the Formation of Furan in Foods. *Food Chemistry*, 126, 1512–1513.

Wei, F., & Tanokura, M. (2015). Organic Compounds in Green Coffee Beans. In V. R. Preedy, *Coffee in Health and Disease Prevention* (pp. 83–91). Academic Press, Elsevier Inc.

Wnorowski, A., & Yaylayan, V. A. (2000). Influence of Pyrolytic and Aqueous-Phase Reactions on the Mechanism of Formation of Maillard Products. *Journal of Agricultural and Food Chemistry*, 48, 3549–3554.

Yaylayan, V. A., Keyhani, A. (2000). Origin of Carbohydrate Degradation Products in L-Alanine/D-[13C]Glucose Model Systems. *Journal of Agricultural and Food Chemistry*, 48, 2415–2419.

Zheng, L. W., Chung, H., & Kim, Y. S. (2015). Effects of Dicarbonyl Trapping Agents, Antioxidants, and Reducing Agents on the Formation of Furan and Other Volatile Components in Canned-Coffee Model Systems. *Food Research International*, 75, 328–336.

3. Conclusion

The following conclusions can be drawn from the results presented in this dissertation and their subsequent discussion in the respective research manuscripts:

- The developed HPLC-DAD method offered a validated fast and selective method for the simultaneous analysis of FFA, 2-FA, HMF and HMFA in coffee extracts and brews in under 15 minutes with no disturbing co-elution.
- No differences were observed from various extraction techniques applied for the extraction of FFA, 2-FA, HMF and HMFA from coffee grounds. Water exhibited optimal extraction performance, while methanol was not appropriate for this task.
- Under similar roasting conditions, coffee produced more FFA than other seeds, beans, or cereal grains. FFA formation kinetics depended on the roasting conditions applied to coffee, where a maximum is reached quickly after onset of roasting followed by a constant decrease with prolonged roasting probably due to evaporation (up to 57% of total FFA produced from coffee were retrieved from the volatile part of the roasted system). The roasting of Arabica and Robusta species resulted in different levels of FFA (except at the maximum time point). The residual water content in coffee did not bring a significant different to the levels of FFA produced.

- FFA in ground coffee was found to be stable through 8 weeks of storage independently of the temperature during storage. Coffee brewing through boiling introduced significant reduction to the level of FFA in the ready-to-drink cup compared to filter drip and espresso, whereas espresso brewing was the most efficient method to clean-up FFA from ground coffee. Also, mimicking coffee ingestion by the incubation of coffee brews with simulated gastrointestinal fluids (gastric or intestinal) did not affect the levels of FFA.

- The comparison between the level of exposure to FFA, 2-FA, HMF and HMFA from coffee brews, pointed out clearly that FFA is the predominant furan derivatives in coffee brew, followed by HMF (almost 50% of the concentration of FFA), while 2-FA or HMFA were found in concentrations no more than 23% of FFA's in the same brew. It is also obvious that a wide variation in the concentration (up to 22-fold) of these furan derivatives can be obtained from different coffee samples and different brewing methods.

- The mitigation of FFA, 2-FA, HMF, and HMFA in a sucrose-alanine and a sucrose-alanine-microcrystalline cellulose model system revealed that polyphenols (e.g. derivatives of hydroxyl-cinnamates and hydroxyl-benzoates) as well as other non-phenolic antioxidants (e.g. quinic acid and EDTA) had the greatest mitigation capacity when added to the model systems, whereas flavonoids and sodium sulfite presented a moderate mitigation capacity. The number and availability of phenol groups of polyphenols were important parameters affecting their mitigation

capacity. Microcrystalline cellulose lowered the mitigation capacity of all tested agents compared to the microcrystalline cellulose-free model system.

- Mitigation agents were incorporated in a coffee model system before a similar roasting process in order to verify the mitigation activity observed in the other simplified model systems. An analogous mitigation trend was observed (except for 2-FA) between coffee and chemical model systems, where taurine was the most effective agent for FFA mitigation and sodium sulfite the most effective one for the mitigation of 2-FA and HMF, suggesting that sucrose and free amino acids play an important role in the formation of furan derivatives from coffee. A minor mitigation capacity on all furan derivatives was shown by other mitigation agents such as catechin and EDTA. Matrix interferences are believed to be the reason leading to the minimized mitigation capacity of all agents tested.

- The furan derivative-specific mitigation of some tested agents inferred that each furan derivative is formed by different pathways or from different intermediates.

4. References

Anese M, Suman M. (2013). Mitigation strategies of furan and 5-hydroxymethylfurfural in food. *Food Research International*. 51: 257–264.

Anese M. (2015). Furan and other furanic compounds in coffee: Occurrence, mitigation strategies, and importance of processing. In: Preedy VR. (editor), *Processing and Impact on Active Components in Food*. Academic Press, Elsevier Inc. pp. 541-547.

Bagdonaite K, Derler K, Murkovic M. (2008). Determination of acrylamide during roasting of coffee. *Journal of Agricultural and Food Chemistry*. 56: 6081-6086.

Brands CMJ, Van Boekel MAJS. (2001). Reactions of monosaccharides during heating of sugar-casein systems: Building of a reaction network Model. *Journal of Agricultural and Food Chemistry*. 49: 4667-4675.

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

Cämmerer B, Wedzicha BL, Kroh LW. (1999). Nonenzymatic browning reactions of retro-aldol degradation products of carbohydrates. *European Food Research and Technology*. 209: 261–265.

Chinnici F, Masino F, Antonelli A. (2003). Determination of furanic compounds in traditional balsamic vinegars by ion-exclusion liquid

chromatography and diode-array detection. *Journal of Chromatographic Science*. 41: 305-310.

Constantinou C, Koutsidis G. (2016). Investigations on the effect of antioxidant type and concentration and model system matrix on acrylamide formation in model Maillard reaction systems. *Food Chemistry*. 197: 769–775.

Curtis TY, Postles J, Halford NG. (2014). Reducing the potential for processing contaminant formation in cereal products. *Journal of Cereal Science*. 59: 382-392.

Ding WX. (2014). Drinking coffee burns hepatic fat by inducing lipophagy coupled with mitochondrial β-oxidation. *Hepatology*. 59: 1235-1238.

Edris AE, Murkovic M, Siegmund B. (2007). Application of headspace-solid-phase microextraction and HPLC for the analysis of the aroma volatile components of treacle and determination of its content of 5-hydroxymethylfurfural (HMF). *Food Chemistry*. 104: 1310-1314.

Farah A. (2012). Coffee constituents. In: Chu YF. (editor), *Coffee: Emerging health effects and disease prevention*. John Wiley & Sons Inc. pp. 21-58.

Franca AS, Oliveira LS, Oliveira RCS, Agresti PCM, Augusti R. (2009). A preliminary evaluation of the effect of processing temperature on coffee roasting degree assessment. *Journal of Food Engineering*. 92: 345-352.

Garg SK. (2016). Green Coffee Bean. In: Gupta RC. (editor), *Nutraceuticals*. Academic Press. Elsevier Inc. pp. 653-667.

Glatt H, Schneider H, Murkovic M, Monien BH, Meinl W. (2012). Hydroxymethyl-substituted furans: Mutagenicity in *Salmonella typhimurium* strains engineered for expression of various human and rodent sulphotransferases. *Mutagenesis*. 27: 41-48.

Høie AH, Monien BH, Sakhi AK, Glatt H, Hjertholm H, Husøy T. (2015). Formation of DNA adducts in wild-type and transgenic mice expressing human sulfotransferases 1A1 and 1A2 after oral exposure to furfuryl alcohol. *Mutagenesis*. 30: 643-649.

Hu G, Hernandez M, Zhu H, Shao S. (2013). An efficient method for the determination of furan derivatives in apple cider and wine by solid phase extraction and high performance liquid chromatography diode array detector. *Journal of Chromatography A*. 1284: 100-106.

[JECFA] Joint FAO/WHO Expert Committee on Food Additives. (2001). 55th meeting: Evaluation of certain food additives and contaminants. *WHO Technical Report Series.* 91.

[JECFA] Joint FAO/WHO Expert Committee on Food Additives. (2012). 76th meeting: Safety evaluation of certain food additives. *WHO Food Additives Series.* 67.

Martins SIFS, Jongen WMF, Van Boekel MAJS. (2001). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology*. 11: 364–373.

Mesias M, Morales FJ. (2015). Analysis of furan in coffee. In Preedy VR. (editor*), Coffee in Health and Disease Prevention*. Academic Press, Elsevier Inc. pp. 1005-1012.

Moon JK, Shibamoto T. (2009). Role of roasting conditions in the profile of volatile flavor chemicals formed from coffee beans. *Journal of Agricultural and Food Chemistry*. 57: 5823-5831.

Moon JK, Shibamoto T. (2010). Formation of volatile chemicals from thermal degradation of less volatile coffee components: Quinic acid, caffeic acid, and chlorogenic acid. *Journal of Agricultural and Food Chemistry*. 58: 5465–5470.

Monien BH, Herrmann K, Florian S, Glatt H. (2011). Metabolic activation of furfuryl alcohol: Formation of 2-methylfuranyl DNA adducts in *Salmonella typhimurium* strains expressing human sulfotransferase 1A1 and in FVB/N mice. *Carcinogenesis*. 32: 1533-1539.

Murkovic M, Bornik MA. (2007). Formation of 5-hydroxymethyl-2-furfural (HMF) and 5-hydroxymethyl-2-furoic Acid during roasting of coffee. *Molecular Nutrition & Food Research*. 51: 390-394.

Murkovic M, Derler K. (2006). Analysis of amino acids and carbohydrates in green coffee. *Journal of Biochemical and Biophysical Methods*. 69: 25–32.

Murkovic M, Pichler N. (2006). Analysis of 5-hydroxymethylfurfual in coffee, dried fruits and urine. *Molecular Nutrition & Food Research*. 50: 842-846.

Murkovic M, Swasti YR. (2013). 5-Hydroxymethyl-furfural and furfuryl alcohol: Occurrence, exposure and detection. In: Plasencia FP, Ciesarova Z. (editors), *Chemical Food Safety and Health*. Nova Science Publishers. pp. 43-55.

Navarro M, Morales FJ. (2017). Effect of hydroxytyrosol and olive leaf extract on 1,2-dicarbonyl compounds, hydroxymethylfurfural and advanced glycation endproducts in a biscuit model. *Food Chemistry*. 217: 602–609.

Okaru AO, Lachenmeier DW. (2017). The food and beverage occurrence of furfuryl alcohol and myrcene - two emerging potential human carcinogens? *Toxics*. 5, 9.

Oral RA, Dogan M, Sarioglu K. (2014). Effects of certain polyphenols and extracts on furans and acrylamide formation in model system, and total furans during storage. *Food Chemistry*. 142: 423–429.

Palmers S, Grauwet T, Celus M, Wibowo S, Kebede BT, Hendrickx ME, Van Loey A. (2015). A kinetic study of furan formation during storage of shelf-stable fruit juices. *Journal of Food Engineering*. 165: 74-81.

Perez Locas C, Yaylayan VA. (2008). 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.

Petisca C, Pérez-Palacios T, Farah A, Pinho O, Ferreira IMPLVO. (2013). Furans and other volatile compounds in ground roasted and espresso coffee using headspace solid-phase microextraction: Effect of roasting speed. *Food and Bioproducts Processing*. 91: 233-241.

Pérez-Palacios T, Petisca C, Melo A, Ferreira IMPLVO. (2012). Quantification of furanic compounds in coated deep-fried products simulating normal preparation and consumption: Optimisation of

HS-SPME analytical conditions by response surface methodology. *Food Chemistry*. 135: 1337–1343.

Poisson L, Blank I, Dunkel A, Hofmann T. (2017). The chemistry of roasting-decoding flavor formation. In: Folmer B. (editor), *The Craft and Science of Coffee*. Academic Press. Elsevier Inc. pp. 273-309.

Rodrigues NP, Bragagnolo N. (2013). Identification and quantification of bioactive compounds in coffee brews by HPLC–DAD–MS^n. *Journal of Food Composition and Analysis*. 32: 105-115.

Sachse B, Meinl W, Sommer Y, Glatt H, Seidel A, Monien BH. (2016). Bioactivation of food genotoxicants 5-hydroxymethylfurfural and furfuryl alcohol by sulfotransferases from human, mouse and rat: A comparative study. *Archives of Toxicology*. 90: 137-148.

Silva P, Silva CL, Perestrelo R, Nunes FM, Câmara JS. (2017). A useful strategy based on chromatographic data combined with quality-by-design approach for food analysis applications: The case study of furanic derivatives in sugarcane honey. *Journal of Chromatography A*. 1520: 117-126.

Totlani VM, Peterson DG. (2007). Influence of epicatechin reactions on the mechanisms of Maillard product formation in low moisture model systems. *Journal of Agricultural and Food Chemistry*. 55: 414-420.

Varelis P, Hucker B. (2011). Thermal decarboxylation of 2-furoic acid and its implication for the formation of furan in foods. *Food Chemistry*. 126: 1512–1513.

Wei F, Tanokura M. (2015a). Chemical changes in the components of coffee beans during roasting. In: Preedy VR. (editor), *Coffee in Health and Disease Prevention*. Academic Press, Elsevier Inc. pp. 83-91.

Wei F, Tanokura M. (2015b). Organic compounds in green coffee beans. In: Preedy VR. (editor), *Coffee in Health and Disease Prevention*. Academic Press, Elsevier Inc. pp. 149-162.

Wnorowski A, Yaylayan VA. (2000). Influence of pyrolytic and aqueous-phase reactions on the mechanism of formation of Maillard products. *Journal of Agricultural and Food Chemistry*. 48: 3549-3554.

Yaylayan VA, Keyhani A. (2000). Origin of carbohydrate degradation products in L-alanine/D-[^{13}C]glucose model systems. *Journal of Agricultural and Food Chemistry*. 48: 2415-2419.

Zheng LW, Chung H, Kim YS. (2015). Effects of dicarbonyl trapping agents, antioxidants, and reducing agents on the formation of furan and other volatile components in canned-coffee model systems. *Food Research International*. 75: 328–336.

5. Curriculum Vitae

Personal information:

- Name: Abdullatif Albouchi
- Place of Birth: Kuwait City, State of Kuwait.
- Date of Birth: 22nd October 1985
- Nationality: Syrian
- Address: Pflanzengasse 7, 8020 Graz, Austria.
- Email: abdullatifl.albouchi@outlook.de

Education:

- 2015 – 2018: PhD student in Functional Food Group

  Institute of Biochemistry, Graz University of Technology

- 2008 – 2014: Master Degree in Pharmaceutical Sciences with specialization in Pharmaceutical Quality Control

  Department of Pharmaceutical Chemistry and Quality Control, Faculty of Pharmacy, Damascus University, Damascus, Syria.

- 2003 – 2008: Bachelor Degree in Pharmacy and Pharmaceutical Chemistry,

  Faculty of Pharmacy, Damascus University, Damascus, Syria.

Fellowship:

- Erasmus Mundus Avempace Plus full degree scholarship (2015 – 2018).

6. National and international participations

Oral presentations:

- 'Furfuryl Alcohol Formation during Roasting of Coffee', Chemical Reactions in Foods VIII, Prague, Czech Republic, 15-17 Feb. 2017.
- 'Furfuryl alcohol Formation during Coffee Roasting', Young AGErs Symposium II, Berlin, Germany, 24-25 Aug. 2017.
- 'Mitigation of Furfuryl Alcohol in Coffee and Related Model Systems', Euro Food Chem XIX, Budapest, Hungary, 4-6 Oct. 2017.
- 'Mitigation of Furan Derivatives in Coffee and Related Model Systems', 19th Joint Scientific Meeting of the NAWI Graz Doctoral Schools of Molecular Biosciences and Biotechnology & Molecular Biology and Biochemistry, 9 Feb. 2018, Graz, Austria.
- 'Mitigation of Furan Derivatives in Coffee and Related Model Systems', Österreichische Lebensmittelchemikertage, Seggauberg, Austria, 25-27 Apr. 2018.

Posters:

- 'Effect of Preservation Methods on the Levels of Furfuryl Alcohol in Coffee', Österreichische Lebensmittelchemikertage, St. Pölten, Austria, 8-10 Jun. 2016.
- 'The Effect of Coffee Roasting, Brewing and Human Gastrointestinal Fluids on Levels of Furfuryl Alcohol', 18th Joint Scientific Meeting of the NAWI Graz Doctoral Schools of Molecular Biosciences and Biotechnology & Molecular Biology and Biochemistry, Graz, Austria, 10 Jul. 2017.