Graz University of Technology

DISSERTATION

Furan Derivatives: Its Occurrence in Foods, Contribution to Melanoidin Formation, Metabolism

Dissertation zur Erlangung des akademischen Grades Doktorin der Naturwissenschaften (Dr.rer.nat.) an der Fakultät für Technische Chemie, Verfahrentechnik und Biotechnologie der Technischen Universität Graz

vorgelegt von
Yuliana Reni Swasti
Geboren am 06.09.1980 in Sleman/Indonesien

Graz, im August 2012

Deutsche Fassung:
Beschluss der Curricula-Kommission für Bachelor-, Master- und Diplomstudien vom 10.11.2008
Genehmigung des Senates am 1.12.2008

STATUTORY DECLARATION

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 marked all material which has been quoted either literally or by content from the used sources.

Graz, am 23.08.2012 ……
(signature)

CONTENTS

Section 2: Characterisation of the Polymerization of Furfuryl Alcohol during Roasting of Coffee

Section 3: Metabolites of Furan Derivatives in Human Urine

ACKNOWLEDGEMENTS

During my PhD study in Functional Food Group, Institute of Biochemistry, Graz University of Technology, several people supported me to complete my dissertation. Therefore, I would like to express my deep gratitude to:

1. My supervisor - Ao.Univ.-Prof. Dipl.-Ing. Dr.techn. Michael Murkovic – who has given me the opportunity to do doctoral research in his group. I am also deeply grateful for his instruction with passion in dealing with the instruments in his laboratory, for his ideas during my study and for his invitation of sightseeing during my stay in Graz. Last but not least, I thank him for his tasty cooking and his attention for my family in Indonesia.
2. Asea uninet for awarding scholarship for my doctoral study in Austria
3. Ao.Univ.-Prof. Dipl.-Ing. Dr.techn. Erich Leitner for the GC-MS analysis.
4. Ao.Univ.-Prof. Dipl.-Ing. Dr.techn. Georg Gübitz, Ing. Angelika Schaefer, Dr.techn. M.Eng. Endry Nugroho Prasetyo for giving me opportunity to do analysis with the Agilent Ion Trap SL.
5. Ph.D Univ.-Doz. Guo Xinghua for his instruction to do analysis with the AB Sciex Q Trap LC/MS/MS.
6. Dr. Trine Husøy from the Institute of Public Health in Oslo/Norway for providing the food samples and for fruitful discussions on the metabolism of the furan derivatives.
7. Univ.-Prof. Dr. Kevin Francesconi for his willingness to be my second expert referee.

8. Prof. Dr. Rainer Bischoff, Dr. Peter Horvatovich, and Fari Pratomosiwi, ST MT for helping with the matlab.
9. Alma and all master students (Tatjana, Baha, Vanessa, Barbara, Tanja, Wolfgang, Patricia) in Functional Food Group Institute of Biochemistry TU Graz for creating comfortable atmosphere in the working place.
10. My mother (Valeria Nani Suwarni), my father (Robertus Bambang Sutopo), my sister (Florentina Yuni Arini) for their prayer and support during my study.
11. Father Rafael Tri Wijayanto, Pr for his prayer and support during my study.
12. Martinus Ariya Seta for his prayer, support and patient throughout my study.
13. Indonesien community in Graz for creating home-like atmosphere.

SUMMARY

Furan could be formed by heating of L–ascorbic acid, amino acids, reducing sugars, and fatty acids. Nevertheless, the mechanism of formation of furan derivatives differs among each other but all are formed by heating of one or two of the precursors. Furan and its derivatives give a positive benefit to the sensory properties of heated food but also have toxic and in some cases mutagenic effects. Moreover, the polymerization of furfuryl alcohol as furan derivative contributes to the formation of the brown colour in heated foods, besides Maillard and caramelization reactions. During heating of food, furfuryl alcohol is formed through degradation of quinic acid or 1,2–enediols. Furfuryl alcohol is a mutagenic compound. In acid conditions it is able to polymerize and form aliphatic polymers that show a brown colour. In addition, some of those furans still remain in the liver or kidney which can be metabolized forming toxic or mutagenic compounds that bind to proteins or DNA. In this research project it was shown that the HPLC using gradient elution with methanol and water can be used for the identification and quantification of HMF, furfuryl alcohol, and furfural in a single run. Instant coffee powder, ready–to–drink filter coffee, and cappuccino contain 5–hydroxymethylfurfural (HMF), furfuryl alcohol, and furfural. Dried plums and raisins also contain HMF and furfural. Crisp bread contains furfuryl alcohol and HMF. Besides that, goat cheese contains furfuryl alcohol and cola beverage contains HMF. The analysed samples were provided by the Norwegian

Institute of Public Health investigating the exposure to these substances in Norway.

Furthermore, here we show that furfuryl alcohol polymerizes in a model system by incubation in 1 M HCl at room temperature. Some of the reaction products are oligomers with dimers, trimers, tetramers, and pentamers having a methylene linkage being identified. The degree of polymerization and the amount of those furfuryl alcohol oligomers increases with increasing reaction time. The results of this model system were used to characterize the polymerization of furfuryl alcohol during roasting of coffee. The coffee was roasted at 210 °C for 2, 3, 4, 5, and 6 min using a home coffee roaster. Furfuryl alcohol and its dimer were found in coffee after 2 and 3 min of roasting reaching a maximum amount after 4 min; probably due to further reactions the dimeric furfuryl alcohol concentration starts to decrease after 4 min. We propose that the polymers of furfuryl alcohol contribute to the brown colour of roasted foods. In urine, the 5–hydroxymethyl–2–furoic acid as metabolite of HMF and 2–furoic acid as metabolite of furfuryl alcohol and furfural can be analysed by HPLC separation and 5-methyl furoic acid as metabolite of 5-methylfurfural and 5-methyl furfuryl alcohol only can be analysed by LC/MS/MS after alkaline treatment to hydrolyse the glycine conjugates.

ZUSAMMENFASSUNG

Furan kann durch Erhitzen von L-Ascorbinsäure, Aminosäuren, reduzierenden Zuckern und Fettsäuren gebildet werden. Die Bildung von Derivaten des Furans bei der Erhitzung von Lebensmitteln folgt jedoch anderen Mechanismen. Furan und dessen Derivate wirken sich positiv auf die sensorischen Eigenschaften von erhitzten Lebensmitteln aus; sie zeigen jedoch auch eine toxische und mutagene Wirkung. Darüber hinaus trägt die Polymerisation von Furfurylalkohol als Furan-Derivat zur Bildung der braunen Farbe in erhitzten Lebensmitteln neben der Maillard Reaktion und Karamelisierung bei. Beim Erhitzen von Lebensmitteln, wird Furfurylalkohol durch den Abbau von Chinasäure beziehungsweise von 1,2-Endiolen gebildet. Insbesondere kann Furfurylalkohol durch eine metabolitsche Aktivierung zu einer mutagenen Verbindung werden, die mit der DNS reagieren kann. In sauren Bedingungen ist er in der Lage zu aliphatischen Polymeren zu reagieren, welche eine braune Farbe zeigen können. In diesem Forschungsprojekt konnte durch den Einsatz der HPLC unter Verwendung einer Gradientenelution mit Methanol und Wasser HMF, Furfurylalkohol und Furfural in einem Lauf Identifiziert und Quantifiziert werden. Löskaffee, ready-to-drink Filterkaffee, sowie Cappuccino enthalten 5-Hydroxymethylfurfural (HMF), Furfurylalkohol und Furfural. Getrocknete Pflaumen und Rosinen enthalten ebenfalls HMF und Furfural. Knäckebrot enthält Furfurylalkohol und HMF. Daneben enthält Ziegenkäse Furfurylalkohol und ein Cola-Getränk HMF. Die Proben stammen aus einer Untersuchung des norwegischen Institutes of Public Health, bei der die Exposition zu diesen Verbindungen bestimmt werden soll.

Darüber hinaus zeigen wir hier, dass Furfurylalkohol in einem Modellsystem durch Inkubation in 1 M HCl bei Raumtemperatur polymerisiert. Einige der Reaktionsprodukte sind Dimere, Trimere, Tetramere und Pentamere mit Methylen-Brücken. Der Polymerisationsgrad und die Menge dieser gebildeten Furfurylalkohol-Oligomere nahmen mit der Reaktionszeit zu. Die Ergebnisse dieses Modellsystem wurden verwendet, um die Polymerisation von Furfurylalkohol beim Rösten von Kaffee zu charakterisieren. Dazu wurde Kaffee bei 210 °C für 2, 3, 4, 5 und 6 min mit einem Heim-Kaffeeröster geröstet. Furfurylalkohol und dessen Dimer wurden in nach 2 bzw. 3 min Rösten gefunden, wobei die maximale Konzentration nach 4 min erreicht wurde; danach sank die Konzentration des Dimers wieder ab, was wahrscheinlich auf eine weitere Reaktion der dimeren Furfurylalkohols zurückzuführen ist. Eine Umlagerung des Polymers führt in weiterer Folge zu einer Braunfärbung. Die Metabolisierung von HMF and Furfural in den Nieren führt zu 5-Hydroxymethyl-2-furancarbonsäure und 2-furancarbonsäure welche im Urin ausgeschieden werden und ebenfalls mit HPLC analysiert werden konnten. Die 5-Methyl-2-furancarbonsäure konnte nur mittels LC/MS/MS und nach alkalischer Hydrolyse – zur Spaltung der Glycin-Konjugate – bestimmt werden.

SECTION 1

FURAN DERIVATIVES IN FOOD PRODUCTS

I. INTRODUCTION

Furan could be formed by heating L–ascorbic acid, amino acids, reducing sugar, fatty acids, and dehydroascorbic acid (Yaylayan, 2006; Becalski and Seaman, 2005). Nevertheless, the formation of furan derivatives differs among each other but it is formed by heating of one or two precursors at high temperature. The formation of 5–methylfurfural is an exception because it can be formed by degradation of 5–hyroxymethylfurfural (Nikolov and Yaylayan, 2011). Furan and its derivatives contribute to the sensory properties of heated foods and it easily metabolized in gastrointestinal tract and eliminated through urine. However, some of those furans and its derivatives still remain in the liver or kidneys and will be metabolized forming toxic or mutagenic substances that have the ability to bind to the DNA.

In this thesis, the methods for identification of furan and its derivatives are described. A fast and accurate method for identification and quantification in foods was developed and a series of foods were analysed. Here presented results form the basis for further evaluation of the exposure and toxicity of these compounds.

II. LITERATURE REVIEW

2.1. Furan

Furans are formed by thermal degradation of carbohydrates and from the Maillard reaction during food processing. They are present in nearly all food aromas (Vermin, 1982). Alkylfurans usually possess sweet, burnt and pungent odours. If the substituents contain aldehydes, ketones or alcohols, they are usually burnt and caramellic (Fors, 1983).

The furan precusor is 3–deoxyglucosone (Wnorowski and Yaylayan, 2000). The amount of furan in brewed arabica coffee from Vietnam, India, Cameroun, Santos, Salvador, and Ethiopia depend on the type of brewing method. Coffee brewed in an espresso machine does not contain any furan (Pera et al., 2009).

Mostly, furan forms from heated L–ascorbic acid at 250 °C through oxidative and non oxidative reactions with aldotetrose and 2–deoxy aldotetrose as intermediate which undergo cyclization. In addition, furan can be formed from 2–furoic acid by decarboxylation; heated reducing sugars through retro–aldol cleavage followed by oxidation and reduction reaction that produced low amount of furan; heated alanine, threonine and aspatic acid only produced furan precursors, but heated serine and cysteine produced furan through aldol condensation (Yaylayan, 2006). Besides that, polyunsaturated fatty acids also produce furan through oxidative reactions (Perez Locas and Yaylayan, 2004) (Fig. 2.1.1). Furthermore, Becalski and Seaman (2005) found that furan is formed by

heating ascorbic acid derivatives (e.g. dehydroascorbic acid) heated to 118 °C produces more furan than ascorbic acid itself. Limacher et al. (2008) published that the formation of furan mostly due to the moiety of C3–C6 glucose and C3–C6 moiety of fructose and also C2–C5 moiety of fructose; While C2 – C5 and C1 – C4 moiety of glucose and also C1 – C4 only give lesser contribution in furan formation. Furan also can be formed through interaction between glucose or fructose fragments or between reducing sugar fragments and amino acid fragments.

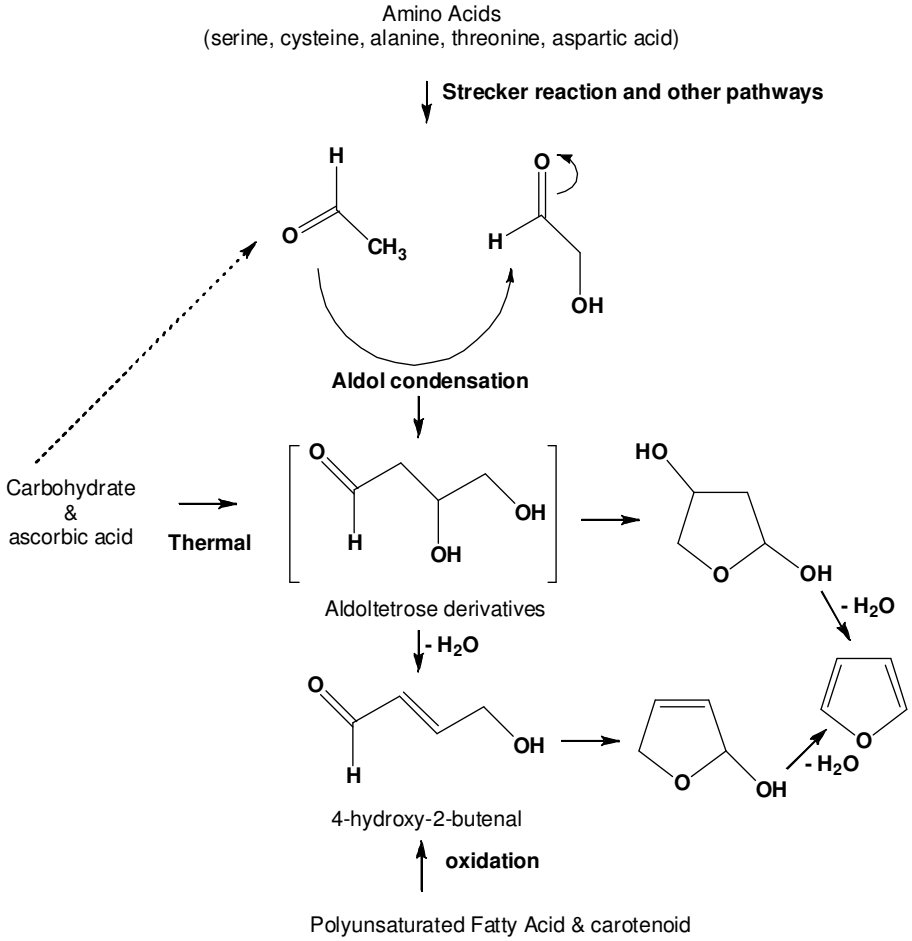


Figure 2.1.1. The formation of furan (Perez Locas & Yaylayan, 2004; Becalski & Seaman, 2005)

Ca. 80 % of furan taken up by foods can be eliminated through urine within 24 h (Burka et al., 1991). Lee et al. (1994) found that furan is mutagenic in the strain of *S. typhimurium* TA 100. Moreover, Peterson et al. (2000) found that the *Z*–2–butene–1,4–dial – a metabolite of furan – is mutagenic in *S. typhimurium* (TA 104) at non–toxic concentrations. *Z*–2–butene–1,4–dial is formed after furan is metabolized by cytochrome P–450 which opens the furan ring (Chen et al., 1995; Ravindrananth et al., 1984).

2.2. 5–Hydroxymethylfurfural (HMF)

Fructose heated to 250 °C produces higher amount of HMF than glucose because the cyclic structure of fructose (frucfuranosyl cation) can readily be dehydrated to form HMF; on the other hand, glucose has to be transformed first to produce open ring structure of glucose. However, the production of HMF from sucrose is higher than from glucose and fructose because at high temperatures the glycosidic bond is cleaved by forming a fructofuranosyl cation and glucose (Perez Locas and Yaylayan, 2008) (Fig. 2.2.1). The average exposure to HMF from coffee consumption is 5.26 mg/d with a coffee consumption of e.g. 8.57 g/capita/d in Spain (Arribas–Lorenzo and Morales, 2010).

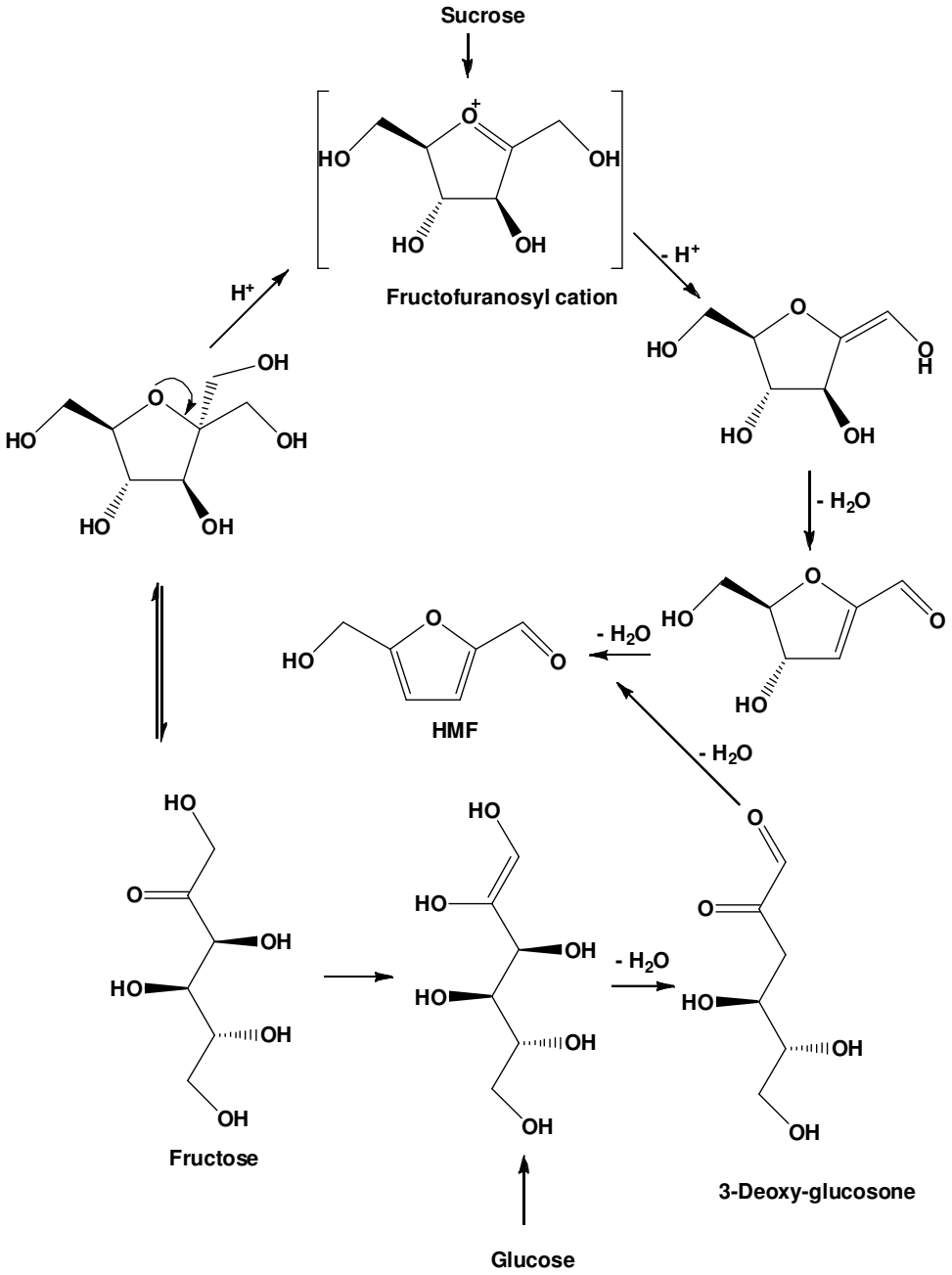


Figure 2.2.1. The Formation of HMF (Perez Locas & Yaylayan, 2008)

5–Hydroxymetylfurfural can undergo polymerisation producing a dimer during heating in absence of glycine through vinylogous aldol addition (Nikolov

and Yaylayan, 2011); dimeric HMF was also found to be part of the building block melanoidin in glucose system (Guan et al., 2011). The presence of glycine not only prevents the formation of dimeric HMF but also prevents the formation of HMF and 5–methylfurfural (Nikolov and Yaylayan, 2011). Fallico et al. (2008) found that HMF in chestnut honey treated at 50 °C for 8 days undergoes degradation; the degradation is faster at 50 °C than at 25 °C. Analysis of HMF using isocratic elution with mobile phase 80 % acetic acid (0.2 %, pH 3) and 20 % methanol in HPLC give a higher response than it was analysed using NMR (Campo et al., 2010).

HMF is easily metabolized in the gastrointestinal tract and 60 – 80 % is excreted renally during 48 h after administration (Godfrey et al., 2000). Gremond et al. (1987) found that HMF can be eliminated in the range of 95 – 100 % from the administered dose through urine in 24 h as 5–hydroxymethyl–2–furoic acid and N–(5–hydroxymethyl–2–furoyl)–glycine. He also found that shortly after administration, HMF is found in liver, but mostly in kidney and bladder. Under this condition, kidneys have a higher risk toward reactive metabolites of HMF (5–sulfooxymethyl)–2–furfural or SMF activity than liver. Bahkiya et al. (2009) found that the organic anion transporters (OATs) contributed to the SMF transportation into kidneys and the toxicity of SMF to renal proximal tubule cells leading to animal death at single dose 250 mg/kg. Nevertheless, Durling et al. (2009) found that DNA damage caused by HMF is not related to sulfotransferase activity and the ability of HMF to cause DNA damage is weak.

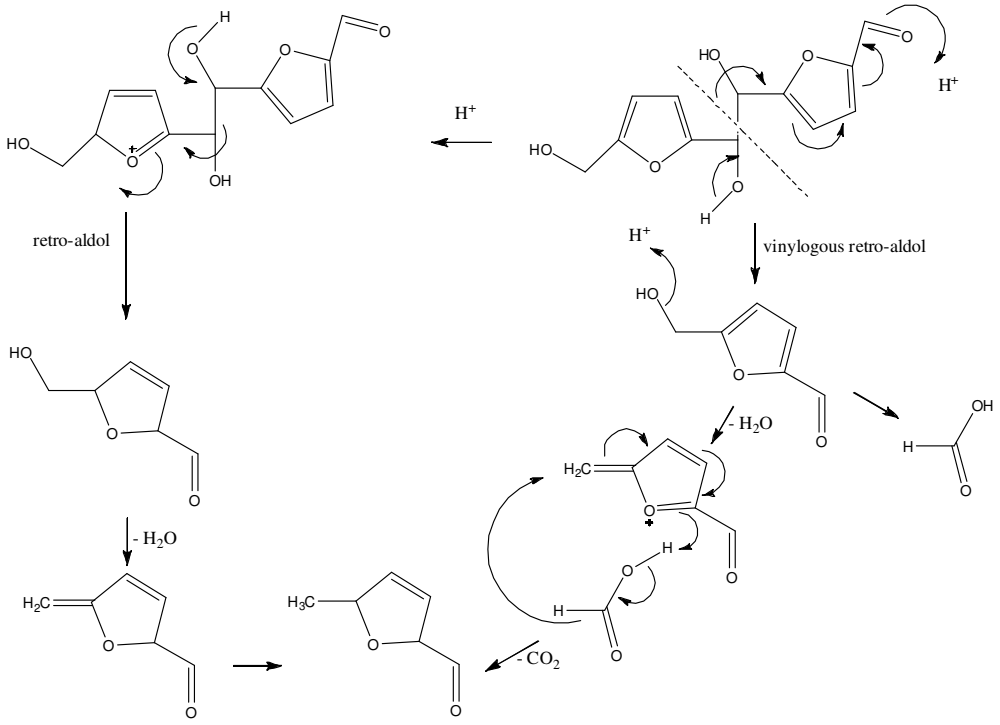
Janzowski et al. (2000) HMF causes genotoxic and mutagenic effects in vitro at high mmol concentration. HMF causes hepato–celular adenoma in mice which were treated with 188 and 375 mg HMF/kg for 3 months (NTP, 2008). Monien et al. (2009) found that the sulfuric ester of the HMF, 5–sulfoxymethylfurfural (SMF), is more carcinogenic than HMF. SMF can react with DNA or proteins where it is generated. In humans sulfuric esters are formed by sulfotransferases that are present in liver and also extrahepatic (Janzowski et al. 2000); human sulfotransferases transforming HMF are more active than murine enzymes (Glatt and Sommer, 2006). SMF also found in the blood of mice which were exposed to HMF (Glatt and Sommer, 2006). Nevertheless, 80 – 100 mg/kg bw/d of HMF does not give adverse reactions in several animals (Abraham et al., 2011). Ulbricht et al. (1984) claim that 10 mg HMF/l is not acute toxic in rats and dogs by parenteral administration of sterilized solution containing hexose, whereas 75 mg HMF/l are severe toxic.

2.3. Furfural

The reaction between furfural and lysine at high temperatures produces a yellow pigment, furpipate, in abundance (Murata et al., 2007). Hofmann (1998) found that interaction of furfural and L–lysine leads to ring opening of the furfural with a subsequent condensation of three furfural molecules producing a red colour. Hofmann et al. (1999) found that furfural has a higher ability to induce brown colour than HMF.

2.4. 5–Methylfurfural

5–Methylfurfural formed from degradation of dimeric HMF at high temperatures without the presence of amino acids through retro–aldol reaction after protonation or through a vinylogous retro–aldol reaction (Nikolov and Yaylayan, 2011) (Fig. 2.4.1).



2.4.1. The formation of 5–Methylfurfural (Nikolov & Yaylayan, 2011)

2.5. 2–Methylfuran

2–Methylfuran (2–MF) is formed from amino acids (alanine, threonine) which undergo a Strecker reaction and subsequently aldol condensation. This furan derivative is also formed from linolenic acid which undergoes heat treatment at high temperatures (Märk et al., 2006) (Fig. 2.5.1). Limacher et al.

(2008) said that the formation of 2–MF is formed through cyclization and dehydration of D–glucose and also by Strecker degradation followed by cyclization and dehydration of 2,5-dideoxypentose under roasting conditions; D–glucose contributes less than Strecker aldehydes to the formation of 2–MF.

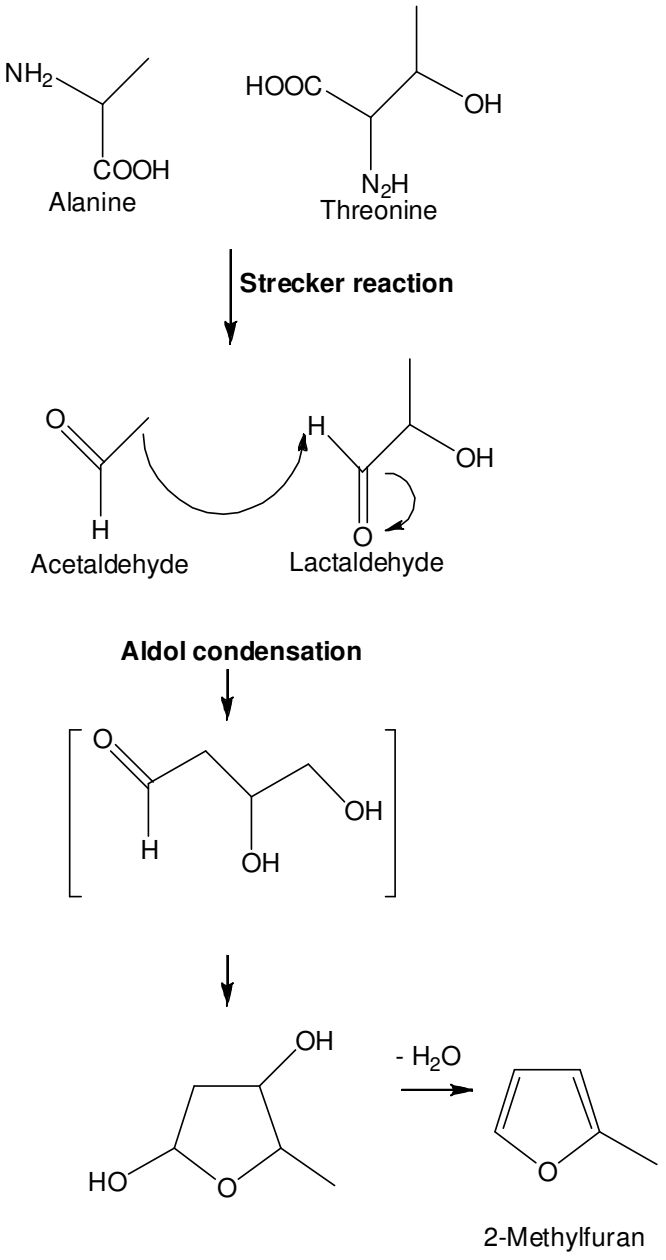


Figure 2.5.1. The formation of 2–methylfuran (Märk et al., 2006)

The formation of 2–methylfuran is lower than the amount of furan when reducing sugar or amino acid are heated to 200 °C for 10 min. However, the amount of 2–MF will increase drastically (higher than the amount of furan) if the system contains amino acids and reducing sugars. In the pressure cooking at pH 7 and pH 4, the amount of furan and 2–MF are lower than in 200 °C; at pH 4 the amount of those two furan derivatives are lower than at pH 7 (Limacher et al., 2008). 2–MF causes liver, lung, and kidney necrosis in rodents by binding covalently to microsomal proteins (Ravindranath and Boyd, 1985).

2.6. Furan derivatives in various food products

Food products	**Furan derivatives**
	HMF (ppm)
Honey Rosemary	20.9 ± 2.7[a]
Honey Eucalyptus	3.3 ± 0.6[a]
Breakfast cereals honey rings	41.0 ± 3.4[a]
Breakfast cereals puff rice	12.6 ± 1.9[a]
Breakfast cereals Puffed corn	36.6 ± 8.9[a]
Breakfast cereals Bran flakes	46.2 ± 8.5[a]
Orange juice	10.6 ± 1.7[a]
Apple juice	2.9 ± 0.8[a]
Biscuits honey biscuits	6.0 ± 0.9[a]
Toasted biscuits	3.8 ± 1.2[a]
Bran biscuits	5.1 ± 1.3[a]
Plum jam	12.3 ± 1.7[a]
Raspberry jam	15.8 ± 0.9[a]
Peach jam	2.7 ± 0.8[a]
Orange marmalade	47.1 ± 8.0[a]
Strawberry jam	15.9 ± 1.6[a]
Instant coffee	1093 ± 33[a]
Instant decaffeinated coffee	983 ± 25[a]
Chocolate with milk A	87.4 ± 10.1[a]
Chocolate dark A	42.1 ± 9.3[a]
Chocolate white	98.9 ± 13.5[a]
Grilled pork loin	0.66 db[b]
Rice stew	3.57 db[b]
Paella	21.0 db[b]
Boiled potatoes	0.04 db[b]
Fried potatoes	1.06 db[b]
Fried anchovies	0.71 db[b]
Breakfast cereal	133 ± 10[c]
Fibre enriched breakfast cereal	63 ± 4[c]
White sugar	3.9[d]
Demerara sugar	83.5[d]
Caramel	1500[d]
Red balsamic vinegar	1.5[d]
White balsamic vinegar	3.9[d]
Infected apple	0.5[d]
Apricot juice	0.867 ± 0.16[e]
Cranberry juice	1.77 ± 0.187 [e]
Red grape juice	1.92 ± 0.0942[e]
Beer	0.698 ± 0.0289[e]
Peach juice	0.551 ± 0.0227[e]
Multivitamin tropical juice	1.95 ± 0.051[e]

Food product	**Furan derivatives HMF (ppm)**
Plum–lemon juice	5.98 ± 0.0358[e]
Caramel candy	1.33 ± 0.0471[e]
Butter snack	2.45 ± 0.0329[e]
Imkerei Gössler honey	2.61 ± 0.108[e]
Aceto Balsamico	59.1± 0.760[e]
Acid with raspberry aroma	4.9 ± 0.1 [e]
Pineapple Juice	3.28 ± 0.1 [e]
Malt Coffee	38.4 ± 1.03[e]
Honey	0.01[q]
	Furan (ppb)
Orange juice	0.121[f]
Apple juice	0.023[f]
Baby foods banana	0.066[f]
Baby foods three fruit	0.044[f]
Olive oil (Franti)	9.0 ± 0 .2[g]
Olive oil (Carapelli)	4.0 ± 0.6[g]
Corn oil	2.3 ± 0.3[g]
Palm oil	64.4 ± 1.5[g]
Freshly brewed coffee	19.2 ± 0.3[g]
Baby food ‘‘spinach’’	172.7 ± 8.0[g]
Baby food ‘‘beef and vegetables’’	83.3 ± 8.2[g]
Baby food ‘‘garden vegetables’’	75.1± 1.7[g]
Baby food ‘‘carrots’’	100.2 ± 5.1[g]
Brewed instant coffee	35.0 ± 2.0[h]
Multi–floral honey	4.8 ± 0.2[h]
Baby food chicken with rice	15.7 ± 1.3[h]
canned and jarred fruit juices	13.1[i]
canned and jarred apple sauce	11.1[i]
canned and jarred peaches	17.7[i]
canned and jarred pineapples	4.8[i]
canned and jarred mixed fruits	27.2[i]
canned and jarred asparagus	5.5[i]
canned and jarred beans	60.3[i]
canned and jarred beets	100.4[i]
canned and jarred carrots	43.9[i]
canned and jarred corn	36.1[i]
canned and jarred mushrooms	17.2[i]
canned and jarred peas	40.3[i]
canned and jarred potatoes	65.2[i]
canned and jarred tomatoes and pasta sauce	51.9[i]

Food products	**Furan derivatives Furan (ppb)**
canned and jarred other vegetables	9.6[i]
canned and jarred tomato/vegetable juices	8.5[i]
canned and jarred condiments and sauces	60.7[i]
canned and jarred soups with vegetables	60.1[i]
canned and jarred soups without vegetables (including chilli)	259.0[i]
canned and jarred baked beans	580.6[i]
canned and jarred pasta	396.4[i]
canned and jarred peanut butter	10.6[i]
canned and jarred luncheon meats	49.7[i]
canned and jarred salmon	13.4[i]
canned and jarred sardines	33.5[i]
canned and jarred shellfish	171.0[i]
canned and jarred tuna	21.8[i]
canned and jarred coffee (brewed)	30.4[i]
Coffee powder, 100% Arabica	918[j]
Coffee drink	86.7[j]
Ketchup (Czech Republic)	11.8[j]
Soya sauce (Slovak Republic)	122[j]
Canned beef goulash (Czech Republic)	78.2[j]
Canned sauce for pasta (Czech Republic)	59.2[j]
Canned pork goulash with sauerkraut (Slovak Republic)	36.8[j]
Canned baked beans with sausage (Czech Republic)	58.4[j]
Jarred mixed fruit jam	3.1[k]
Beers	1 – 9.1[l]
	Furan (ppm)
Baked beans	48 – 50[m]
Leek and potato soup	13 – 14[m]
Tomato soup	37 – 39[m]
Vegetable soup	42 – 45[m]
Baby beverages	2[n]
Pasta	35[n]
Baby food carrot	23.8[o]
Baby food carrot, potato, beetroot, rice and beans	77.4[o]
Baby food beef, carrot and potato	95.5[o]
Baby food beef, carrot and potato (pieces)	11.7[o]
Baby food beef, carrot, potato and pasta	10.7[o]
Baby food beef, carrot, potato and pasta (pieces)	17.8[o]
Baby food beef, carrot, potato and arracacha root	26.3[o]
Baby food beef, carrot, potato, rice and egg	24.8[o]

Food products	**Furan derivatives Furan (ppm)**
Baby food chicken, carrot, potato and pasta	34.5[o]
Baby food chicken, potato, spring greens and spinach	39.2[o]
Baby food chicken, carrot and potato (pieces)	20.0[o]
Baby food turkey, carrot, potato and arracacha root	12.8[o]
Baby food spaghetti with bolognese sauce	23.9[o]
Baby food beef stroganoff	44.8[o]
Baby food beef stew	28.2[o]
Baby food chicken risotto	32.6[o]
Baby food apple and prune	2.5[o]
Baby food papaya and orange juice	3.0[o]
Baby food banana and oat (pieces)	5.7[o]
Baby food banana and oat	< 2.4[o]
Baby food pear	< 2.4[o]
Baby food apple and orange juice	< 2.4[o]
	5–Methyfurfural
Baked sweet potato	0.9[p] (ppb)
Red balsamic vinegar	1.9[d] (ppm)
White balsamic vinegar	0.8[d] (ppm)
	2–Methylfuran (ppb)
Canned and jarred fruit juices	1.6[i]
Canned and jarred apple sauce	7.7[i]
Canned and jarred peaches	7.1[i]
Canned and jarred pineapples	2.9[i]
Canned and jarred mixed fruits	5.0[i]
Canned and jarred asparagus	2.5[i]
Canned and jarred beans	11.1[i]
Canned and jarred beets	9.0[i]
Canned and jarred carrots	8.9[i]
Canned and jarred corn	8.9[i]
Canned and jarred mushrooms	10.2[i]
Canned and jarred peas	18.2[i]
Canned and jarred potatoes	1.8[i]
Canned and jarred tomatoes and pasta sauce	24.8[i]
Canned and jarred other vegetables	13.3[i]
Canned and jarred tomato/vegetable juices	7.6[i]
Canned and jarred condiments and sauces	8.3[i]
Canned and jarred soups with vegetables	11.5[i]
Canned and jarred soups without vegetables (including chilli)	49.4[i]
canned and jarred baked beans	91.3[i]
Food products	**Furan derivatives 2–Methylfuran (ppb)**
canned and jarred pasta	41.9[i]

Food products	**Furan derivatives 2–Methylfuran (ppb)**
Canned and jarred luncheon meats	58.9[i]
Canned and jarred salmon	65.4[i]
Canned and jarred sardines	18.0[i]
Canned and jarred shellfish	149.0[i]
Canned and jarred tuna	24.8[i]
Canned and jarred coffee (brewed)	60.8[i]
	Furfural (ppm)
Honey	50.6[d]
Caramel	239.2[d]
Red balsamic vinegar	0.5[d]
White balsamic vinegar	0.4[d]
Apple juice (clear)	0.3[d]
Apple juice (cloudy)	0.2[d]
Infected apple	0.8[d]

[a] Teixidó et al., 2011
[b] Delgado–Andrade et al., 2010
[c] Rufian–Henares and Delgado–Andrade, 2010
[d] Gaspar and Lucena, 2009
[e] Golubkova, 2011
[f] Sarafraz–Yazdi et al., 2012
[g] Lancker et al., 2009
[h] Altaki et al. 2009
[i] Becalski et al., 2010
[j] Vranová et al. 2007
[k] Kim et al., 2010
[l] EFSA, 2009
[m] Roberts et al., 2008
[n] Lachenmeier et al., 2009
[o] Arisseto et al., 2010
[p] Wang and Kays, 2000
[q] Spano et al. 2009

III. MATERIALS AND METHODS

3.1. Materials

Furfuryl alcohol (FA) ≥99 % was purchased from Fluka (Sigma–Aldrich, Switzerland), methanol HPLC grade was purchased from Mallinckrodt Baker (The Netherlands), 5–(hydoxymethyl)furfural was purchased from Fluka Chemikals (Buchs, Switzerland), furfural was purchased from Roth (Karlsruhe, Germany), 5–methylfurfural, 2,5–dimethylfuran, 2–methylfuran were purchased from Sigma Aldrich (Steinheim, Germany), 5–methyl–2–furanmethanol purchased from ABCR (Karlshuhe, Germany), furan was purchased from Fluka Chemikals (Buchs, Switzerland). Food samples are provided by Norwegian Institute of Public Health,

3.2. Methods

0.3 g sample (n : 3) were mixed with 1 ml methanol and extracted with vortex genie 2 for 10 min at low speed and then centrifuged with 14.000 rpm at 4 °C for 10 min. The samples were then analysed with the LC Agilent 1100. LC conditions: LiChrospher 100 RP–18 (125 × 3 mm, 5 μm) as column from Agilent Technologies, 5 μl injection volume, 0.6 ml/min solvent flow rate, gradient elution until 15 min: 25 % MeOH, 75 % water, DAD at 228 nm for furfuryl alcohol analysis, 277 nm for furfural analysis, 284 nm for 5–hydroxy-methylfurfural analysis, and 293 nm for 5–methyl–furfural.

IV. RESULTS AND DISCUSSION

4.1. Furan Derivatives in Coffee

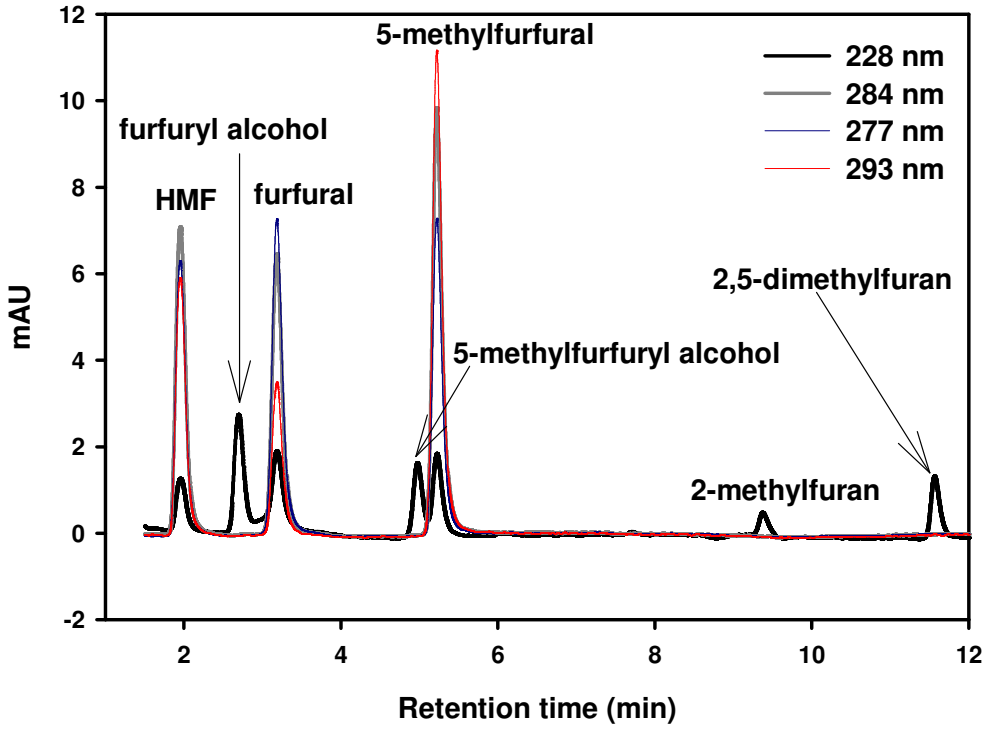


Figure 4.1.1. Chromatogram of gradient elution furan derivatives standard

Gradient elution in HPLC separation was used in this furan derivatives analysis; using this method we were able to separate HMF, furfuryl alcohol, furfural, 5–methyl furfuryl alcohol, 2–methylfuran, 5–methylfurfural, 2,5–dimethylfuran standard with different maximum absorbance in ultra violet range (Fig. 4.1.1). Nevertheless, only HMF, furfuryl alcohol, and furfural could be detected in the food samples due to the low concentration and volatility of other furan derivatives.

Table 4.1.1. HMF, fufural, and furfuryl alcohol content in coffee products

Coffee products	HMF (µg/g)	Furfural (µg/g)	Furfuryl Alcohol (µg/g)
Filtermalt instant coffee powder	7.17 ± 0.49	2.75 ± 0.18	13.0 ± 0.2
Instant coffee powder type 1	39.0 ± 1.4	–	2.05 ± 0.1
Instant coffee powder type 2	71.6 ± 2.9	–	5.53 ± 0.1
Ready–to–drink filter coffee type 1	4.87 ± 0.3	0.64 ± 0.1	4.53 ± 0.8
Ready–to–drink filter coffee type 2	2.02 ± 0.5	1.1 ± 0.4	4.31 ± 0.3
Ready–to–drink filter coffee type 3	3.74 ± 0.2	1.42 ± 0.2	16.8 ± 3.0
Double cappuccino	0.39 ± 0.03	0.26 ± 0.01	3.98 ± 0.6
Cappuccino ready–to–drink	1.39 ± 0.2	0.53 ± 0.2	1.79 ± 0.3

Most coffee products contained HMF, furfuryl alcohol, and furfural except 2 instant coffees powder that did not contain any furfural although its HMF content were the highest compared to other coffee products. High content of HMF in instant coffee was also reported by Husøy et al. (2008) and 80 % of analysed foods contained HMF (Murkovic and Pichler, 2006). Moreover, ready–to–drink filter coffees have a higher content of HMF, furfuryl alcohol, and furfural than cappuccino (Tab. 4.1.1).

4.2. HMF Content in Various Food Products

Table 4.2.1. HMF content in various food products

Food products	HMF (µg/g)
Crisp bread type 1	0.24 ± 0.02
Crisp bread type 2	–
Crisp bread type 3	–
Dried plum	16.8 ± 2.8
Raisins	5.96 ± 0.4
Mixed fruit cereal	2.84 ± 0.04
Yoghurt + mixed fruit cereal	1.23 ± 0.1
Honey	0.63 ± 0.1
Cola drink	0.26 ± 0.03

Besides coffee products, baked products such as crisp bread contain HMF but not all the crisp breads contain HMF although the different products were produced by the same company. Crisp bread type 1, which has the lowest amount of whole wheat compared to crisp bread type 2 and crisp bread type 3, contains HMF. A higher HMF content was found in dried plums and raisins. Raw plums has a higher content of total sugar (9.92 g total sugar/100g) than raw grapes (6.989 g total sugar/100 g), therefore the HMF production will be higher in dried plums than in raisins during the drying process because heating of fructose, glucose, and sucrose will produce HMF (Perez Locas and Yaylayan, 2008). Moreover, cereal samples contain HMF because the cereal sample was a mixed fruit cereal and which also contained raisins. In the yoghurt plus cereal sample HMF was also found because the cereal was also a mixed fruit cereal with raisins which will contribute to the total HMF content (Tab. 4.2.1).

HMF also found in honey samples, but the amount is below the maximum level of HMF allowed in honey (15 mg/kg) (Bogdanova et al., 2004) and also below the

high range of HMF allowed by Codex 1997 (20 – 40 mg/kg). HMF could be found in honey because the composition of sugar in honey mostly fructose and glucose with the ratio fructose/glucose mostly below 1 (Kaškoniene et al., 2010) and normally honey undergo heat treatment. Heat treatment in honey is used to prevent contamination from mesophiles aerobics, fungi, yeast, *Clostridium botulinum* that could occur during extraction. Therefore a heat treatment is needed to eliminate contamination which does not produce toxic compounds. The heat treatment should be short and using not too high temperatures with a quick cooling afterwards (Tosi et al., 2002). HMF was also found in honey by Teixidó et al. (2011). Gaspar and Lucena (2009) found furfural in honey. The presence of HMF in cola beverages may also be due to the heat treatment for preservation and the high sugar content in these drinks (Tab. 4.2.1).

4.3. Furfuryl Alcohol Content in Various Food Products

Table 4.3.1. Furfuryl alcohol content in various food products

Food products	**Furfuryl alcohol (µg/g)**
Crisp bread type 1	0.08 ± 0.003
Norwegian goat cheese	8.39 ± 0.3
Meat balls in sauce	2.59 ± 0.1

The types of crisp bread that contain HMF also contain furfuryl alcohol. The concentration of furfuryl alcohol in crisp bread type 1 is lower than the HMF concentration. Moreover, the meat balls in sauce contain furfuryl alcohol because the production used heat and acidic conditions and also there is sugar content that can cause furfuryl alcohol production. The Norwegian goat cheese also content

furfuryl alcohol because the production of this type of cheese used high temperature for quite a long time for the caramelization of the lactose forming its typical brown colour and caramel taste (Tab. 4.3.1).

4.4. Furfural in Various Food Products

Table 4.4.1. Furfural content in various food products

Food products	Furfural (µg/g)
Dried plum	0.56 ± 0.1
Raisins	0.29 ± 0.01
Buns with cinnamon and sugar	0.18 ± 0.01
Mixed fruit cereal	0.15 ± 0.003
Pastasauce	0.17 ± 0.01

Dried plums, raisins, and breakfast cereals also contain furfural but the furfural content is lower than the HMF content. The lower furfural content in raisins compared to dried plums also may due to the comparable lower total sugar content in raisin. Buns and pasta sauce contain furfural with no HMF or furfuryl alcohol present (Tab. 4.4.1).

V. CONCLUSION

The HPLC analysis using gradient elution with methanol and water allows the identification and quantification of HMF, furfuryl alcohol, furfural in a single run.

VI. REFERENCE

Teixidó, E., Núñez, O., Santos, F.J. & Galceran, M.T., (2011), 5–Hydroxymethylfurfural Content in Foodstuffs Determined by Micellar Electrokinetic Chromatography., Food Chem., 126, 1902 – 1908.

Delgado–Andrade, C., Seiquer, I., Haro, A., Castellano, R. & Navarro, M.P., (2010), Development of The Maillard Reaction in Foods Cooked by Different Techniques. Intake of Maillard–Derived Compounds, Food Chem., 122, 145 – 153.

Rufian–Henares, J.A. & Delgado–Andrade, C., (2009), Effect of Digestive Process on Maillard Reaction Indexes and Antioxidant Properties of Breakfast Cereals, Food Research International, 42, 394 – 400.

Gaspar, E.M.S.M. & Lucena, A.F.F., (2009), Improved HPLC Methodology for Food Control–Furfurals and Patulin as Markers of Quality, Food Chem., 114, 1576 – 1582.

Golubkova, T., (2011), Bildung von Potentiell Toxischen Furanderivaten in Lebenmitteln., Diplomarbeit, Institut für Biochemie TU Graz. Austria. pp. 38 – 40

Sarafraz–Yazdi, A., Abbasian, M. & Amiri, A., (2012), Determination of Furan In Food Samples Using Two Solid Phase Microextraction Fibers Based on Sol–Gel Technique With Gas Chromatography–Flame Ionisation Detector., Food Chem., 131, 698 – 704.

Lancker, F., Adams, A., Owczarek, A., Meulenaer, B. & Kimpe, N., (2009), Impact of Various Food Ingredients on the Retention of Furan in Foods, Mol. Nutr. Food Res., 53, 1505 – 1511.

Roberts, D., Crews, C., Grundy, H., Mills, C. & Matthews, W., (2008), Effect of Consumer Cooking on Furan in Convenience Foods., Food Additives & Contaminants: Part A., 25, 25 – 31.

Lachenmeier, D.W., Reusch, H. & Kuballa, T., (2009), Risk Assessment of Furan in Commercially Jarred Baby Foods, Including Insights Into Its Occurrence and Formation in Freshly Home–Cooked Foods For Infants and Young Children., Food Additives & Contaminants: Part A, 26, 776 – 785.

Arisseto, A.P., Vicente, E. & Toledo, M.C.F., (2010), Determination of Furan Levels in Commercial Samples of Baby Food From Brazil and Preliminary Risk Assessment, Food Additives & Contaminants: Part A, 27, 1051 – 1059.

Altaki, M.S., Santos, F.J. & Galceran, M.T., (2009), Automated Headspace Solid–Phase Microextraction Versus Headspace for the Analysis of Furan in Foods by Gas Chromatography–Mass Spectrometry, Talanta, 78, 1315 – 1320.

Becalski, A., Hayward, S., Krakalovich, T., Pelletier, L., Roscoe, V. & Vavasour, E., (2010), Development of an Analytical Method and Survey of Foods for Furan, 2–Methylfuran and 3–Methylfuran with Estimated Exposure, Food Additives & Contaminants: Part A, 27, 764 – 775.

Vranová, J., Bednáriková, A. & Ciesarová, Z., (2007), In–House Validation of A Simple Headspace Gas Chromatography Mass Spectrometry Method for Determination of Furan Levels In Food. J. Food Nutr. Res., 46, 123 – 127.

Kim, T., Kim, S. & Lee, K., (2010). Analysis of Furan in Heat–Processed Foods Consumed in Korea Using Solid Phase Microextraction–Gas Chromatography/Mass Spectrometry (SPME–GC/MS), Food Chem., 123,1328 – 1333.

Wang, Y. & Kays, S.J., (2000), Contribution of Volatile Compounds to the Characteristic Aroma of Baked 'Jewel' Sweetpotatoes, J. Amer. Soc. Hort. Sci. 125, 638 – 643. 2000.

EFSA., (2009). Results on the Monitoring of Furan Levels in Food, EFSA Scientific Report, 304, 1 – 23.

Vernin, G., Heterocyclic Aroma Compounds in Foods: Occurrence and Organoleptic Properties, in: The Chemistry of Heterocyclic Flavoring and Aroma Compounds (ed. G. Vernin), Ellis Horwood Publishers, Chichester, pp. 72 – 150.

Fors, S., (1983), Sensory Properties of Volatile Maillard Reaction Products and Related Compounds, in The Maillard Reaction in Foods and Nutrition (eds. G. R. Waller and M. S. Feather), ACS Symposium Series 215, American Chemical Society, Washington, D.C., pp. 185 – 286.

Limacher, A., Kerler, J., Davidek, T., Schmalzried, F. & Blank, I., (2008), Formation of Furan and Methylfuran by Maillard–Type Reactions in Model Systems and Food. J. Agric. Food Chem., 56, 3639 – 3647.

Perez Locas, C. & Yaylayan, V.A., (2004), Origin and Mechanistic Pathways of Formation of the Parent Furans: A Food Toxicant. J. Agric. Food Chem., 52, 6830 – 6836.

Pera, L.L., Liberatore, A., Avellone, G., Fanara, S., Dugo, G. & Agozzino, P., (2009), Analysis of Furan in Coffee of Different Provenance by Head–Space Solid Phase Microextraction Gas Chromatography–Mass Spectrometry: Effect of Brewing Procedures. Food Additives and Contaminants, 26, 2009, 786 – 792.

Durling, L.J.K., Busk, L. & Hellman, B.E, (2009), Evaluation of The DNA Damaging Effect of The Heat–Induced Food Toxicant 5–Hydroxymethylfurfural (HMF) in Various Cell Lines with Different Activities of Sulfotransferases. Food Chem. Toxicol., 47, 880 – 884.

Bakhiya, N., Monien, B., Frank, H., Seidel, A. & Glatt, H., (2009), Renal Organic Anion Transporters Oat1 and Oat3 Mediate the Cellular Accumulation of 5 Sulfooxymethylfurfural, A Reactive, Nephrotoxic Metabolite of The Maillard Product 5–Hydroxymethylfurfural, Biochem. Pharmacol., 78, 414 – 419.

Glatt, H.R. & Sommer, Y., (2006), Health Risks by 5–Hydroxymethylfurfural (HMF) and Related Compounds. In: Skog K, Alexander J, editors. Acrylamide and Other Health Hazardous Compounds in Heat–Treated Foods. Cambridge: Woodhead Publishing, pp. 328 – 57.

Godfrey, V.B., Chen, L.J., Griffin, R.J., Lebetkin, E.H. & Burka, L.T., (1999), Distribution and Metabolism of (5–Hydroxymethyl)Furfural in Male F344 Rats and B6C3F1 Mice After Oral Administration. J. Toxicol. Environ. Health A. 57:199 – 210.

Fallico, B., Arena, E. & Zappala, M., (2008), Degradation of 5–Hydroxymethylfurfural in Honey. J. Food Sci., 73, C625 – 31.

Monien, B.H., Frank, H., Seidel, A. & Glatt, H., (2009). Conversion of the Common Food Constituent 5–Hydroxymethylfurfural into a Mutagenic and Carcinogenic Sulfuric Acid Ester in the Mouse in Vivo. Chem. Res. Toxicol., 22, 1123 – 1128.

National Technology Program, (2008), NTP Technical Report on The Toxicology and Carcinogenesisstudies of 5–(Hydroxymethyl)–2–Furfural(CAS NO. 67–47–0) in F344/N RATS and B6C3F1 Mice. Service U.S. Department of Health and Human Services.

Guan, Y.G, Wu, X.L., Yu, S.J. & Xu, X.B., (2011), Proposed Formation Mechanism, Antioxidant Activity and Mda–Mb–231 Cells Survival Analysis of Two Glucose–Ammonium Sulfite Caramel Colour Melanoidins Fractions. Carbohydr. Polym., 86, 948 – 955.

Nikolov, P.Y. & Yaylayan, V.A., (2011), Thermal Decomposition of 5(Hydroxymethyl)–2–furaldehyde (HMF) and Its Further Transformations in the Presence of Glycine, J. Agric. Food Chem., 59, 10104 – 10113.

Janzowski, C., Glaab, V., Samimi, E., Schlatter, J. & Eisenbrand, G., (2000), 5–Hydroxymethylfurfural: Assessment of Mutagenicity, DNA–damaging Potential and Reactivity Towards Cellular Glutathione, Food Chem. Toxicol., 38, 801 – 809.

Perez Locas, C. & Yaylayan, V.A., (2008), Isotope Labelling Studies on the Formation of 5–(Hydroxymethyl)–2–furaldehyde (HMF) from Sucrose by Pyrolysis–GC/MS. J. Agric. Food Chem., 56, 6717 – 6723.

Campo, G., Berregi, I., Caracena, R. & Zuriarrain, J., (2010), Quantitative Determination of Caffeine, Formic Acid, Trigonelline and 5–(hydroxymethyl)furfural in Soluble Coffees by 1H NMR Spectrometry, Talanta 81, 367 – 371.

Arribas–Lorenzo, G. & Morales, F.J., (2010), Estimation of Dietary Intake of 5–Hydroxymethylfurfural and Related Substances from Coffee to Spanish Population. Food Chem. Toxicol., 48, 644 – 649.

Hofmann, T., Bors, W. & Stettmaier, K., (1999), Studies on Radical Intermediates in the Early Stage of the Non–enzymatic Browning Reaction of Carbohydrate and Amino Acids, J. Agric. Food Chem., 47, 379 – 90.

Murata, M., Tutsuka, H. & Ono, H., (2007), Browning of Furfural and Amino Acids, and a Novel Yellow Compound, Furpipate, Formed in Lysine and Furfural, Biosci. Biotechnol. Biochem., 71, 1717 – 1723.

Hofmann, T., (1998), Characterization of the Chemical Structure of Novel Colored Maillard Reaction Products from Furan–2–carboxaldehyde and Amino Acids, J. Agric. Food Chem., 46, 932 – 940.

Märk, J., Pollien, P., Lindinger, C., Blank, I. & Märk, T., (2006), Quantitation of Furan and Methylfuran Formed in Different Precursor Systems by Proton Transfer Reaction Mass Spectrometry. J. Agric. Food Chem., 54, 2786 – 2793.

Ravindranath, V. & Boyd, M.R., (1985), Metabolic Activation of 2–Methylfuran by Rat Microsomal Systems. Toxicology and Applied Pharmacology, 78, 370 – 376.

Becalski, A. & Seaman, S., (2005), Furan Precursors in Food: A Model Study and Development of a Simple Headspace Method for Determination of Furan. J. AOAC Int., 88, 102 – 106.

Yaylayan, V., (2006), Precursors, Formation and Determination of Furan in Food. J. Verbr. Lebensm., 1, 5 – 9.

Abraham, K., Gürtler, R., Berg, K., Heinemeyer, G., Lampen, A. & Appel, K.E., (2011), Toxicology and Risk Assessment of 5–Hydroxymethylfurfural in Food. Mol. Nutr. Food. Res., 55, 667 – 678.

Ulbricht, R.J., Northup, S.J. & Thomas, J.A, (1984), A Review of 5–Hydroxymethylfurfural (HMF) in Parenteral Solution. Fundam. Appl. Toxicol., 4, 843 – 853.

Spano, N., Ciulu, M., Floris, I., Panzanelli, A., Pilo, M.I., Piu, P.C, Salis, S. & Sanna, G., (2009). A Direct RP–HPLC Method for the Determination of Furanic Aldehydes and Acids in Honey, Talanta, 78, 310 – 314.

Husøy, T., Haugen, M., Murkovic, M., Jöbstl, D., Stølen, L.H., Bjellaas, T., Rønningborg, C., Glatt, H., & Alexander, J., Dietary Exposure to 5–Hydroxymethylfurfural from Norwegian Food and Correlations With Urine Metabolites of Short–Term Exposure. Food Chem. Toxicol., 46, 3697 – 3702.

Murkovic, M. & . Pichler, N., (2006), Analysis of 5–Hydroxymethylfurfural in Coffee, Dried Fruits and Urine. Mol. Nutr. Food Res., 50, 842 – 846.

Bogdanov, S., Ruoff, K. & Oddo, L.P., (2004), Physico–Chemical Methods for the Characterization of Unifloral Honeys: A Review. Apidologie , 35, S4 – S17.

Kaškoniene, V., Venskutonis, P.R. & Čeksterytė, V., (2010), Carbohydrate Composition and Electrical Conductivity of Different Origin Honeys from Lithuania. LWT – Food Science and Technology, 43, 801 – 807.

Tosi, E., Ciappini, M., Ré, E. & Lucero, H., Honey Thermal Treatment Effects on Hydroxymethylfurfural Content. Food Chem., 77, 71 – 74.

Codex Alimentarius Commision, (1997). Joint FAO/WHO Food Standards Programme. Geneva, Switzerland.

Lee, H., Bian, S. S., & Chen, Y.L., (1994), Genotoxicity of 1,3–dithiane and 1,4–dithiane in the CHO/SCE Assay and the Salmonella/microsomal Test. Mutation Research, 21, 213 – 218.

Chen, L.J., Hecht, S.S., & Peterson, L.A., (1995). Identification of cis–2–butene–1,4–dial as A Microsomal Metabolite of Furan. Chem. Res. Toxicol., 8, 903 – 906.

Ravindrananth, V., Boyd, M.R., & Burka, L.T., (1984), Reactive Metabolites from the Bioactivation of Toxic Methylfurans. Science, 224, 884 – 886.

Peterson, L.A., Naruko, K.C., & Predecki, D.P., (2000), A Reactive Metabolite of Furan, Cis–2–Butene–1,4–Dial, is Mutagenic in the Ames Assay. Chem. Res. Toxicol., 13, 531 – 534.

Burka, L.T., Washburn, K.D., Irwin, R.D., (1991), Disposition of [^{14}C] furan in the male F344 Rat. J. Toxicol. Environ. Health, 34, 245 – 257.

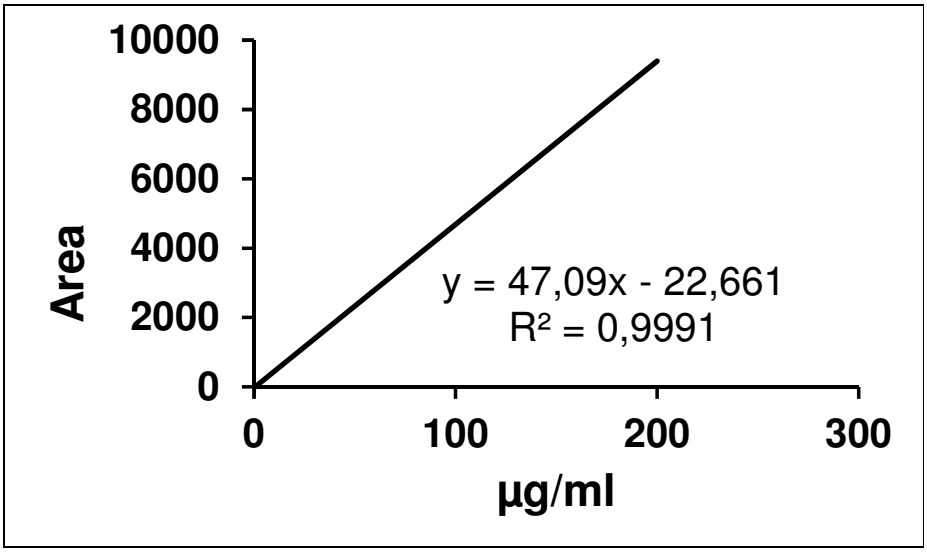
Appendix

1. Concentration versus area standards

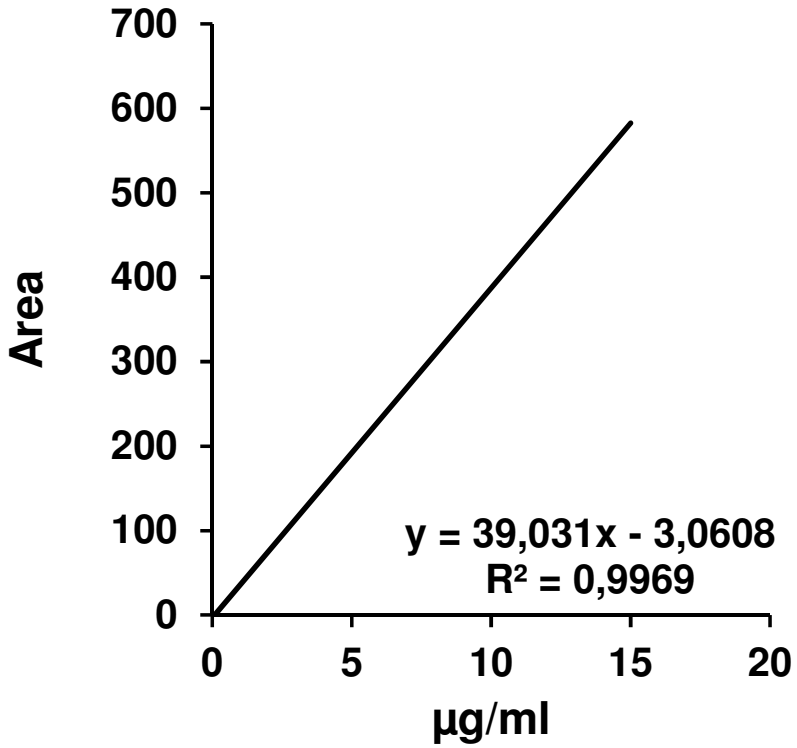
HMF		Furfuryl Alcohol		Furfural	
Concentration (µg/ml)	Area	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
0.05	3.47	0.01	0.1	0.01	1.14
10	585.14	1	32.95	0.5	12.59
50	2194.41	5	203.2	5	296.4
100	4584.82	10	365.19	10	642.35
200	9473.59	15	593.61	15	1116.42

2. Standard curves of HMF, Furfuryl acohol, and furfural

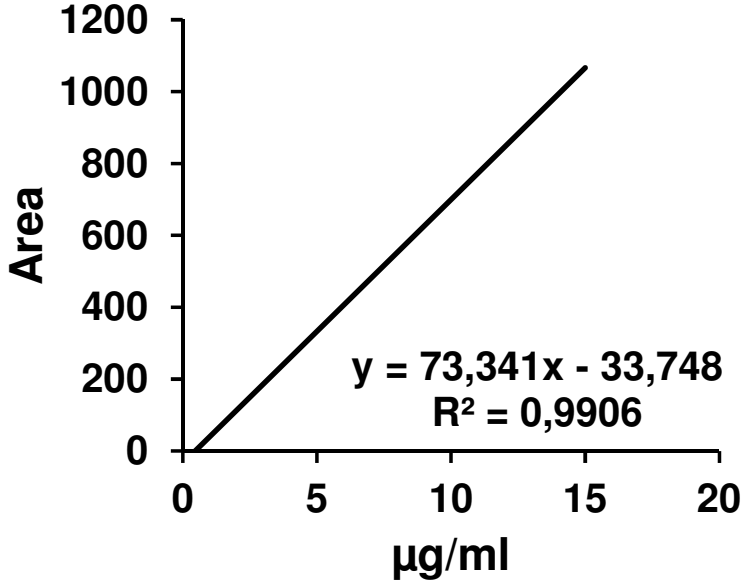
a. HMF Standard curve



b. Furfuryl alcohol standard curve



c. Furfural standard curve



3. Furan derivatives in Norwegian foods

Food products	Furan derivatives (µg/g)		
	HMF	Furfural	Furfuryl alcohol
Instant coffee powder type 1	7.17 ± 0.49	2.75 ± 0.18	13.0 ± 0.2
Instant coffee powder type 2	39.0 ± 1.4	–	2.05 ± 0.13
Instant coffee powder type 3	71.6 ± 2.9	–	5.53 ± 0.12
Filter coffee type 1	4.87 ± 0.34	0.64 ± 0.05	4.53 ± 0.80
Filter coffee type 2	2.02 ± 0.50	1.1 ± 0.37	4.31 ± 0.27
Filter coffee type 3	3.74 ± 0.17	1.42 ± 0.21	16.8 ± 3.0
Double cappucino	0.39 ± 0.03	0.26 ± 0.007	3.98 ± 0.60
Cappuciono	1.39 ± 0.18	0.53 ± 0.19	1.79 ± 0.31
Crisp bread Wasa husman	–	–	–
Crisp bread Rug–sprø originale	–	–	–
Crisp bread Wasa solruta spelt/dinkel	0.24 ± 0.02	–	0.08 ± 0.003
Saltstenger	–	–	–
Honney	0.63 ± 0.08	–	–
Peanuts	–	–	–
Chocolate bar with peanut	–	–	–
Chocolate bar	–	–	–
Soft tortilla	–	–	–
Bread type 1	–	–	–
Bread type 2	–	–	–
Buns with cinnamon and sugar	–	0.18 ± 0.006	–
Chips	–	–	–
Pancake	–	–	–
Cookies with chip & hazelnut	–	–	–
Mix fruit cereal	2.84 ± 0.04	0.15 ± 0.003	–
Pizza type 1	–	–	–
Pizza type 2	–	–	–
Pasta	–	–	–
Potatoes baked in oven	–	–	–
Boiled potatoes	–	–	–
Mashed potatoes	–	–	–
Cheese	–	–	–
Norwegian goat cheese	–	–	8.39 ± 0.34
Yoghurt	–	–	–
Yoghurt with mixed fruit cereal	1.23 ± 0.11	–	–
Milk	–	–	–
Fish in tomatosauce	–	–	–
Lever paste	–	–	–

Food products	Furan derivatives (µg/g)		
	HMF	Furfural	Furfuryl alcohol
Meat sauce	–	–	–
White sauce	–	–	–
Dried plums	16.8 ± 2.8	0.56 ± 0.06	–
Tomat ketchup	–	–	–
Mayonaise	–	–	–
Pasta sauce	–	0.17 ± 0.008	–
Stew	–	–	–
Meat balls in sauce	–	–	2.59 ± 0.11
Meat soup	–	–	–
Vegetable soup	–	–	–
Raspberry jam	–	–	–
Strawberry jam	–	–	–
Raisins	5.96 ± 0.44	0.29 ± 0.005	–
Kaviar	–	–	–
Orange juice	–	–	–
Fuite juice	–	–	–
Cola Drink	0.26 ± 0.03	–	–
Beverage	–	–	–
Beer type 1	–	–	–
Beer type 2	–	–	–

4. Chromatogram of HMF in coffee products

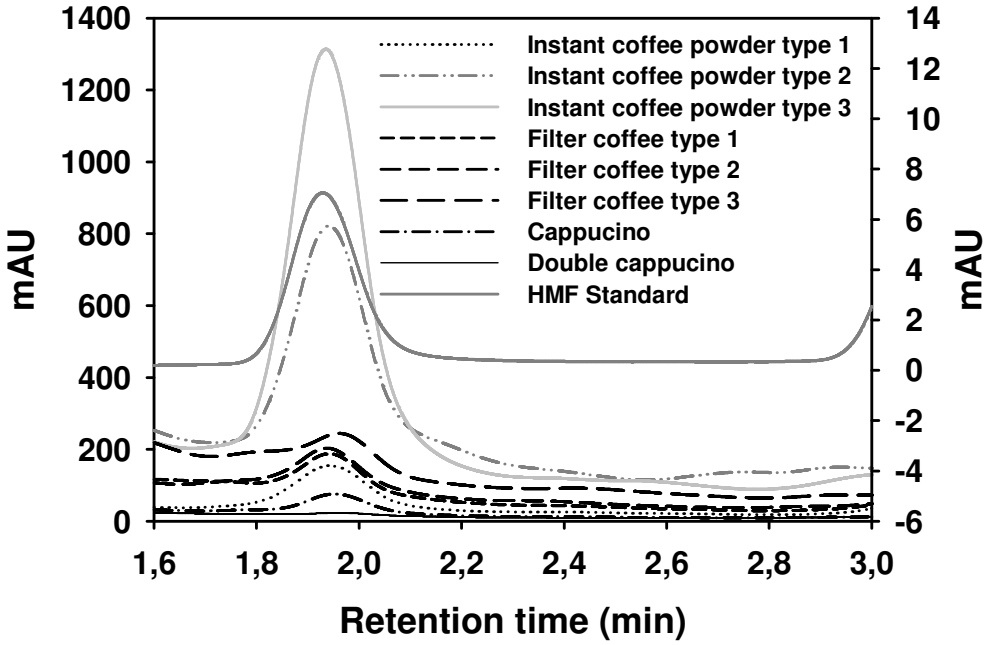


Figure 1. HMF content in coffee

5. Furfuryl alcohol content in coffee products

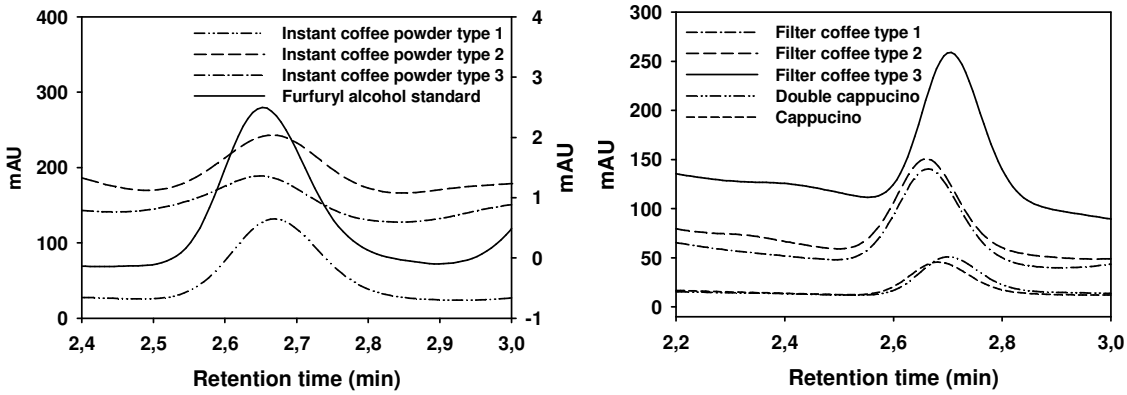


Figure 2. Furfuryl alcohol content in coffee

6. Furfural content in coffee products

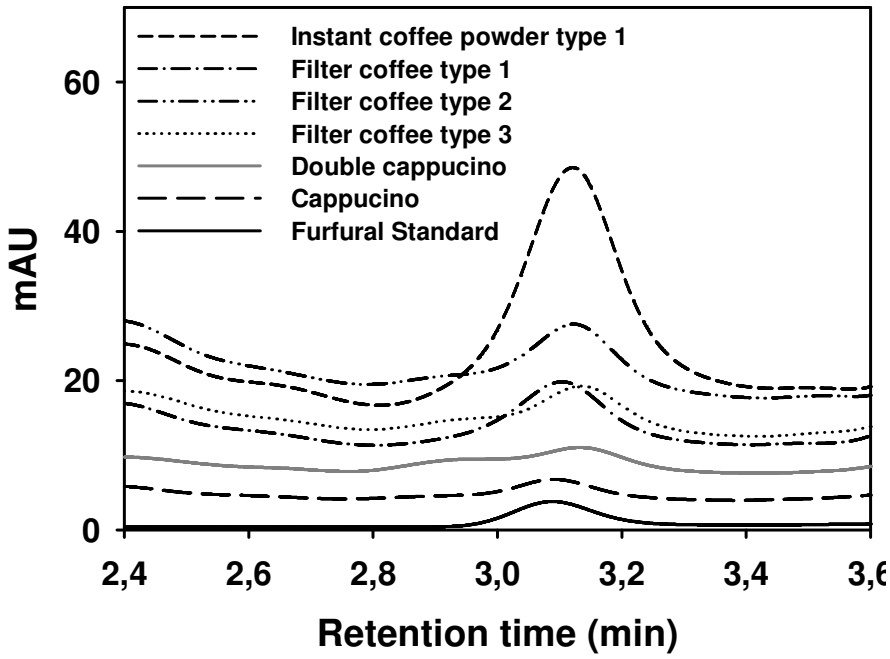


Figure 3. Furfural content in coffee products

7. HMF content crisp bread and mix fruit cereal

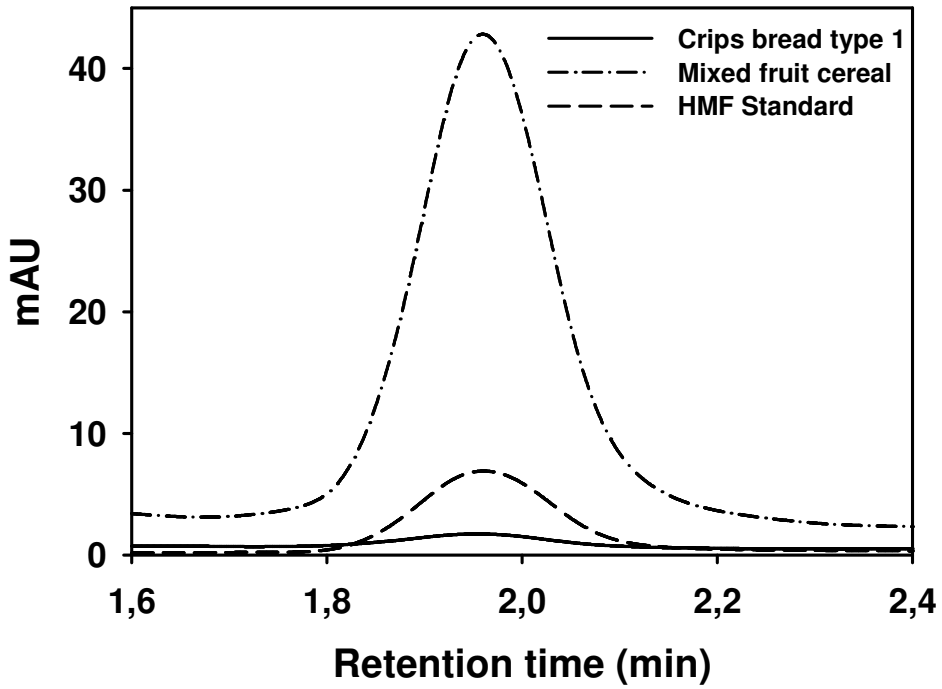


Figure 4. HMF in crispbread and cereal

8. HMF content in honey, yoghurt plus mixed fruit cereal, and cola drink

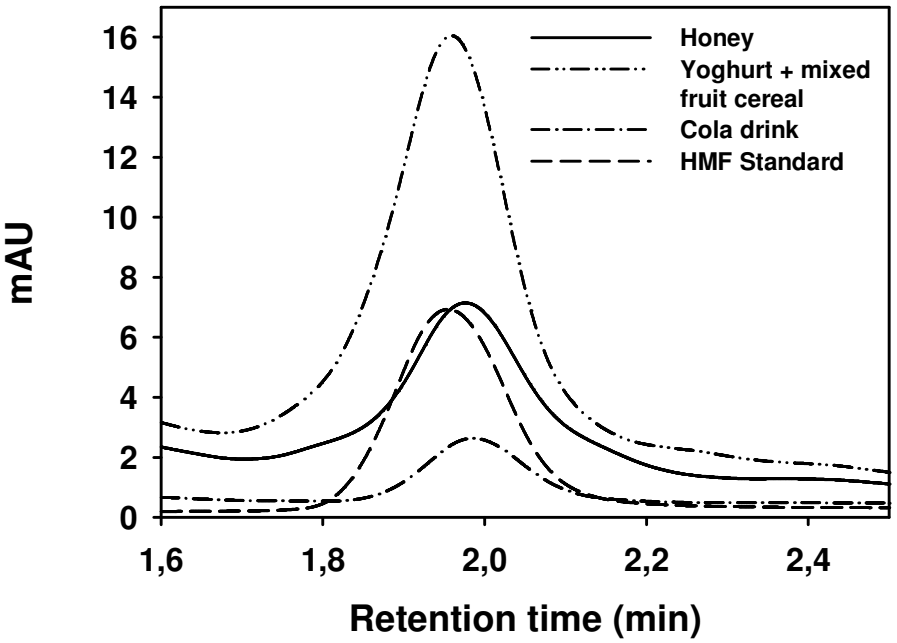


Figure 5. HMF content in honey, yoghurt+mixed fruit cereal, and cola drink

9. HMF in raisin and dried plum

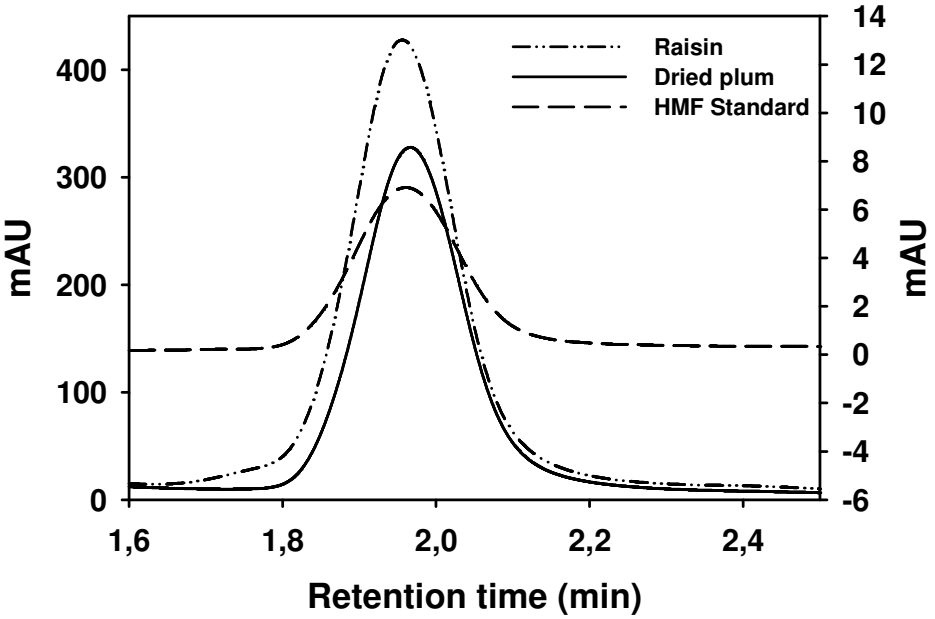


Figure 6. HMF in raisin and dried plum

10. Furfuryl alcohol in food products

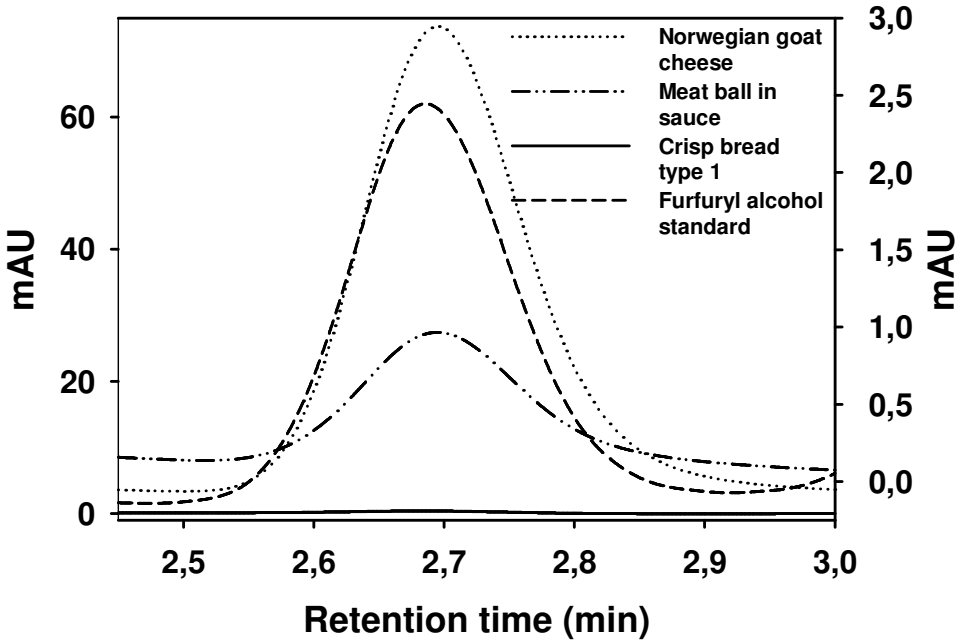


Figure 8. Furfuryl alcohol in food products

11. Furfural in other food products

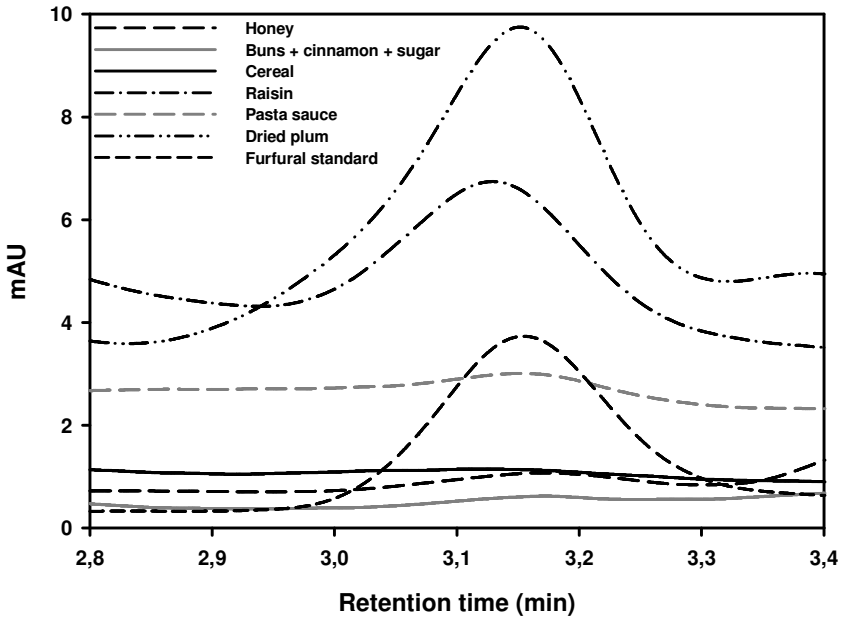


Figure 7. Furfural in other food products

SECTION 2

CHARACTERISATION OF THE POLYMERIZATION OF FURFURYL ALCOHOL DURING ROASTING OF COFFEE

I. INTRODUCTION

The formation of the brown colour in heated foods is a result of the caramelization and the Maillard reaction. In many foods, the brown colour is a positive quality trait like in bread, coffee, or fried potatoes. In the course of these reactions not only the colour changes but also aroma active substances are formed giving the heated foods a typical appearance to the consumer. Besides the wanted substances some toxic compounds are also formed during heating such as heterocyclic amines, acrylamide, and furan (Galceran & Puignou, 2006; Tareke et al., 2002; Perez Locas & Yaylayan, 2004).

Recently, furfuryl alcohol has attracted the safety research because new biological activation reactions have been identified which are also relevant for furfuryl alcohol activation in a way that this compound can become a DNA–reactive intermediate that has a mutagenic effect (Glatt and Sommer, 2006). Primary alcohol have a higher genotoxic activity compared to secondary and tertiary alcohols (e.g. 1–hydroxymethylpyrene, (+)–1–(α–hydroxyethyl)pyrene, 1–(α–hydroxy)isopropyl–pyrene) (Glatt, 2000). Although it polymerizes, the

concentration of the monomeric furfuryl alcohol is still high in heated food products. Furfuryl alcohol gives a burnt sugar odour, cooked–sugar odour, rubber–like odour, and when furfuryl alcohol interacts with dihydroxybenzene or trihydroxybenzene during roasting of coffee it will produce a bitter taste (Lee et al., 2006; Wang & Kays, 2000; Bonvehì, 2005; Karagu–Yüceer et al., 2002; Kreppenhofer et al., 2011). Nevertheless, furfuryl alcohol is used as a flavouring agent with an acceptable daily intake of 0 – 0.5 mg/kg (Joint FAO/WHO, 2000).

Furfuryl alcohol can be formed by the degradation of 1,2–enediols and quinic acid at high temperatures (Brands & Boekel, 2001; Moon & Shibamoto, 2010). Quantitatively, furfuryl alcohol as a furan derivative is predominating in roasted coffee (Kreppenhofer et al., 2011). Furfuryl alcohol is predicted to have an influence on the formation of brown colour during roasting of coffee. Furfuryl alcohol can polymerize in acid conditions by condensation of the hydroxyl group and the hydrogen atom of the heterocycle at carbon 5 producing a polymer with methylene linkages (Dunlop & Peters, 1953) resulting in a brown coloured polymer (Choura et al., 1996). Dimerization of furfuryl alcohol could also occur by condensation of the two hydroxyl groups of furfuryl alcohol producing dimethylene ether linkages. However, in acid conditions this type of condensation releases formaldehyde to form the methylene linkage (Dunlop & Peters, 1953). The brown colour of the aliphatic furfuryl alcohol polymer could be induced by the loss of one hydrogen atom from a central carbon (Choura et al., 1996). It is also possible that the furfuryl alcohol and its oligomers are introduced into the melanoidins – a high molecular mass and brown coloured product. Other

heterocyclic aromatic ring systems (e.g. pyridines, pyrazines, pyrroles, and imidazoles) also contribute to the melanoidin formation (Nursten, 2005). Generally, melanoidin forms by condensation of amino compounds with products from Amadori rearrangements which undergo sugar dehydration and sugar fragmentation (Hodge, 1953). In coffee beverages, melanoidin contributes up to 25 % of the dry matter (Borrelli et al., 2002).

Here the formation of intermediate furfuryl alcohol oligomers during roasting of coffee will be described. This is important because this reaction could contribute to the browning of coffee during roasting and it is expected that with the increased chain length the mutagenic potential of furfuryl alcohol is reduced.

II. LITERATURE REVIEW

2.1. Formation of Furfuryl Alcohol

Glucose or fructose in high temperature can undergo isomerisation reaction. The key intermediate in these isomerisation reactions, 1,2–enediol, is considered as the starting intermediate of the degradation by β–elimination producing the unstable compound 3–deoxyaldoketose followed by cleavage producing formic acid and a compound with five carbon atoms (De Bruijn et al., 1986). From this C_5 compound – 2–deoxyribose, from monosaccharide heating – furfuryl alcohol is produced, Fig. 2.1.1 (Brands & Boekel, 2001). Besides that, heating of quinic acid at 250 °C for 30 min under nitrogen produces furfuryl alcohol 250 μg/g, (Fig. 2.1.2, Moon & Shibamoto, 2010). Wnorowski and Yaylayan (2000) found that

furfural alcohol forms from an intact glucose skeleton. Quantitatively, furfuryl alcohol as a furan derivative is predominating in roasted coffee (Kreppenhofer et al., 2011).

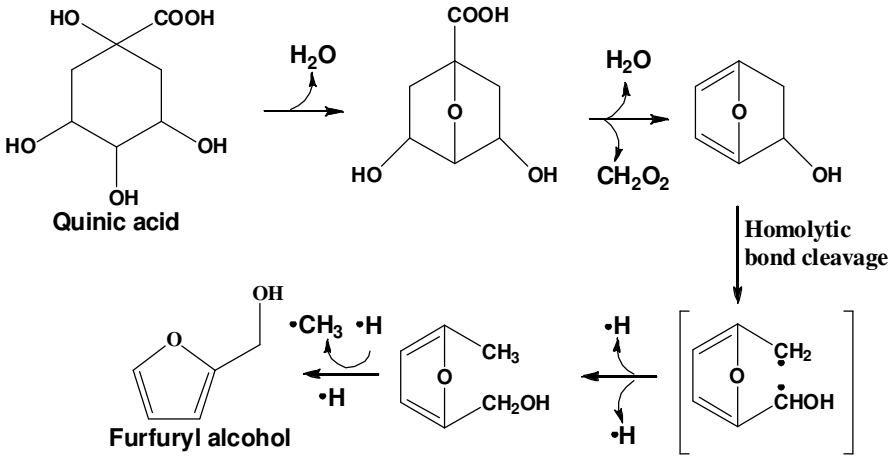


Figure 2.1.1. The production of furfuryl alcohol from degradation quinic acid (Moon & Shibamoto, 2010)

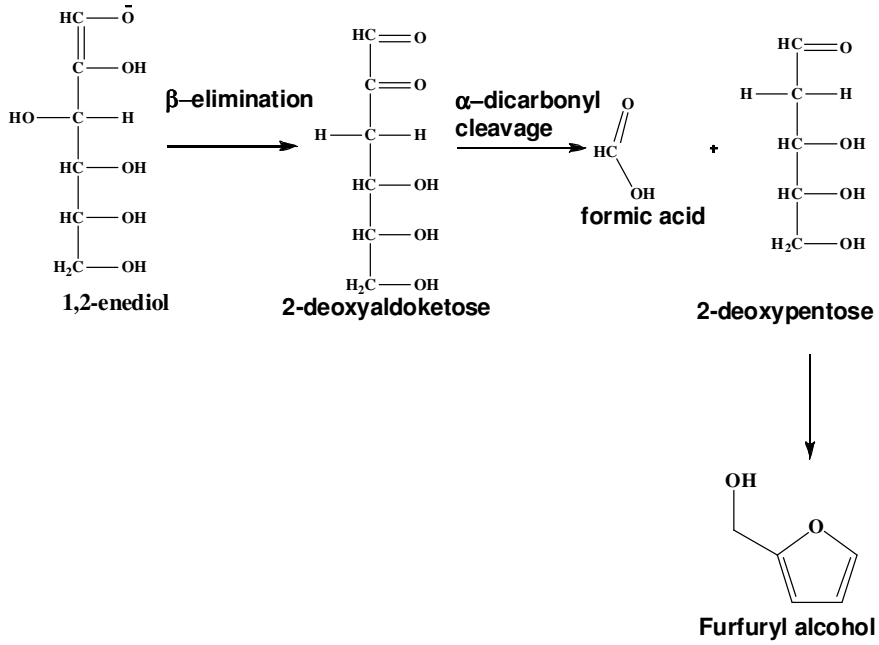


Figure 2.1.2. The production of furfuryl alcohol from degradation of reducing sugars (Brands & Boekel, 2001)

2.2. Polymerisation of Furfuryl Alcohol

Furfuryl alcohol can polymerize in acid conditions by condensation of the hydroxyl group and hydrogen atom of the heterocycle at carbon 5 producing a polymer with methylene linkages (Dunlop & Peters, 1953) resulting in a brown coloured polymer (Choura et al., 1996). The energy activation during early condensation is low and can be attributed to a self condensation mechanism (Guigo et al., 2007). Moreover, polymerisation of furfuryl alcohol could occur by interaction of furfuryl alcohol with the carbenium ion of another furfuryl alcohol molecule. This condensation is kinetically more preferred than between two furfuryl alcohols, (Fig. 2.2.1, Kim et al., 2011). Polymerization of furfuryl alcohol could also occur by condensation of the two hydroxyl groups producing a polymer with dimethylene ether linkages, but in acid conditions this type of condensation releases formaldehyde to form a methylene linkage (Dunlop and Peters, 1953).

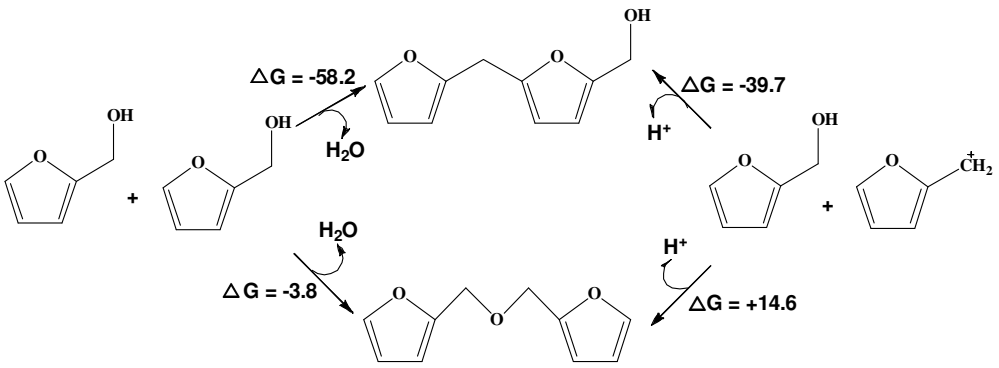


Figure 2.2.1. Condensation of furfuryl alcohol in the polymerization reaction (Kim et al., 2011)

*△G: energy for protonation reaction

Colour formation in furfuryl alcohol polymerisation starts with the elimination of water and followed by hydride–ion exchanges from the carbon of the methylene linkage that will change the colour from intermediate green to dark brown (Choura et al., 1996). The intensity of the colour is in line with increased conjugation of the double bonds during furfuryl alcohol polymerisation, Fig.2.2.2. Besides the colour formation, the viscosity of the solution furfuryl alcohol in acid condition also increases until it reaches a solid state (Bertarione et al., 2008). The increase of viscosity happened by Diels–Alder cyclo additions between oligomers formed in the previous step and this is also reflected by the increase of activation energies (Guigo et al., 2007).

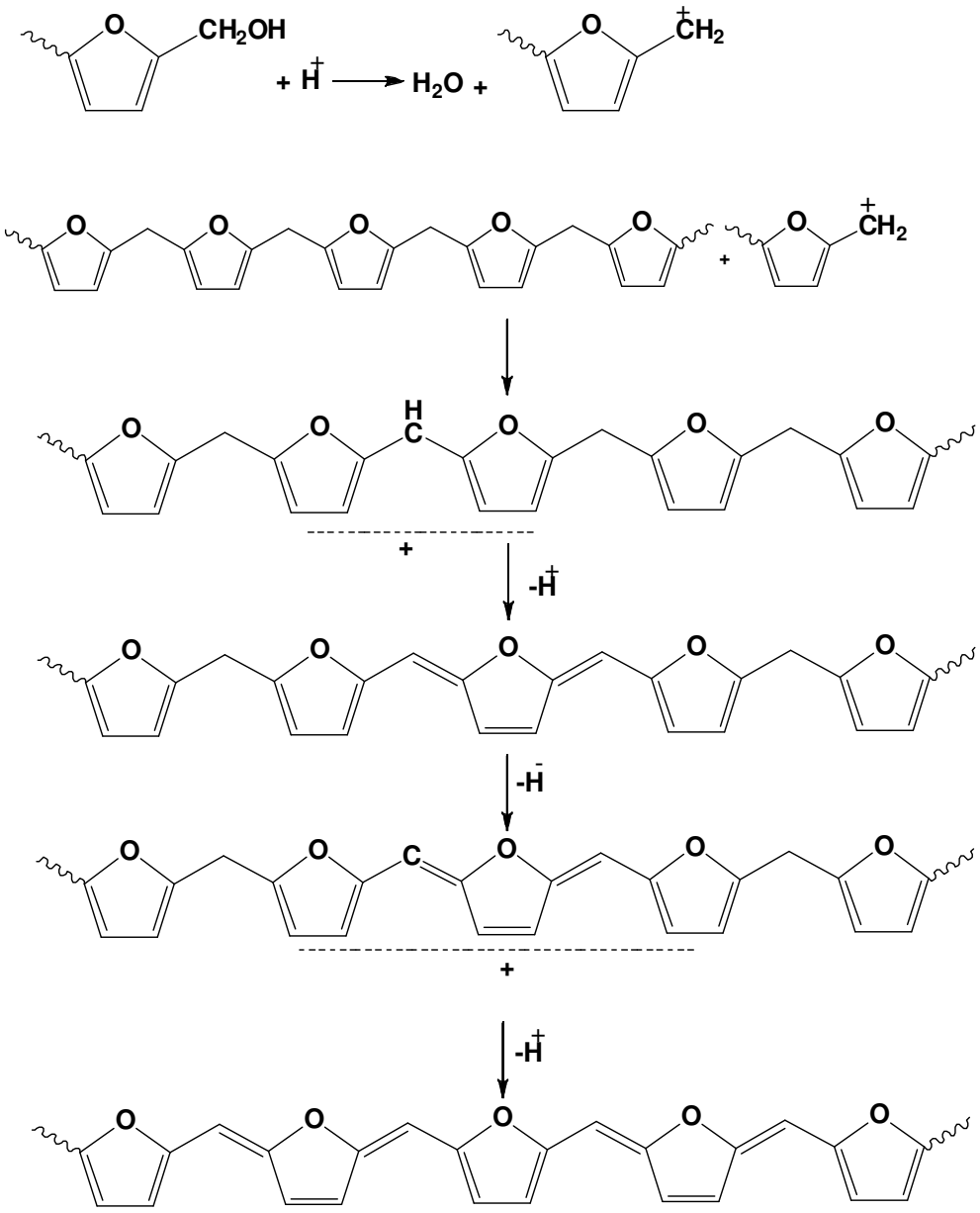


Figure 2.2.2. Formation of the conjugation of polymeric furfuryl alcohol (Bertarione et al., 2008)

2.3. Furfuryl Alcohol in Foods

The concentration of the monomeric furfuryl alcohol is still high in roasted coffee.

Furfuryl alcohol in instant coffee is 267 μg/g and in coffee roasted at 210 °C for 3

min is 564 μg/g (Golubkova, 2011). Medium roasted coffee has higher furfuryl alcohol content than light roasted coffee (Moon & Shibamoto, 2009). Futhermore, furfuryl alcohol is also found in food product like in rice cakes 2–2.3 μg/g (Buttery et al., 1999), bread 187 μg/g (Jensen et al., 2011), honey 1.55 μg/g (Vázquez et al., 2007), toasted almonds cv. Marcona 5.97 $\pm$ 1.09 μg/g, toasted almonds cv. Comuna 8.88 $\pm$ 1.39 μg/g, toasted almonds cv. California 4.40 $\pm$ 1.23 μg/g (Vázquez–Araújo et al., 2008), non fat dried milk stored for 3 months in low humidity at room temperature 14.5 μg/g (Karagu–Yüceer et al., 2002), popcorn 0.0382–0.0821 μg/g (Park & Maga, 2006), corn tortilla chips 0.54 μg/g (Buttery & Ling, 1998), roasted cocoa powder 0.021 μg/g (Bonvehì, 2005), palm sugar was made by using traditional heating process at 210 °C 0.139 μg/g, palm sugar was made by a traditional heating process at 240 °C 0.518 μg/g (Ho et al., 2007), baked "Jewel" sweet potato 0.014 μg/g fresh weight (Wang & Kays, 2000) and citrus honey 0.011 μg/g (Castro–Vázquez et al., 2007). Also, furfuryl alcohol was found in frying oil that was used for beef, veal, chicken product frying (Munro & Danielewska–Nikiel, 2006).

2.4. Health Effects of Furfuryl Alcohol

Estimated furfuryl alcohol intake is 130 μg/kg human body weight (Munro & Danielewska–Nikiel, 2006). Furfuryl alcohol is mutagenic to *Salmonella typhimurium* strains TA100 engineered for the expression of human SULT1A1 because sulfotransferase can activate furfuryl alcohol into the mutagenic compound, 2–sulfooxymethylfuran. The 2–sulfooxymethylfuran is generated

intracellularly in proximity to the DNA leading to the formation of 2–methylfuranyl adducts. The covalent 2–methylfuranyl adducts cause mutagenic effects. The mutagenicity of furfuryl alcohol is dose dependent and increases its mutagenicity from 3 to 200 nmol furfuryl alcohol per plate (Monien et al., 2011). The DNA samples of liver, kidney, and lung contain 2–methylfuranyl adducts in mice that received furfuryl alcohol with the drinking water. Rodents exposed to furfuryl alcohol form tumors that contain 2–methylfuranyl (NTP, 1999).

III. MATERIALS AND METHODS

3.1. Materials

Furfuryl alcohol (FA, ≥99 %) was purchased from Fluka (Buchs, Switzerland), hydrochloric acid (HCl, 32 %) was purchased from Merck (Darmstadt, Germany), methanol HPLC grade was purchased from Mallinckrodt Baker (The Netherlands), 100 % acetic acid was purchased from Roth (Karlsruhe, Germany), and solid phase extraction (SPE) accucat, 200 mg, 3 ml was purchased from Varian (Agilent Technologies, USA), robusta green coffee.

3.2. Methods

3.2.1. Polymerization Furfuryl Alcohol.

40 µl Furfuryl alcohol were mixed with 40 µl aqueous acid (1 M HCl and 40 µl MeOH) and then it was incubated at 22 °C for 6 h. The reaction mixture was then diluted to 25 ml with methanol and then 1 ml of the sample to 5 ml with methanol. The samples were then analysed with the LC–MS Agilent 1100. LC–MS

conditions: LiChrospher 100 RP–18 (125 × 3 mm, 5 μm) as column from Agilent Technologies, 5 μl injection volume (for model system), 10 μl injection volume (for roasted coffee extract), 0.6 ml/min solvent flow rate, gradient elution until 15 min: 25 % MeOH, 73 % water; 2 % acetic acid in water pH 2.5 (constant during 15 min), DAD at 228 nm, ESI, scan mode, positive mode,70 V, mass range 75 – 200 (dimer), 75 – 280 (trimer), 75 – 350 (tetramer), and 75 – 450 (pentamer).

3.2.2. Furfuryl Alcohol Polymer Analysed with Ion Trap.

Oligomers of furfuryl alcohol were measured with an Agilent Ion Trap SL (Palo Alto, Ca, USA) equipped with an electrospray ionisation system. Drying gas flow was set to 10 l/min with a temperature of 350 °C, nebulizer set to 50 psig. The maximum accumulation time was fixed to 300 ms and the loading of the trap was controlled by the instrument with an ICC of 30,000. The electrospray voltage set at +3,500 V and the coupling products were measured in positive ion mode.

3.2.3. Furfuryl Alcohol Polymer Analysed with AB Sciex Q Trap LC/MS/MS

Furfuryl alcohol polymer solutions were injected into AB Sciex Q Trap LC/MS/MS. General analysis conditions: The solvent and gradient elution is the same as used in LC–MS analysis, LiChroCART 55–2 Purospher® STAR (MERCK) as column, flow rate 0.3 ml/min, column temperature 30 °C, curtain gas (CUR) 30 °C, ion spray voltage (IS) 5500, collision gas (CAD) Medium, temperature (TEM) 500 °C, ion gas source 1 (GS1) 30 °C, ion gas source 2 (GS2) 30 °C, resolution 1. Scan and selected ion monitoring (SIM) analysis: Declustering potential (DP) 30 and entrance potential (EP) 10. Product ion (MS2) and multiple reaction monitoring (MRM) analysis: DP 30, EP 10, collision energy

(CE) 30, collision cell exit potential (CXP) 15. MS3 analysis condition: excitation energy (AF2) 70, collision energy 10, scan rate 4000.

3.2.4. Furfuryl Alcohol Polymer with GC–MS

2 ml 2 h polymerized furfuryl alcohol were diluted with 2 ml water and then separated with SPE C18 (conditioned with 5 ml MeOH and washed with 3 ml water). After the sample had passed through the SPE, the cartridge was washed with 1 ml water, 1 ml 10 % MeOH in water, and 1 ml MeOH. The analytes were eluted from the solid phase with a second ml of MeOH. The extracts were injected into GC–MS after changing the solvent to chloroform. For the GC–MS analysis a HP5MS column was used with 0.25 µm thickness and spitless injection.

3.2.5. Fractionation Furfuryl Alcohol Oligomers

The furfuryl alcohol polymerization reaction mixture was purified by taking fractions from the HPLC separation right after the diode array detector. To obtain enough substance this was repeated 5 times. Then, the eluent was evaporated with a flow of nitrogen and redissolved in 300 µl MeOH and analysed by LC–MS.

3.2.6. Determination of Furfuryl Alcohol and Its Polymer in Roasted Coffee

40 g of green coffee were roasted in a household coffee roaster (i–Roaster 40211) at 210 °C for 2, 3, 4, 5 and 6 min. 1 g ground coffee was extracted with 10 ml methanol by vortexing for 2 min. 2 ml of the filtered extract were purified by SPE accucat (conditioned with 5 ml MeOH and 3 ml water). The purified extracts were analysed by LC–MS.

IV. RESULTS AND DISCUSSION

4.1. Polymerization of Furfuryl Alcohol

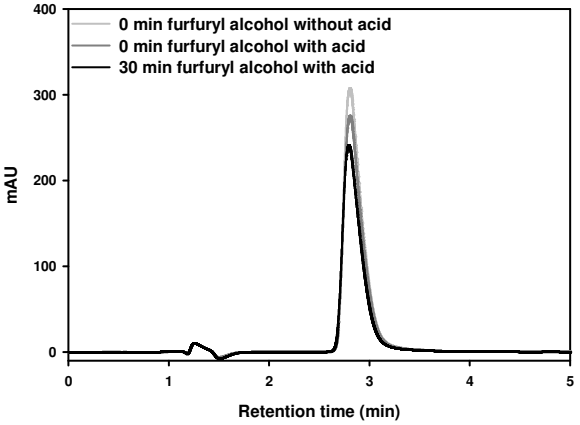


Figure 4.1.1. Furfuryl alcohol content during early polymerisation

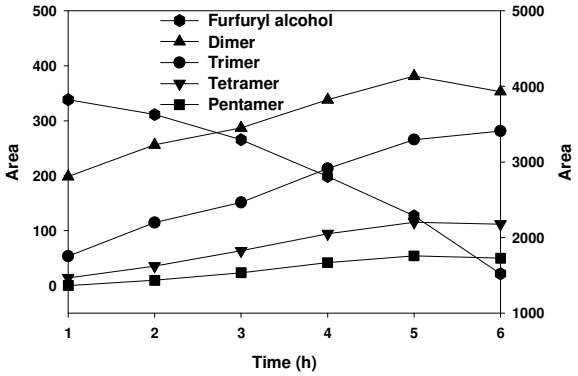


Figure 4.1.2. Kinetic of furfuryl alcohol polymerisation

The polymerisation of furfuryl alcohol starts directly after adding the acid. This is shown by the decreasing amount of furfuryl alcohol (Fig. 4.1.1) and the formation of the oligomers during the incubation (Fig. 4.1.2). At 1 h of polymerisation, tetrameric furfuryl alcohol already occurs in small amounts; however, the pentameric furfuryl alcohol is not yet formed (Fig. 4.1.2). In experiments without addition of acid for 6 h, incubation furfuryl alcohol polymerization was not

observed, whereas incubation with acid addition produced furfuryl alcohol oligomers (Fig. 4.1.3). The furfuryl alcohol oligomers could also be detected by mass spectrometry (Fig. 4.1.4).

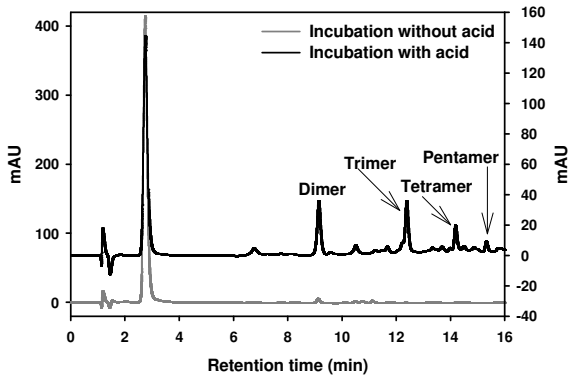


Figure 4.1.3. HPLC chromatogram of furfuryl alcohol polymerization at 22 °C for 6 h

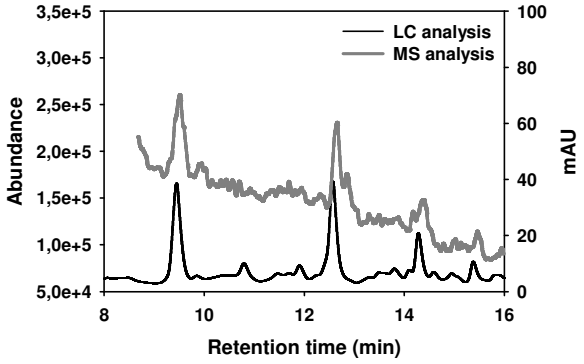


Figure 4.1.4. LC–MS chromatogram of oligomeric furfuryl alcohol

The major peaks 1, 2, 3, and 4 in the chromatogram obtained from the LC–MS analysis are identified as dimer, trimer, tetramer, and pentamer (Fig. 4.1.3) with the fragments of m/z 161 (Fig. 4.3.1.1), m/z 241 (Fig. 4.3.2.1), m/z 321 (Fig. 4.3.3.1), and m/z 401 (Fig. 4.3.3.2), respectively. The polymerization of furfuryl alcohol in acidic conditions proceeds by attaching hydroxyl group and

α–hydrogen of the heterocycle at carbon 5 and water elimination. Those two positions are the most reactive in furfuryl alcohol (Wallon et al., 1971). In addition, the activation energy for the initiation of the furfuryl alcohol polymerization is lower compared to the chain prolongation (Guigo et al., 2007). At the beginning of polymerization, the solution becomes green later gradually brown and finally dark brown in a solid form (Batista and Souza, 2000). The formation of the brown colour of aliphatic furfuryl alcohol oligomers starts with hydride–ion exchange that forms carbenium ions and its colour arises from the formation of conjugated sequences, as a result of successive proton and hydride ion losses (Choura et al., 1996). The polymer itself should not absorb in the visible range since the conjugated system is interrupted by methylene groups between every furan ring.

4.2. Fractionation of Furfuryl Alcohol Oligomers

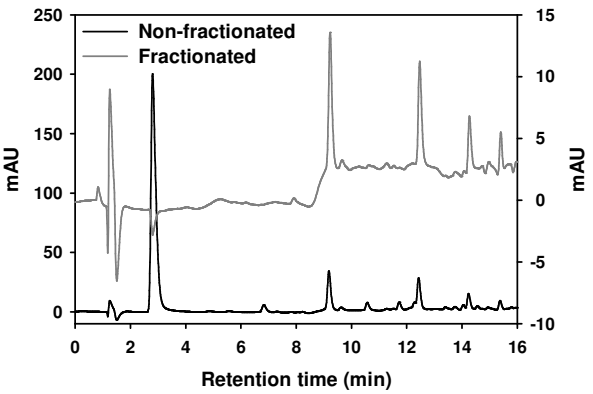


Figure 4.2.1. Fractionated furfuryl alcohol oligomers

The fractionated samples of the furfuryl alcohol oligomers also showed the same retention time as in the direct analysis. These conditions describe that those peaks

are oligomers and non–volatile compounds (Fig. 4.2.1). Moreover, all the peaks, except the oligomers, that were present in the direct analysis disappeared.

4.3. Structural Determination of Furfuryl Alcohol Oligomers

4.3.1. Dimeric Furfuryl Alcohol

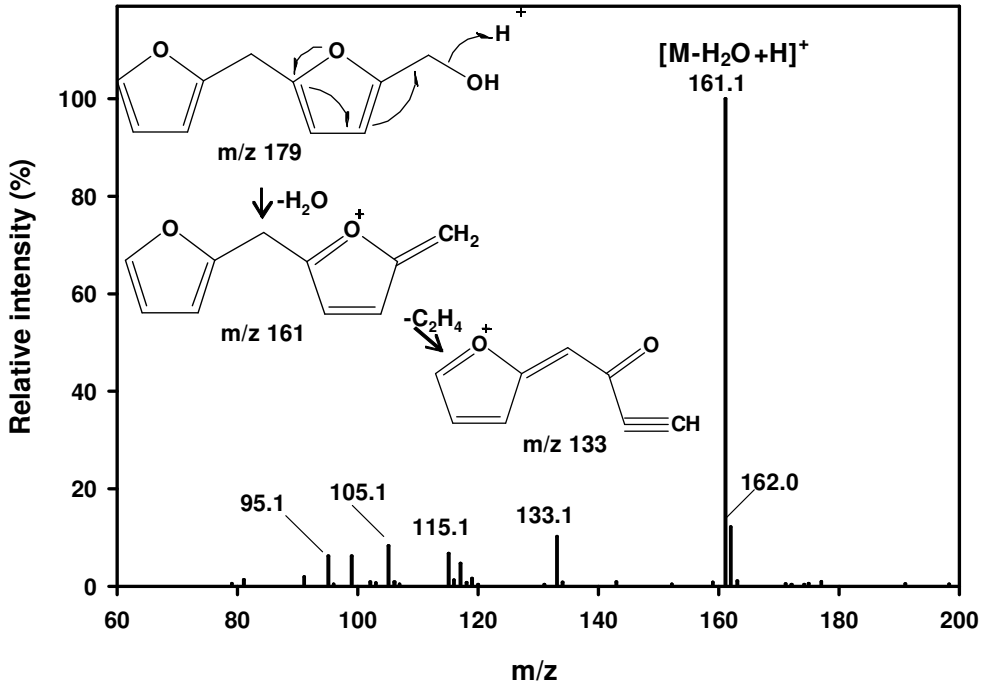


Figure 4.3.1.1. Suggested mechanism fragmentation of dimeric furfuryl alcohol in the ESI–MS

The ion 161 is formed by elimination of water $[M–18+H]^+$. Elimination of water in vicinal alcohols is common and the molecular ion (m/z 179) cannot be observed in the mass spectrum (Cooks, 1971). After elimination of water from the oligomers, an alkene that is attached to the O–bearing carbon is usually eliminated $[M–46+H]^+$ followed by elimination $[M–74+H]^+$ which could be seen in the mass spectrum as m/z 133 and m/z 105 with 70 V fragmentation voltage (Fig. 4.3.1.1).

Further fragmentation $[M–102+H]^+$ can be seen as ion with m/z 77 which is only formed when the fragmentation voltage is as high as 130 V (Fig. 4.3.1.3); at lower fragmentation voltages (e.g. 100 V) this fragment cannot be observed (Fig. 4.3.1.2). The elemental formula of this dimer is $C_{10}H_8O_2$.

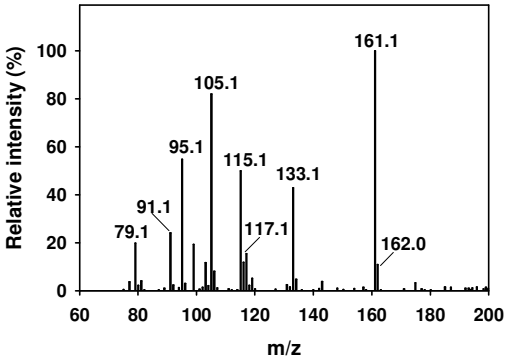


Figure 4.3.1.2. Fragmentation of dimeric furfuryl alcohol at 100 V in ESI–MS

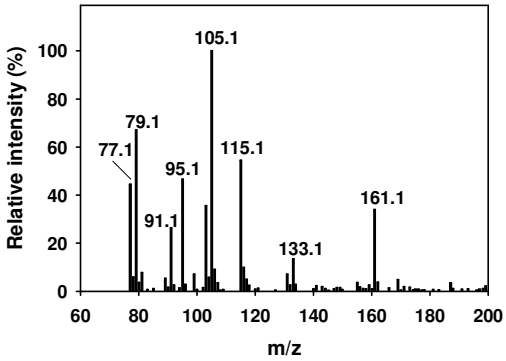


Figure 4.3.1.3. Fragmentation of dimeric furfuryl alcohol at 130 V in ESI–MS

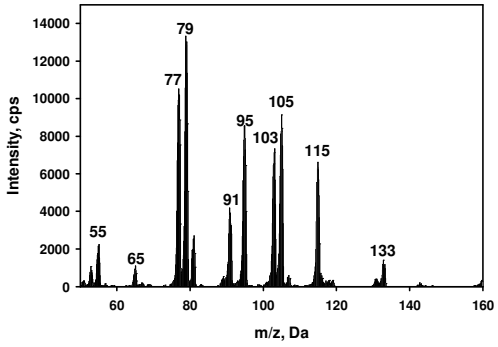


Figure 4.3.1.4. Product ion of dimeric furfuryl acohol in AB Sciex Q Trap LC/MS/MS

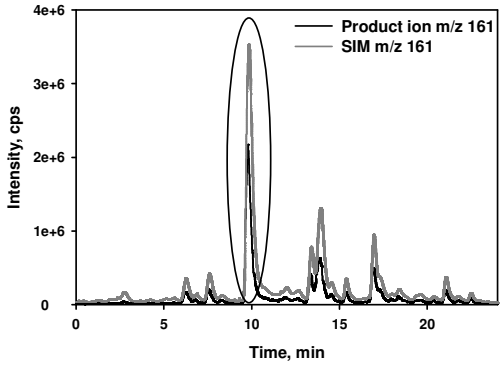


Figure 4.3.1.5. Chromatogram of the product ion of dimeric furfuryl alcohol in AB Sciex Q Trap LC/MS/MS

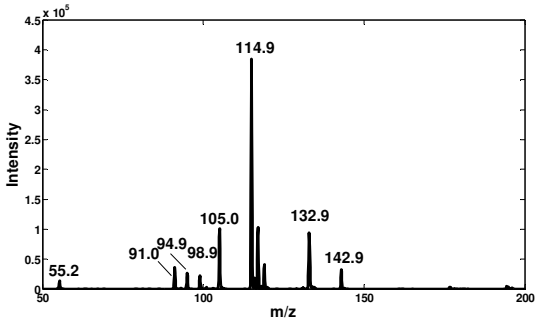


Figure 4.3.1.6. MS2 (product ion of m/z 161) of dimeric furfuryl alcohol in Agilent Ion Trap SL

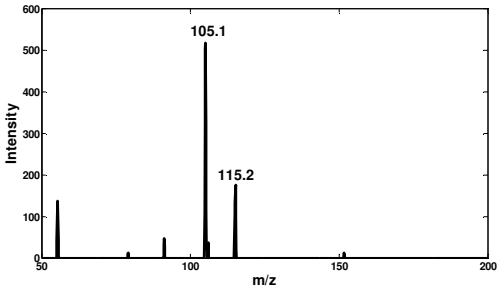


Figure 4.3.1.7. MS3 (fragmentation of m/z 133 from parent ion m/z 161) of dimeric furfuryl alcohol in Agilent Ion Trap SL

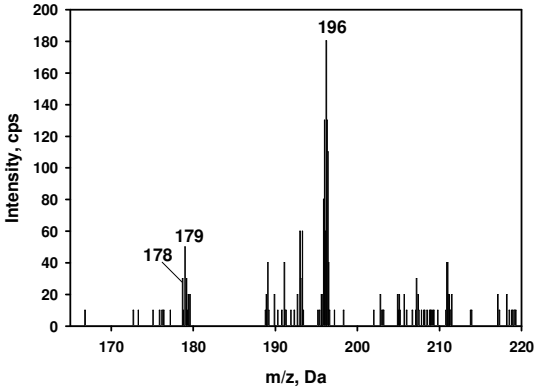


Figure 4.3.1.8. Precursor ion scan of dimeric furfuryl alcohol with AB Sciex Q Trap LC/MS/MS

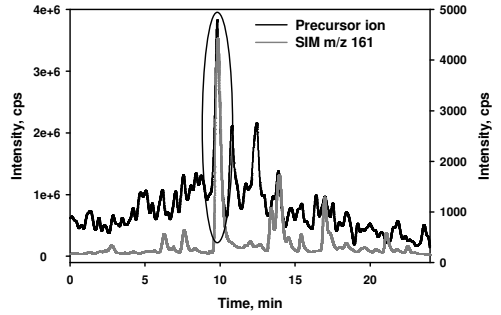


Figure 4.3.1.9. Precursor ion scan of dimeric furfuryl alcohol with AB Sciex Q Trap LC/MS/MS

The fragmentation pattern of dimeric furfuryl alcohol at 130 V in ESI–MS (MSD, Agilent) shows the same pattern as the product ion (m/z 161) of dimeric furfuryl analysed with AB Sciex Q Trap LC/MS/MS (Fig. 4.3.1.4) and it also has the same retention time as the SIM chromatogram of dimeric furfuryl alcohol (m/z 161) (Fig. 4.3.1.5). Moreover, the product ion m/z 161 obtained with the Agilent ion trap SL produces m/z 114.9 or m/z 115 as the most abundant fragment unlikely with the AB Sciex LC/MS/MS which produces m/z 105.1 as the most abundant fragment (Fig. 4.3.1.6). Nevertheless, fragmentation of m/z 113 (fragment

obtained from m/z 161; MS3 m/z 161) produces m/z 105.1 as the most abundant fragment (Figs. 4.3.1.7). In addition, the parent ion of m/z 161 can be analysed by AB Sciex LC/MS/MS which shows as protonated adduct at m/z 179 and as ammonium adduct at m/z 196 (Fig. 4.3.1.8) and it also have the same retention time as SIM m/z 161 (Fig. 4.3.1.9).

4.3.2. Trimeric Furfuryl Alcohol

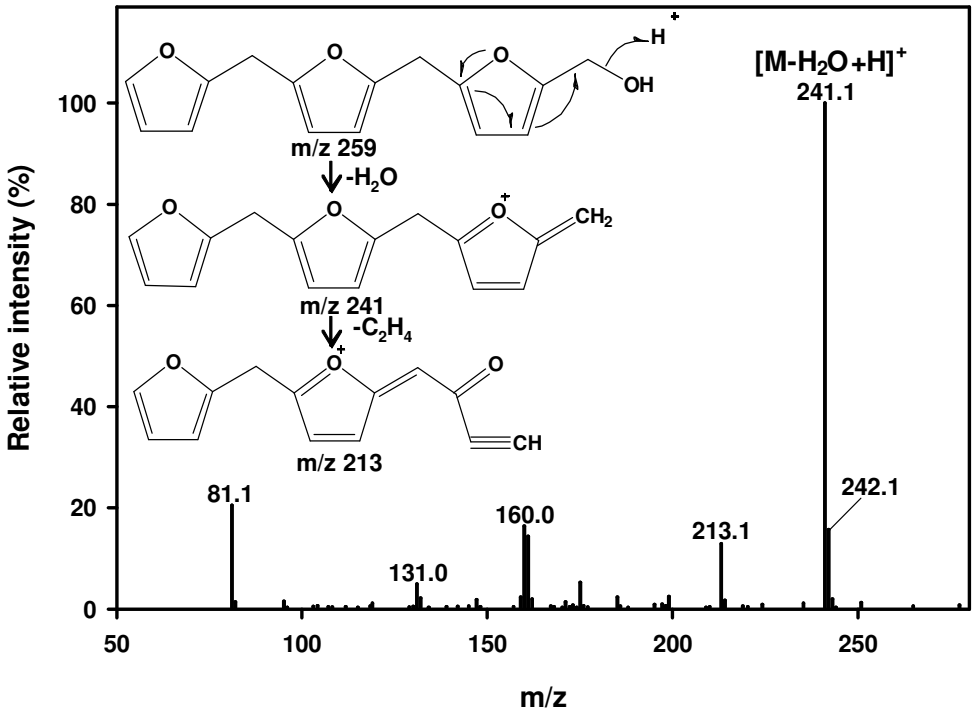


Figure 4.3.2.1. The suggested mechanism fragmentation of trimeric furfuryl alcohol at 100 V in the ESI–MS

Trimeric furfuryl alcohol cannot be fragmented at a fragmentation voltage of 70 V. At least 100 V is needed to obtain collision induced fragments (Fig. 4.3.2.1) and with an increasing fragmentation at 130 V (Fig. 4.3.2.2) produced more

fragments. This oligomeric furfuryl alcohol also loses water during ionization. Analogous to the other oligomers, the molecular ion (m/z 259) cannot be seen. Trimeric furfuryl alcohol fragments into m/z 29 + R_1 + R_2 (59, 73, 87,…) (Cooks, 1971) and it can be seen in the mass spectrum with masses of m/z 241, m/z 213; the subsequent fragmentation m/z 199 is not formed extensively with 100 V fragmentation voltage, but it could be seen at higher voltages (e.g. 130 V) (Fig. 4.3.2.2). The elemental formula of this trimer is $C_{15}H_{12}O_3$

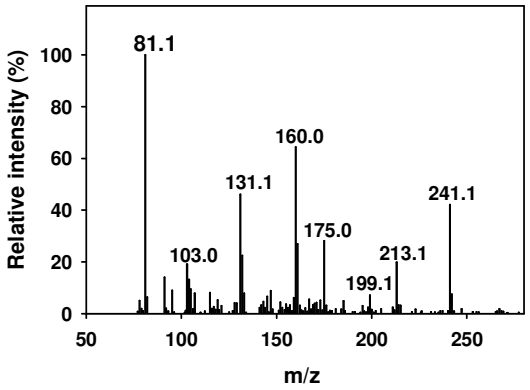


Figure 4.3.2.2. Fragmention of trimeric furfuryl alcohol at 130 V in ESI–MS

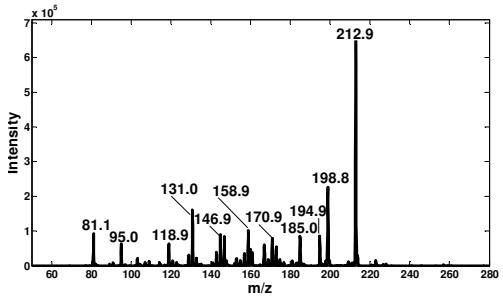


Figure 4.3.2.3. MS2 (product ion of m/z 241) trimeric furfuryl alcohol in Agilent Ion Trap SL

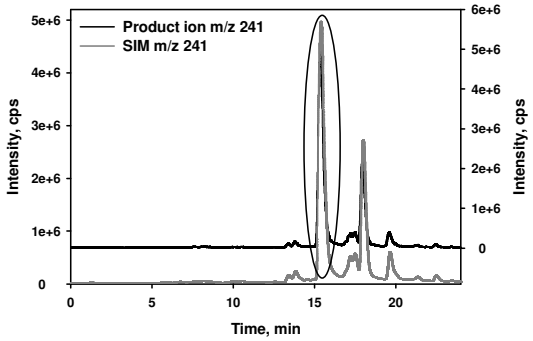


Figure 4.3.2.4. Chromatogram product ion of trimeric furfuryl alcohol in AB Sciex Q Trap LC/MS/MS

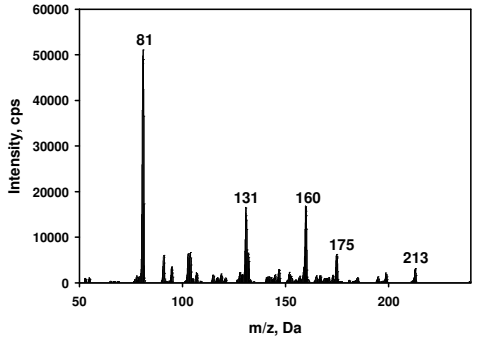


Figure 4.3.2.5. Product ion trimeric of furfuryl acohol in AB Sciex Q Trap LC/MS/MS

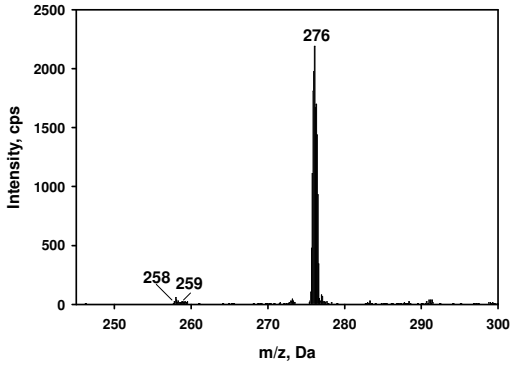


Figure 4.3.2.6. Precursor ion of trimeric furfuryl alcohol in AB Sciex Q Trap LC/MS/MS

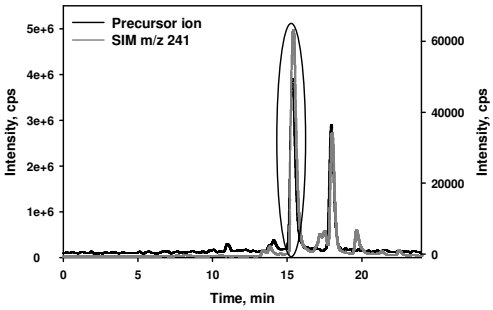


Figure 4.3.2.7. Chromatogram precursor ion of trimeric furfuryl alcohol in AB Sciex Q Trap LC/MS/MS

Moreover, the fragmentation patterns of trimeric furfuryl alcohol in ESI–MS or Agilent ion trap SL (Fig. 4.3.2.3.) or AB Sciex (Fig. 4.3.2.5) are similar but Agilent ion trap SL produce m/z 213 from m/z 241 in very high intensity. The precursor ion or molecular ion of trimeric furfuryl alcohol can also be seen in AB Sciex analysis (Fig. 4.3.2.6), m/z 259, and it has the same retention time as m/z 241 (Fig. 4.3.2.7). The m/z 276 is a trimeric furfuryl alcohol with ammoiun adduct.

4.3.3. Tetrameric and Pentameric Furfuryl Alcohol

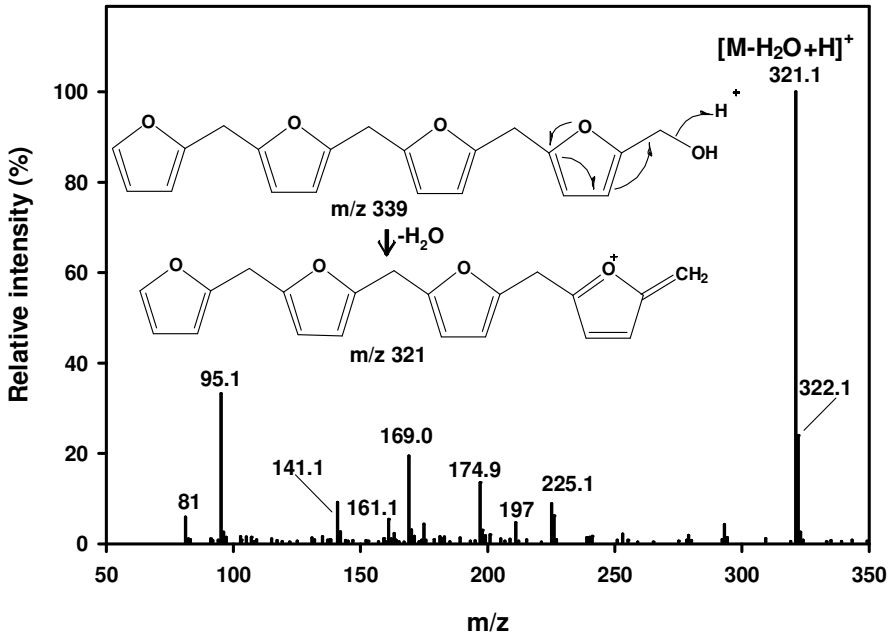


Figure 4.3.3.1. The suggested mechanism fragmentation of tetrameric furfuryl alcohol at 130 V in the ESI–MS

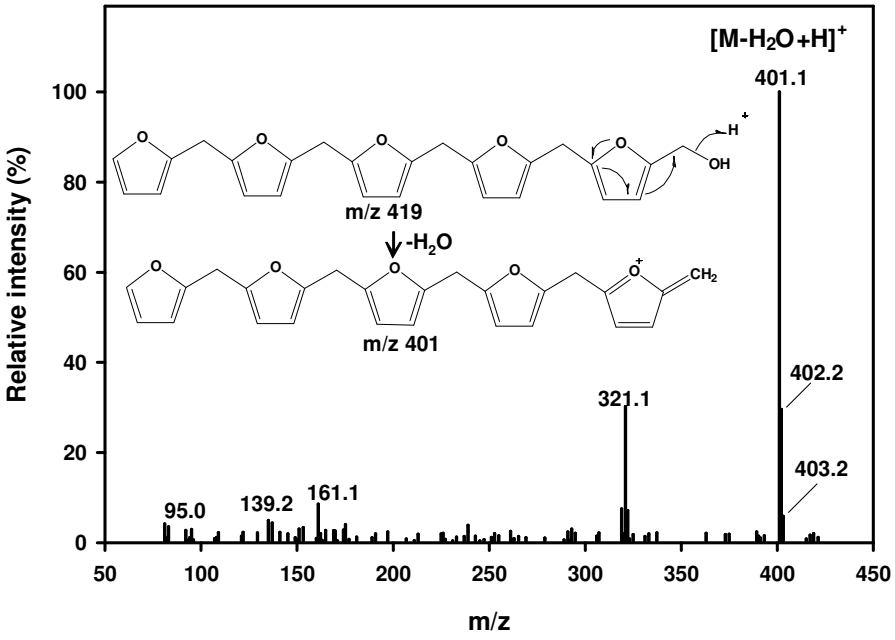


Figure 4.3.3.2. The suggested mechanism fragmentation of pentameric furfuryl alcohol at 100 V in the ESI–MS

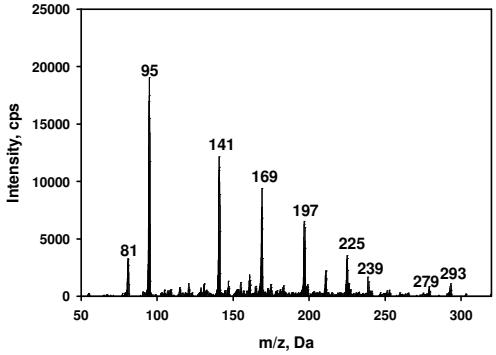


Figure 4.3.3.3. Product ion scan of tetrameric furfuryl alcohol

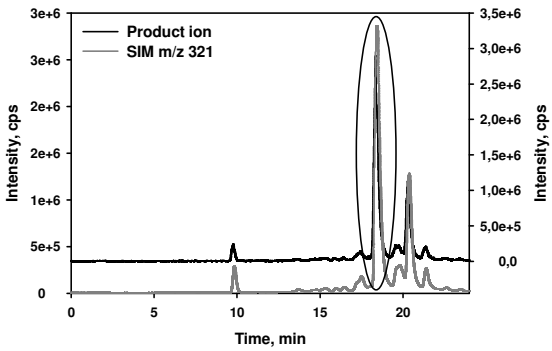


Figure 4.3.3.4. Chromatogram product of ion of tetrameric furfuryl alcohol

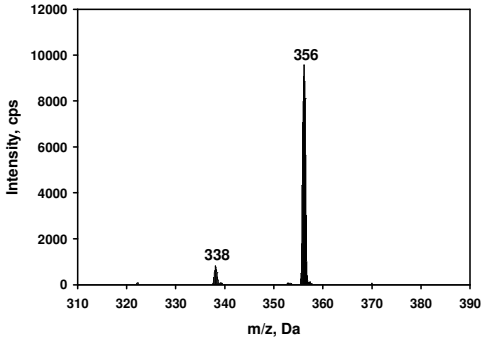


Figure 4.3.3.5. Precursor ion scan of tetrameric furfuryl alcohol

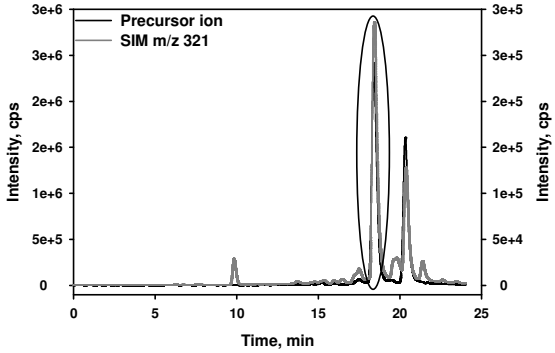


Figure 4.3.3.6. Chromatogram of the precursor ion of tetrameric furfuryl alcohol

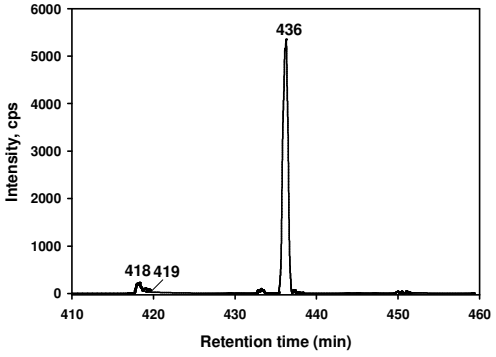


Figure 4.3.3.7. Precursor ion pentameric furfuryl alcohol

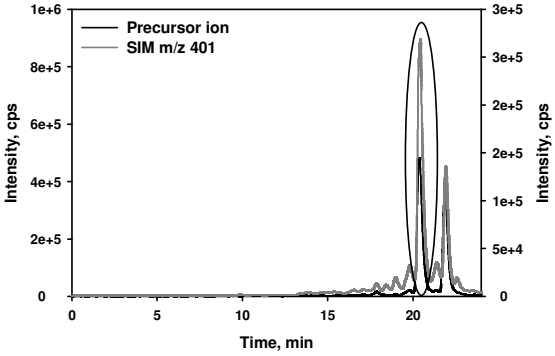


Figure 4.3.3.8. Chromatogram precursor ion of pentameric furfuryl alcohol

Tetrameric and pentameric furfuryl alcohol need a fragmentation voltage of 130 V to induce fragmentation (Figs. 4.3.3.1, 4.3.3.2). The tetrameric and pentameric furfuryl alcohol also looses water during ionization in mass spectrometry analysis.

Moreover, the fragmentation pattern of the tetrameric furfuryl alcohol in LC–MS and in AB Sciex Q Trap LC/MS/MS is similar (Fig. 4.3.3.3) and the product ion also has the same retention time with the SIM m/z 321 (Fig. 4.3.3.4). The molecular ion of tetrameric and pentameric furfuryl alcohol also can be seen in AB Sciex Q Trap analysis and its intensity are higher with the ammonium adduct (Figs. 4.3.3.5, 4.3.3.7) and also it has the same retention as the SIM of tetrameric or pentameric base peaks (Figs. 4.3.3.6, 4.3.3.8).

4.4. The Type of Linkage of Furfuryl Alcohol Oligomers

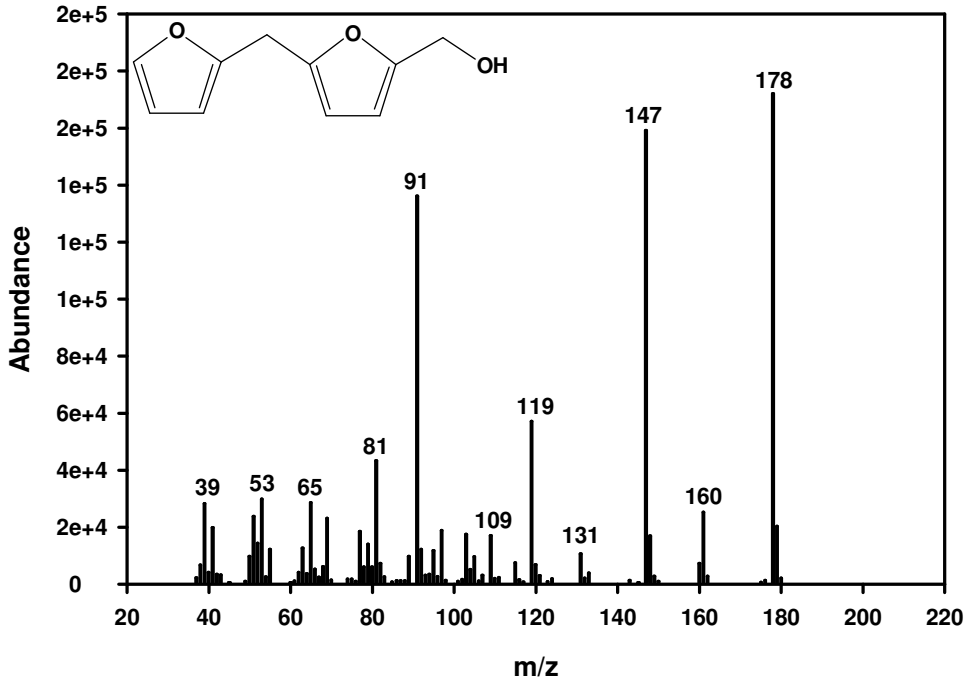


Figure 4.4.1. Dimeric furfuryl alcohol with methylene linkage in GC–MS

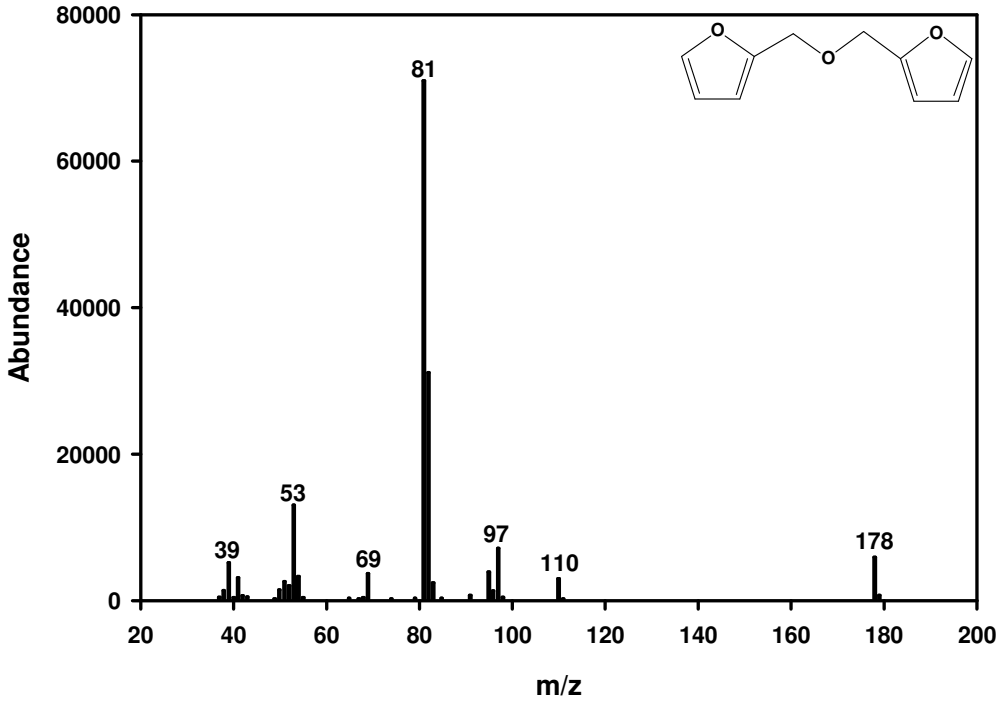


Figure 4.4.2. Dimeric furfuryl alcohol with dimethylene ether linkage in GC–MS

The type of linkage that is formed during polymerization of furfuryl alcohol in 1 M HCl was identified by interpretation of the fragment ions. A dimer of furfuryl alcohol with methylene linkage will eliminate water during mass spectrometric analysis (Fig. 4.4.1). In contrast, the dimer with a dimethylene ether linkage will not eliminate water (Loughran et al., 1972) and it has a different way of fragmentation as can be seen from the dimeric furfuryl alcohol analysed with GC/MS (Fig. 4.4.2).

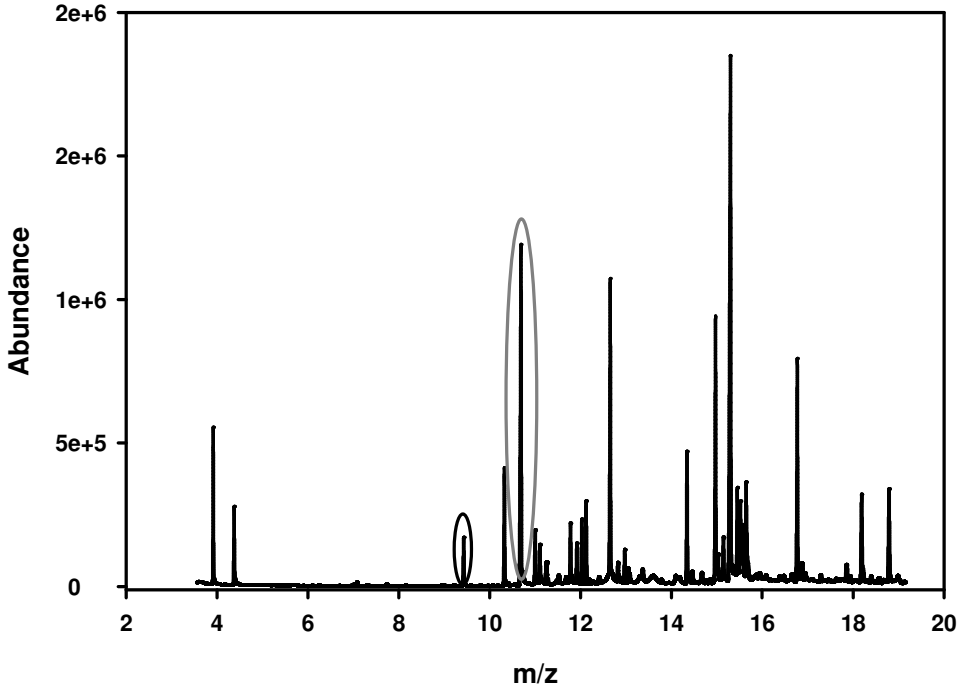


Figure 4.4.3. GC analysis of dimeric furfuryl alcohol

The formation of furfuryl alcohol oligomers with methylene linkages is significantly higher and it seems to be favourable in the furfuryl alcohol polymerization. From GC/MS results it can be concluded that the amount of the dimer with methylene lingkage (grey curve) is higher than that of the dimer with dimethylene ether linkage (black curve) (Fig. 4.4.3). This condition is in line with earlier results that found that methylene linkage is more abundant then dimethylene ether linkage in furfuryl alcohol oligomers (Fawcett and Dadamba, 1982).

4.5. Furfuryl Alcohol and Its Oligomers in Roasted Coffee Beans

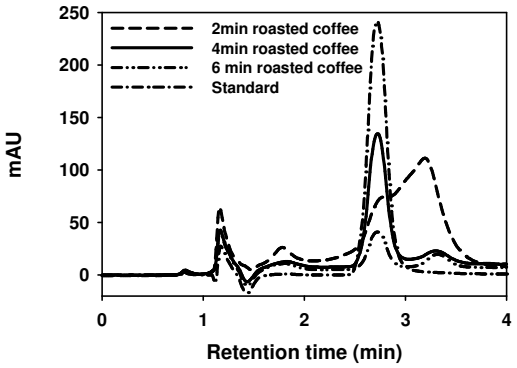


Figure 4.5.1. HPLC chromatogram of the formation of furfuryl alcohol during roasting of coffee at 210 °C

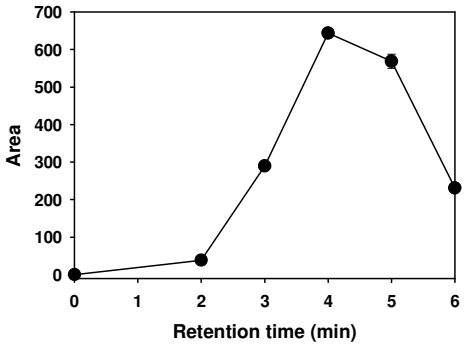


Figure 4.5.2. The kinetic of production of furfuryl alcohol during roasting of coffee at 210 °C measured by LC–UV

Green coffee (unroasted) does not contain any furfuryl alcohol. However, furfuryl alcohol is formed during roasting. After 2 min of roasting at 210 °C the formation of furfuryl alcohol starts with a significant increase with a decline after 5 min (Figs. 4.5.1, 4.5.2). Furfuryl alcohol is formed from glucose or fructose via the intermediate 1,2–enediol (Brands and van Boekel, 2001) or from quinic acid via a different pathway (Moon and Shibamoto, 2010).

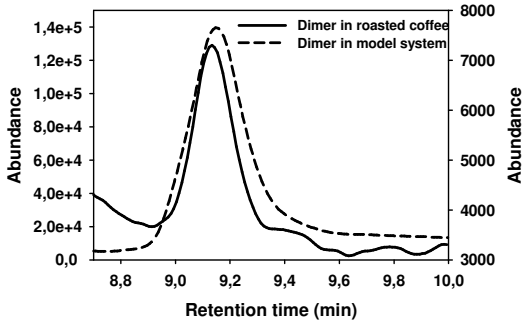


Figure 4.5.3. The chromatogram of dimeric furfuryl alcohol in roasted coffee at 210 °C measured by SIM ESI–MS

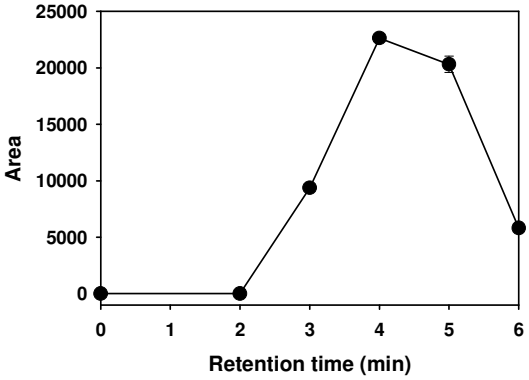


Figure 4.5.4. The kinetic of the production of dimeric furfuryl alcohol during roasting of coffee at 210 °C measured by SIM ESI–MS

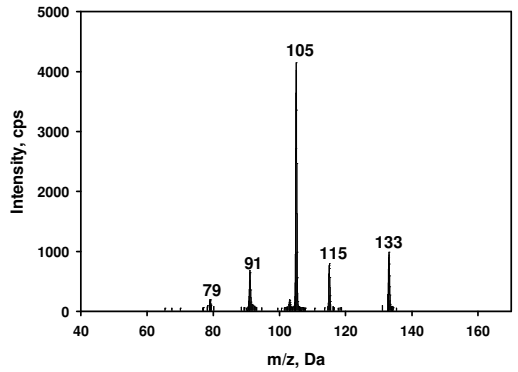


Figure 4.5.5. MS3 of dimeric furfuryl alcohol in model system analysed by AB Sciex Q Trap LC/MS/MS

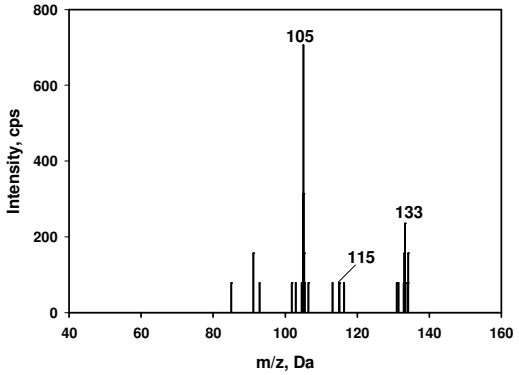


Figure 4.5.6. MS3 of dimeric furfuryl alcohol in roasted coffee analysed by AB Sciex Q Trap LC/MS/MS

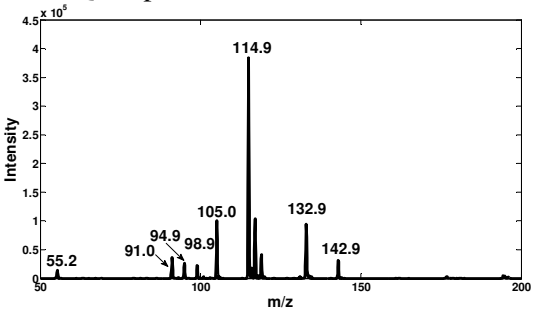


Figure 4.5.7. Product ion scan (MS2) of dimeric furfuryl alcohol in model system analysed by Agilent Ion Trap SL

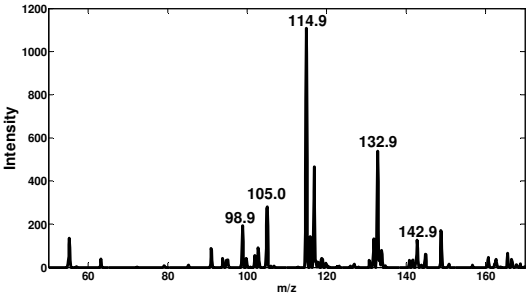


Figure 4.5.8. Product ion scan (MS2) of dimeric furfuryl alcohol in roasted coffee analysed by Agilent Ion Trap SL

The formation of the dimer starts after 3 min of roasting having a maximum at 4 min; longer roasting results in a decrease of the dimer probably due to further polymerization or degradation (Figs. 4.5.3, 4.5.4). Moreover, the fragmentation pattern of the dimeric furfuryl alcohol is the same as the fragmentation pattern of the dimeric furfuryl alcohol in model system (Figs. 4.5.5, 4.5.6, 4.5.7, 4.5.8). This means that furfuryl alcohol polymerizes during roasting in acidic conditions. The low pH may arise through degradation of 1,2–enediols that produces formic acid and also through degradation of 2,3–enediols that produce acetic acid during the heat treatment of sugar containing foods (Brands and Boekel, 2001). Therefore, we conclude that furfuryl alcohol is able to polymerize under these conditions and contributes to the formation of the brown colour.

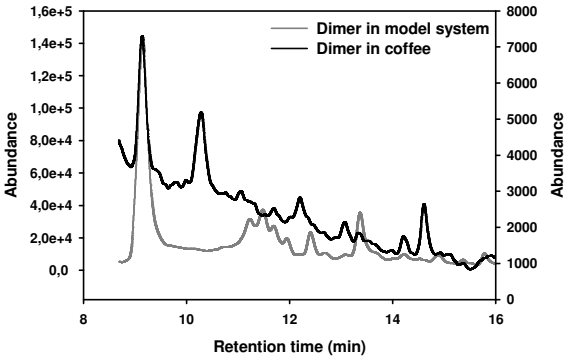


Figure 4.5.9 SIM chromatogram of dimeric furfuryl alcohol roasted coffee in LC–MS

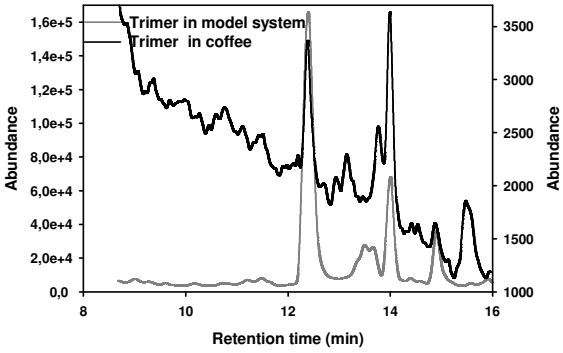


Figure 4.5.10. SIM chromatogram of trimeric furfuryl alcohol roasted coffee in LC–MS

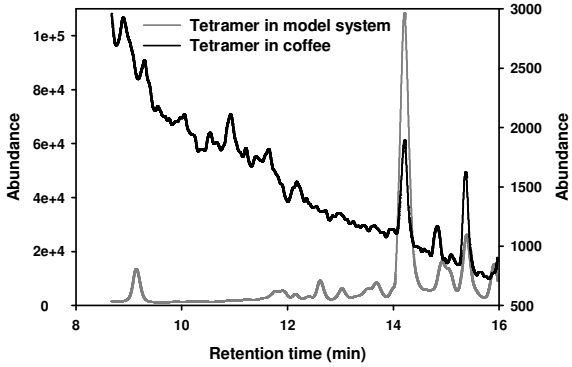


Figure 4.5.11. SIM chromatogram of tetrameric furfuryl alcohol roasted coffee in LC–MS

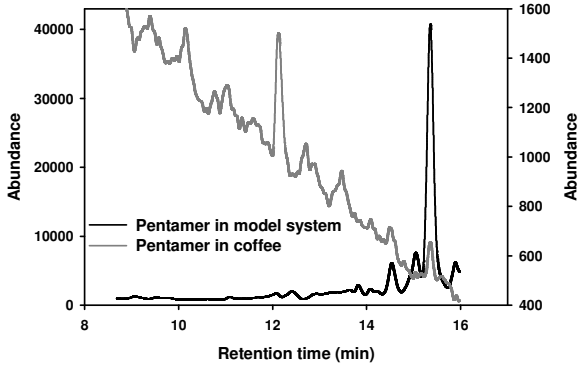


Figure 4.5.12. SIM chromatogram of pentameric furfuryl alcohol roasted coffee in LC–MS

However, in only once sample of coffee – roasted at 210 °C for 4 min –dimeric, trimeric, tetrameric, and a small amount of pentameric furfuryl alcohol was identified (Figs. 4.5.9, 4.5.10, 4.5.11, 4.5.12). Dimeric until pentameric furfuryl alcohol was isolated by Barr and Wallon (1971) during the polymerisation furfuryl alcohol in acidic conditions.

V. CONCLUSION

The polymerization of furfuryl alcohol in acidic conditions leads to the formation of oligomers. The structures that were identified by mass spectrometric experiments contained 2 to 5 units of furfuryl alcohol with methylene linkage. Higher degrees of oligomerization were not found. The concentration of the oligomers increased during the course of the reaction. The dimer of furfuryl alcohol and furfuryl alcohol itself are both found in roasted coffee. The polymers of furfuryl alcohol can also contribute to the brown colour of roasted coffee

VI. REFERENCE

Galceran, M.T. & Puignou, L., (2006), Latest Development in the Analysis of Heterocyclic Amines in Cooks Food. In: Skog, K., Alexander, J.(Eds.), Acrylamide and Other Hazardous Compounds in Heat–Treated Foods. Woodhead Publishing, Cambridge, pp. 68 – 116.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S. & Törnqvist, M., (2002), Analysis of Acrylamide, A Carcinogen Formed in Heated Foodstuffs. J. Agric. Food Chem., 50, 4998 – 5006.

Perez Locas, C. & Yaylayan, V.A., (2004), Origin and Mechanistic Pathways of Formation of the Parent Furans – A Food Toxicant. J. Agric. Food Chem., 52, 6830 – 6836.

Glatt, H. & Sommer, Y., (2006), Health Risk of 5–Hydroymethylfurfural (HMF) and Related Compound. In: Acrylamide and Other Hazardous Compounds in Heat–Treated Foods. Woodhead Publishing, Cambridge, pp. 328 – 357.

Lee, S.M., Seo, B.C. & Kim, Y., (2006), Volatile Compounds in Fermented and Acid–hydrolyzed Soy Sauces. J. Food Sci., 71, 3, 146 – 156.

Wang, Y. & Kays, S.J., (2000), Contribution of Volatile Compounds to the Characteristic Aroma of Baked 'Jewel' Sweetpotatoes. J. Amer. Soc. Hort. Sci. 125, 638 – 643.

Bonvehì, J.S., (2005), Investigation of Aromatic Compounds in Roasted Cocoa Powder. Eur. Food Res. Technol., 221, 19 – 29.

Karagu–Yüceer, Y., Cadwallader, K.R. & Drake, M., (2002), Volatile Flavour Components of Stored Non–fat Dry Milk. J. Agric. Food Chem., 50, 305 – 312.

Kreppenhofer, S., Frank, O. & Hofmann, T., (2011), Identification of (Furan–2yl) Methylated Benzene Diols and Triols as a Novel Class of Bitter Compounds in Roasted Coffee. Food Chem., 126, 441 – 449.

Joint FAO/WHO expert committee on food additives, (2000). Fifty–fifth Meeting. Geneva. FAO and WHO.

Brands, C.M.J. & Boekel, M.A.J.S., (2001), Reactions of Monosaccharides during Heating of Sugar–Casein Systems: Building of a Reaction Network Model. J. Agric. Food Chem., 49, 4667 – 4675.

Moon, J. & Shibamoto, T., (2010), Formation of Volatile Chemicals from Thermal Degradation of Less Volatile Coffee Components: Quinic Acid, Caffeic Acid, and Chlorogenic Acid. J. Agric. Food Chem., 58, 5465 – 5470.

Choura, M., Belgacem, N.M. & Gandini, A., (1996), Acid–catalysed Polycondensation of Furfuryl Alcohol: Mechanisms of Chromospheres Formation and Cross–Linking. Macromolecules, 29, 3839 – 3850.

Nursten, H., (2005), The Maillard Reaction Chemistry, Biochemistry and Implications. The Royal Society of Chemistry, United Kingdom.

Borrelli, R.C., Visconti, A., Mennella, C., Anese, M., Fogliano, V., (2002), Chemical Characterization and Antioxidant Properties of Coffee Melanoidins. J. Agric. Food Chem., 50, 6527 – 6533.

De Bruijn, J. M.., Kieboom, A. P. G., Van Bekkum, H., Van Der Poel, P. W. 1986. Reactions of Monosaccharides in Aqueous Alkaline Solutions. Sugar Technol. Rev. 13, 21 – 52.

Guigo, N., Mija, A., Vincent, L. and Sbirrazzuoli, N., (2007), Chemorheological Analysis and Model–Free Kinetics of Acid Catalysed Furfuryl Alcohol Polymerization. Phys. Chem. Chem. Phys., 9, 5359 – 5366.

Kim, T., Assary, R.S., Marshall, C.L., Gosztola, D.J., Curtiss, L.A. and Stair, P.C., (2011), Acid–Catalyzed Furfuryl Alcohol Polymerization: Characterizations of Molecular Structure and Thermodynamic Properties. Chem.Cat.Chem., 3, 1451 – 1458.

Bertarione, S., Bonino, F., Cesano, F., Damin, A., Scarano, D. and Zecchina, A., (2008), Furfuryl Alcohol Polymerization in H–Y Confined Spaces: Reaction Mechanism and Structure of Carbocationic Intermediates. J. Phys. Chem. B. 112, 2580 – 2589.

Golubkova, T., (2011), Bildung von Potentiell Toxischen Furanderivaten in Lebenmitteln. Diplomarbeit. Institut für Biochemie TU Graz. Austria. pp. 38 – 40.

Moon, J. and Shibamoto, T., (2009), Role of Roasting Conditions in The Profile of Volatile Flavor Chemicals Formed from Coffee Beans. J. Agric. Food Chem., 57, 5823 – 5831.

Buttery, R.G., Orts, W.J., Takeoka, G.R. & Nam, Y., (1999), Volatile Flavour Components of Rice Cakes. J. Agric. Food Chem., 47, 4353 – 4356.

Jensen, S., Ostdal, H., Skibsted, L.H. & Thybo, A.K., (2011), Antioxidants and Shelf Life of Whole Wheat Bread. Journal of Cereal Science, 53, 292 – 297.

Vázquez, L., Verdú, A., Miquel, A., Burl, F. & Carbonell–Barrachina, A.A., (2007), Changes in Physico–Chemical Properties, Hydroxymethylfurfural and Volatile Compounds During Concentration of Honey and Sugars in Alicante And Jijona Turrón. Eur. Food Res. Technol., 225, 757 – 767.

Vázquez–Araújo, L., Enguix, L., Verdú, A., Garcıá–Garcıá, E. & Carbonell–Barrachina, A.A., (2008), Investigation of Aromatic Compounds in Toasted Almonds Used for The Manufacture of Turrón. Eur. Food Res. Technol., 227, 243 – 254.

Park, D. & Maga, J.A., (2006), Identification of Key Volatiles Responsible For Odour Quality Differences in Popped Popcorn of Selected Hybrids. Food Chem., 99, 538 – 545.

Buttery, R.G. and Ling, L.C., (1998), Additional Studies on Flavor Components of Corn Tortilla Chips. J. Agric. Food Chem., 46, 2764 – 2769.

Ho, C.W., Aida, W.M.W., Maskat, M.Y. & Osman, H., (2007), Changes in Volatile Compounds of Palm sap (Arenga Pinnata) During the Heating Process for Production of Palm Sugar. Food Chem., 102, 1156 – 1162.

Castro–Vázquez, L., Díaz–Maroto. L.C. & Pérez–Coello, MS., (2007), Aroma Composition and New Chemical Markers of Spanish Citrus Honeys. Food Chem., 103, 601 – 606.

Takeoka, G., Perrino Jr, C. & Buttery, R., (1996), Volatile Constituents of Used Frying Oils. J. Agric. Food Chem., 44, 654 – 660.

Munro, I. C. and Danielewska–Nikiel, B. 2006. Comparison of Estimated Daily Intakes of Flavouring Substances with No–Observed–Effect Levels. Food Chem. Toxicol., 44,758 – 809.

Monien, B.H., Hermann, K., Florian, S. and 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 Advance Access, 32, 1533 – 1539.

NTP., (1999), Toxicology and Carcinogenesis Studies of Furfuryl Alcohol (CAS No.98–00–0) in F344/N Rats and B6C3F1 Mice (inhalation studies). Natl. Toxicol. Program Tech. Rep. Ser. NTP – Department of Health and Human Services, 482.

Batista, P.S. & Souza, M.F., (2000), Furfuryl Alcohol Conjugated Oligomer Pellicle Formation. Polymer, 41, 8263 – 8269.

Wallon, S.B., Barr, J.B. & Petro, B.A. (1971). A Study of Furfuryl Alcohol Resin Components by Gel Permeation Chromatography. J. Chromatogr, 54, 33 – 41.

Fawcett, A.H. & Dadamba, W., (1982), Characterisation of Furfuryl Alcohol Oligomers by 1H and 13C NMR Spectrometry. Die Makromolekulare Chemie, 183, 2799 – 2809.

Pretsch, E., Badertscher, M. & Affolter, C., (2009), Structure Determination of Organic Compounds. Springer, pp.354.

Loughran, E.D., Wewerka, E.M. & Hammons, G.J., (1972), A Mass Spectrometer Study of Substituted Furfuryl Compounds. J. Heterocycl. Chem., 9, 57 – 65.

Wnorowski, A. & Yaylayan, V.A., (2000), Influence of Pyrolytic and Aqueous–Phase Reactions on the Mechanism of Formation of Maillard Products. J. Agric. Food Chem., 48, 3549 – 3554.

Cooks, R.G., (1971), in The Chemistry of the Hydroxyl Group, ed. S. Patai, Interscience, London, pp. 1045.

Glatt, H., (2000), Sulfotransferases in the Bioactivation of Xenobiotics. Chemico–Biological Interactions, 129, 141 – 170.

Hodge, J.E., (1953), Dehydrated Foods. Chemistry of Browning Reactions in Model Systems, J. Agric. Food Chem., 1, 928 – 943.

Dunlop, A.P. & Peters, F.N., (1953), In: The Furans. Reinhold Publishing Co., New York.

Barr, J.B. & Wallon, S.B., (1971), The Chemistry of Furfuryl Alcohol Resins J. Appl. Polym. Sci., 15, 1079 – 1090.

Appendix

1. The formation of oligomers in model system

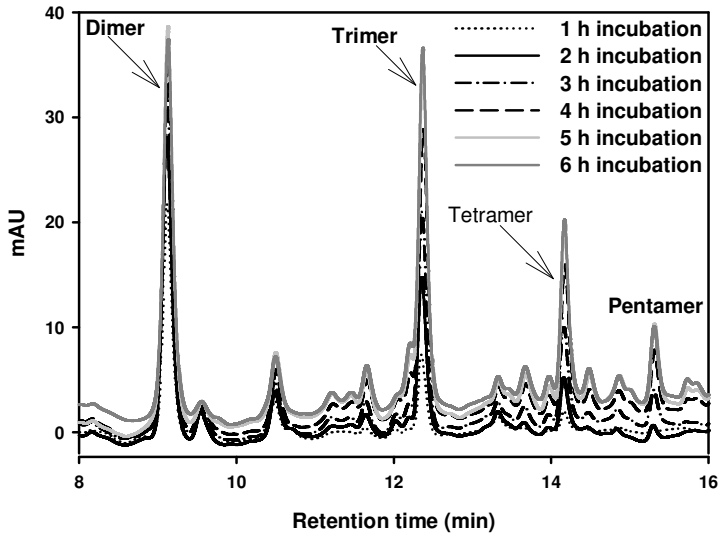


Figure 1. HPLC separation chromatogram of furfuryl alcohol oligomers

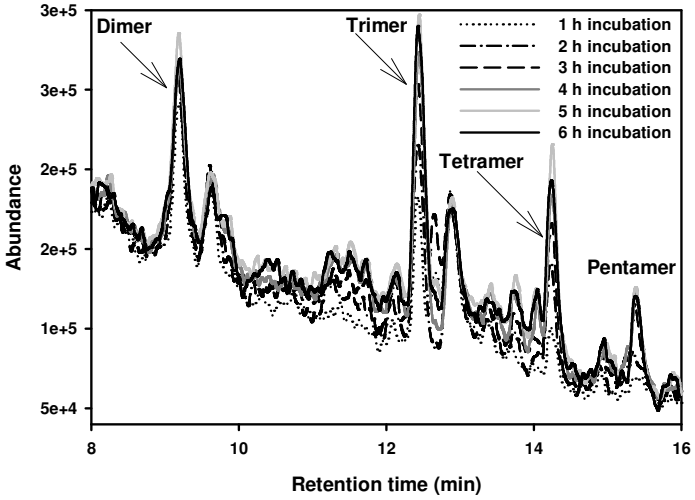


Figure 2. SIM chromatogram of furfuryl alcohol oligomers

2. Furfuryl alcohol oligomers and roasted coffee samples in Agilent Ion Trap SL analysis

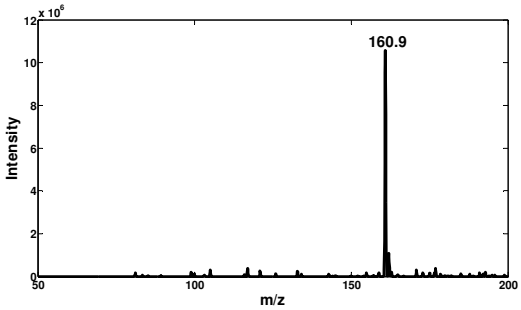


Figure 3. MS1 of dimeric furfuryl alcohol in Agilent Ion Trap SL

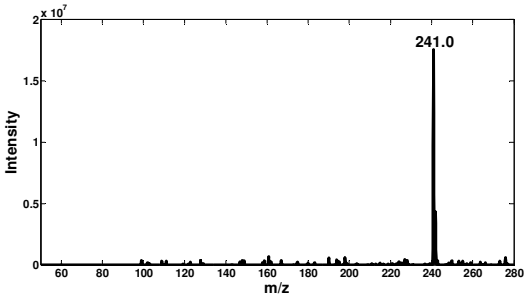


Figure 4. MS1 of trimeric furfuryl alcohol in Agilent Ion Trap SL

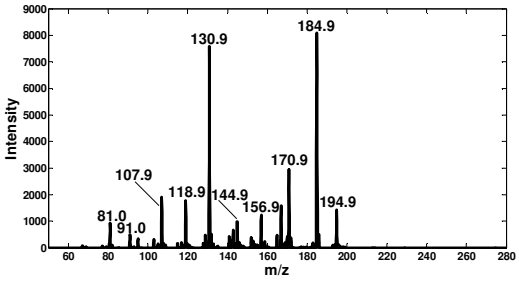


Figure 5. MS3 of trimeric furfuryl alcohol in ion trap analysis

3. Furfuryl alcohol oligomers and roasted coffee samples in AB Sciex Q Trap LC/MS/MS

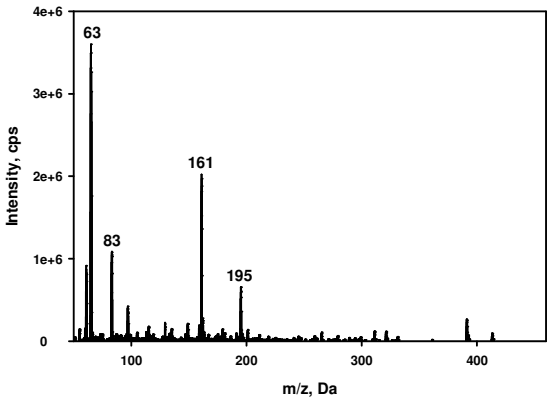


Figure 7. Scan chromatogram of dimeric furfuryl alcohol

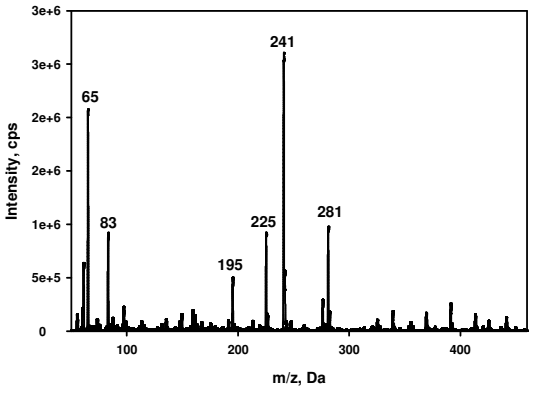


Figure 8. Scan chromatogram of trimeric furfuryl alcohol

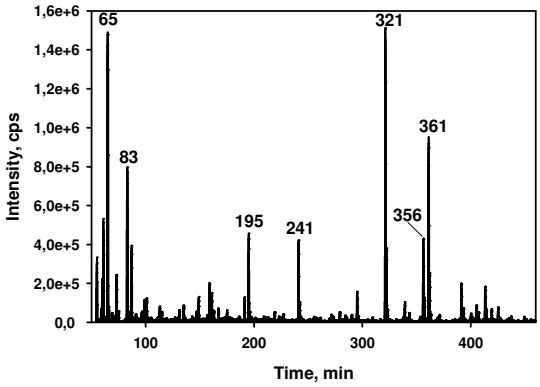


Figure 9. Scan chromatogram of tetrameric furfuryl alcohol

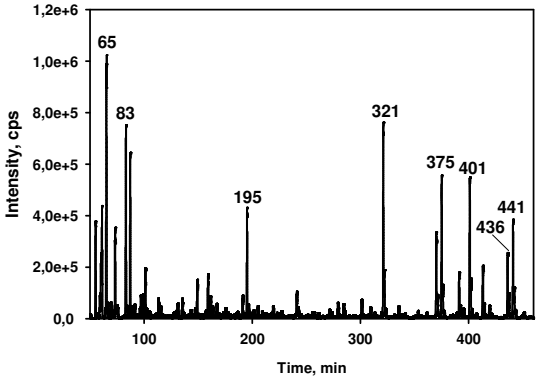


Figure 10. Scan chromatogram of pentameric furfuryl alcohol

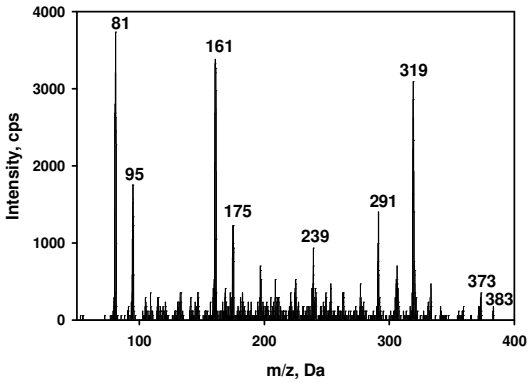


Figure 11. Product ion of pentameric furfuryl alcohol

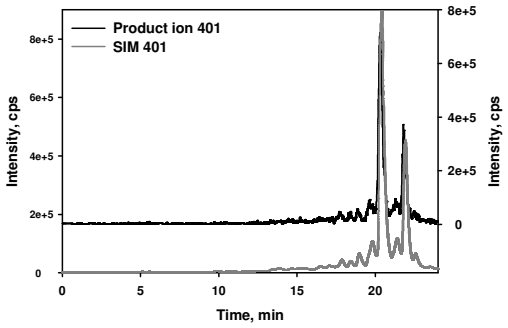


Figure 12. Product ion chromatogram of pentameric furfuryl alcohol

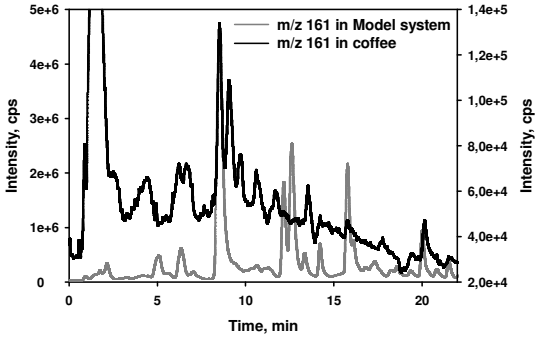


Figure 13. Dimeric furfuryl alcohol in roasted coffee analysed by AB SCiex Q Trap LC/MS/MS

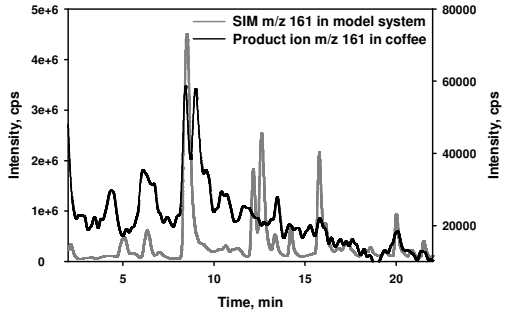


Figure 14. Chromatogram product ion dimeric furfuryl alcohol in AB SCiex Q Trap LC/MS/MS

SECTION 3

METABOLITES OF FURAN DERIVATIVES IN HUMAN URINE

I. INTRODUCTION

Heat processing is widely used for foods preparation. Besides the sensory and textural properties that are desired, some toxic and also mutagenic compounds also occur, such as 5–hydroxymethylfurfural (HMF), furfuryl alcohol, furfural, 5–methylfurfuryl alcohol, 5–methylfurfural. Those compounds are furan derivatives that occur in the present of carbohydrate, protein, acid and heat treatment. Murkovic and Pichler (2006) found that 80 % foods already analysed contain HMF. HMF is easily metabolized and 95 – 100 % from the administration dose will be eliminated through urine within 24 h. The major metabolite of HMF is N–(5–hydroxymethyl–2–furoyl)glycine (Germond et al., 1987). Furfuryl alcohol and furfural are also easily metabolized and the metabolite mostly conjugated with glycine. Moreover, 83 – 88 % from the administration dose of furfuryl alcohol and furfural are eliminated through urine within 72 h (Nomeir et al., 1992). Besides that, 5–methylfurfuryl alcohol and 5–methylfurfural metabolite (5-methyl furoic acid) is also predicted to be present.

In this chapter, the methods of separation of conjugated furan derivative metabolites with glycine and the methods to identify those furan derivative metabolites will be described. It is important since it is necessary to know all possible of these metabolites and compare it to the dietary intake. Then the metabolic flow of the furan derivative will be clearer.

II. LITERATURE REVIEW

2.1. Alcohol Metabolism

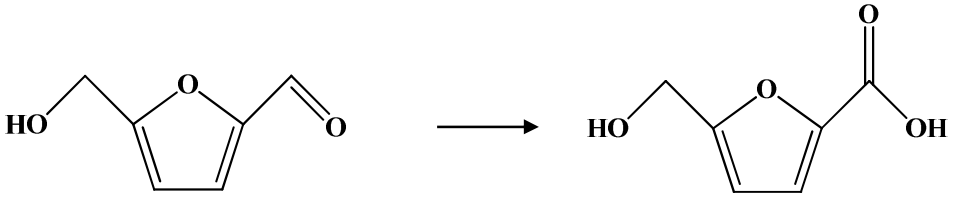
Elimination alcohol mostly takes place in liver through oxidative pathway by alcohol dehydrogenase (ADH) perform the major role in the first pathway at low to moderate level substrate and it will loose hydrogen and electrons during the reaction (Cederbaum, 1995; Crabb, 1995; Cornell et al., 1979; Havre et al, 1977); ADH needs the coenzyme NAD^+ in its oxidation reaction (Lieber, 1976). Oxidation of alcohol not only occurs in liver, but also in the gastrointestinal tract with lower ethanol metabolism than in liver and the enzymes that take part are ADH and the microsomal ethanol oxidizing system (MEOS) with some bacteria contributing to this degradation (Seitz et al., 1994). The activity of this oxidation pathway is influenced by the diet, endocrine activity, type of ADH isoenzyme, and smoking habits (Crabb et al, 1987). The concentration of the substrate and product during oxidation through ADH activity is in line with the elimination of ethanol (Bosron et al., 1983). The oxidation reaction of ethanol can be seen in the chemical reaction below.

$CH_3CH_2OH + NAD^+ \longrightarrow CH_3CH{=}O + NADH + H^+$ (Lieber, 1976)

2.2. HMF Metabolism in Human

HMF is not metabolized completely, but there are still 0.75 % unmetabolized because HMF found in urine after 6 hours of eating of 20 g roll with plum jam that contain 24 mg HMF (Murkovic and Pichler, 2006). Using NMR analysis, N–(5–hydroxymethyl–2–furoyl)glycine is the major HMF metabolite found in rat urine (Germond et al., 1987).

The human body able to metabolize HMF to HMFA and furan–2,5–dicarboxylic acid in the range of 38–78 % of the HMF (Jellum et al. 1973). The conversion of HMF to HMFA is catalysed by aldehyde dehydrogenase, Fig. 2.2.1 (Kopmann et al., 2010). Prior et al. (2006) found that the recovery of HMF after administration of dried plum juice (3994 mmol HMF/ml and 486 mmol HMF/ml) to humans within 6 h is 46.2 % and 14.2 %, respectively; The major metabolite was 5–hydroxymethyl–2–furoic acid and followed by N–(5–hydroxmethyl–furoyl)glycine. Besides that, HMFA reaches the maximum content after 30 min of administration and declines at 60 min after administration. The recovery of the HMF metabolite within 48 h after oral administration in rat and mice is higher than in human (60 – 80 %; Godfrey et al., 1999). Moreover, Germond et al. (1987) found that 95 – 100 % of the HMF metabolites can be excreted after 24 h of administration in rats. The human body is able to metabolize only ca. 50 % of the HMF that is infused and excreted in oxidized form (HMFA). The metabolite that was excreted was not in conjugated form (Eldjarn et al., 1974).

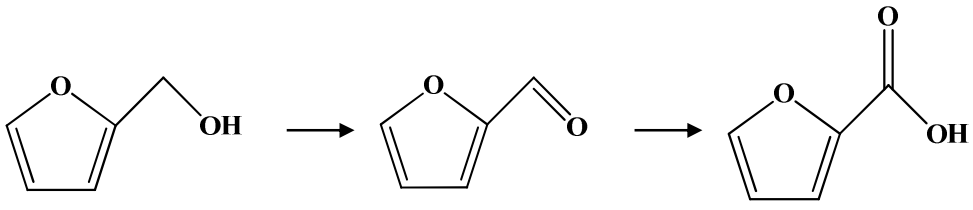


HMF HMFA

Figure 2.2.1. The metabolism mechanism of HMF (Kopmann et al., 2010)

2.3. Furfural and Furfuryl Alcohol Metabolism in Humans

Furfural and furfuryl alcohol can be absorbed up to 86 – 89 % in the gastrointestinal tract and after 72 h administration the highest level of those furan derivatives can be found in the liver and kidney and also small amounts in the brain. Furfural and furfuryl alcohol are then excreted as furoylglycine (73 – 80 %), furoic acid (1 – 6 %), furanacrylic acid (3 – 8 %) up to 83 – 88 % through urine and 2 – 4 % through feces and by exhaling CO_2 (Nomeir et al., 1992). Parkash and Cadwell (1994) found that furfural is also effectively metabolized to furoylglycine and furanacryloylglycine with a recovery more than 90 %. Kopmann et al.(2010) found that firstly furfuryl alcohol will be converted to furfural by the activity of alcohol dehydrogenase and then furfural is converted to 2-furoic acid by aldehyde dehydrogenase activity, Fig. 2.3.1.



Furfuryl alcohol **Furfural** **2–Furoic acid**

Figure 2.3.1. The metabolism mechanism of furfuryl alcohol and furfural (Kopmann et al., 2010)

2.4. Furoic Acid

Furoic acid is a stable heterocycle because the carboxyl group attaches to it and it also readily esterified but is does not undergo Diels–Alder reactions (Gandini and Belgacem, 1997). Furoic acid is found in heated L–ascorbic acid and in heated carbohydrates as intermediate product (Yaylayan, 2006). Arribas–Lorenzo and Morales (2010) found furoic acid in ground coffee and its level is nearly the same as of HMFA. Furoic acid is also found naturally in the human urine with around 15 mg/g creatinine (ACGIH, 1994). The amount of urinary furoic acid is linier with the furfuryl alcohol exposure in male Wistar rats (Savolainen and Pfäfflli, 1983). Mostly, furoic acid in urine is conjugated with glycine (Tan et al., 2003). Therefore, urine samples need to be hydrolyzed with 8 M NaOH for 1 h at 95 °C and then neutralized with 7.3 M HCl and extracted with ethyl acetate (Pfäffli et al., 1985). Extraction with ethyl acetate for HMFA analysis is more efficient than with solid phase extraction (Mardens et al, 1992).

2.5. 5–Hydroxymethyl–2–Furoic Acid (HMFA)

5–Hydroxymethyl–2–furoic acid (HMFA) is found in roasted coffee arabica and robusta can be formed through aldol condensation and ketalisation reaction between glyceraldehyde and pyruvate which are already present in green coffee. Glyceraldehyde is formed through degradation sucrose at 240 °C for 1 – 5 min (Murkovic and Bornik, 2007). The pyruvate originates from the green coffee (Bähre and Meier, 1999). Estimated combined HMF and HMFA intake is 37 mg/day, but the concentration of HMFA is lower than HMF (Husøy et al., 2008).

Jöbstl et al. (2010) found that the concentration of HMFA ranges from not detectable up to 100 µg HMFA/ml urine and most of the frequent concentration is 10 µg HMFA/ml urine in 300 urine samples. HMF and HMFA are not produce endogenously in human (Husøy et al., 2008); HMFA is of dietary origin (Pettersen and Jellum, 1972). Mrochekand and Rainey, 1972 found that the HMFA is conjugated with glycine in normal patient urine and lymphocytic leukemia patients urine. Moreover, the amount of excreted HMFA from healthy individuals urine is 1 to 25 mg/24 h. In GC/MS analysis, HMFA will firstly loose water and continue to loose the aldehyde group, carboxyl group, and carbonyl group (Mrochekand and Rainey, 1972).

2.6. Positive Effect of Furoic Acid and HMFA in Human Health

Oral administration of 2–furoic acid and HMFA to rats is able to reduce plasma non–esterified fatty acid (NEFA) (Kagami et al., 2008); this ability is inline with nicotinic acid which has a chemical structure alike with those furan derivatives. The decrease of NEFA level will increase insulin activity (Karpe and Frayn, 2004). 2–Furoic acid is also able to reduce serum cholesterol and serum triglyceride in rats and also elevated HDL cholesterol because 2–furoic acid interferes with intracellular enzyme activity. However, 2–furoic acid causes hepatic toxicity with LD_{50} 250 mg/kg in mice (Hall et al. 1993).

III. MATERIALS AND METHODS

3.1. Materials

Hydrochloric acid (HCl) 32 % was purchased from Merck (Darmstadt, Germany), methanol HPLC grade was purchased from Mallinckrodt Baker (The Netherlands), 100 % acetic acid was purchased from Roth (Karlsruhe, Germany), sodium hydroxide was purchased from Riedel–de Haën (Seelze, Germany), ethyl acetate optigrade was purchased from Protochem (Wesel, Germany), 5–hydroxymetyl–2–furoic acid was purchased from Matrix Scientific, 2–furoic acid and 5–metyl–2–furoic acid were purchased from Sigma Aldrich. The urine samples were obtained from volunteers after drinking one cup of normal coffee.

3.2. Methods

3.2.1. 5–Hydroxymethyl–2–furoic Acid and Furoic Acid Analysis

Urine samples were alkalinised and neutralised using the method that was already established by Pfäffli et al. (1985). The neutral urine sample was then extracted 3 times using ethyl acetate to have optimum extraction of the furan derivatives metabolites and then it was dried using nitrogen and diluted again using solution of 5 % methanol and 0.04 % acetic acid in water. 5 µl sample then injected to HPLC Agilent 1100 using gradient elution. HPLC condition: column Lichospher RP–18 (125 × 3 mm, 5 µm), column temperature 25 °C, 255 nm, Solvent A: 5 % methanol and 0.04 % acetic acid in water, Solvent B: 100 % methanol. Gradient condition: 0 – 4 min 100 % solvent A and 0 % Solvent B, 4 – 12 min

0 % solvent A and 100 % solvent B; the post time was 6 min. The diluted standards in water were also analysed by the same treatment as urine.

3.2.2. 5–Methyl–2–Furoic Acid

5–Methyl–2–furoic acid was diluted in water and analysed using HPLC Agilent 1100 which were coupled with AB Sciex Q Trap LC/MS/MS system. Separation was done using 5 µl injection volume, column temperature 25 °C, Column LiChroCART 55–2 Purospher® STAR MERK, isocratic elution 35 % solvent A, 1 % solvent B, 64 % solvent C. Solvent A: 5 % MeOH and 0.04 % acetic acid in water; B: 2 % Acetic Acid in water; C: 100 % MeOH. AB Sciex Q Trap A LC/MS/MS conditions: curtain gas (CUR) 30 °C, ion spray voltage (IS) 5500, collision gas (CAD) Medium, temperature (TEM) 250 °C, ion gas source 1 (GS1) 30 °C, ion gas source 2 (GS2) 30 °C, resolution 1. The urine sample that was already alkalized, neutralized, and extracted was also analysed using HLPC Agilent 1100 which was coupled with AB Sciex Q Trap LC/MS/MS with gradient elution and with 10 µl injection volume. Gradient elution: 0 – 2.5 min 89 % solvent A, 1 % solvent B, 10 % solvent C, 2.5 – 4 min 35 % solvent A, 1 % solvent B, 64 % solvent C, 4 – 5.2 min 2 % solvent B and 98 % solvent C.

IV. RESULTS AND DISCUSSIONS

4.1. Furan Derivative Metabolites with HPLC Analysis

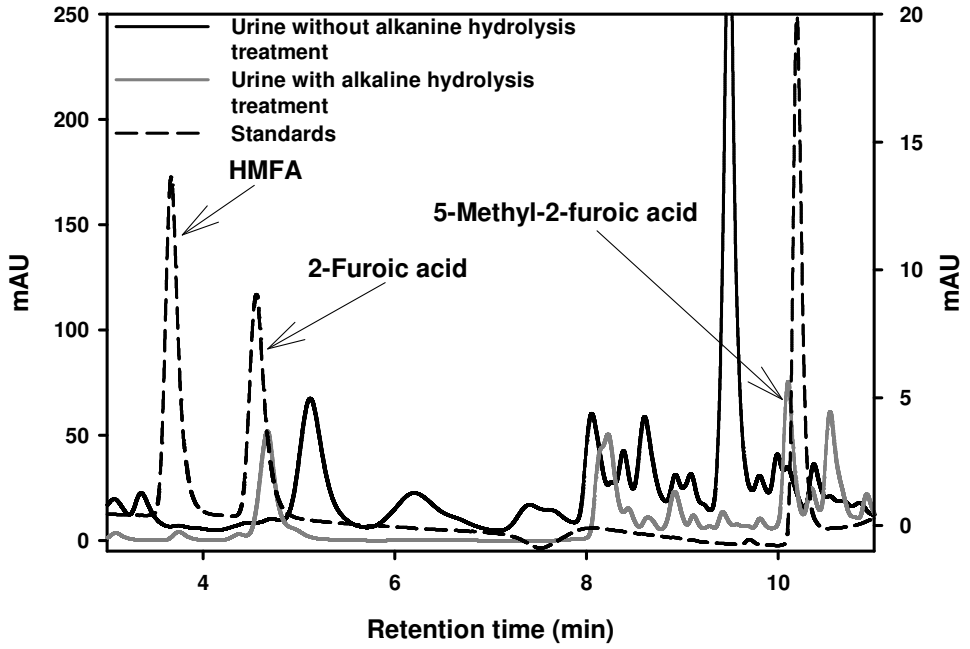


Figure 4.1.1. HPLC chromatogram of urine with and without alkaline hydrolysis treatment

Furfuryl alcohol is predicted to be metabolized in the same way as alcohol in human. The metabolism mostly happens in the liver and starts with oxidation of the hydroxyl group by alcohol dehydrogenase to produce furfural (Cederbaum, 1995; Crabb, 1995; Cornell et al., 1979). Furfural will then undergo further oxidation by the presence of oxygen and water producing 2–furoic acid (Kopmann et al., 2010). Mostly, 2–furoic acid is conjugated with glycine producing furoylglycine and the rest is in the unconjugated form and eventually will be eliminated through urine (Nomeir et al., 1992).

In addition, HMF will be metabolized by oxidation in the presence of oxygen and water to produce HMFA. HMFA will be conjugated with glycine and some of it will be eliminated unconjugated through the urine. The conjugation of HMFA is depending on the availability of glycine (Godfrey et al., 1999).

The furan derivative metabolites from urine only can be analysed after alkaline hydrolysis treatment. Urine without alkaline hydrolysis treatment does not show any furan derivatives metabolite although from the same urine (Fig. 4.1.1). This is may be due the conjugation of furan derivatives metabolite with glycine. Therefore, alkaline hydrolysis treatment is needed to break the conjugation between furan derivative metabolites and glycine.

4.2. Recovery of Furan Derivative Metabolites Diluted in Water with Alkaline Hydrolysis Treatment

Table. 4.2.1. Recovery after alkaline hydrolysis treatment

Standard	Extraction	Area		Recovery (%)
	First	294.914		66.02
	Second	218.584		
	Third	111.988		
HMFA, RT	Fourth	53.3308	678.8168	
	First	339.532		38.46
	Second	59.2686		
	Third	1.97169		
Furoic acid, RT	Fourth	0	400.77229	
	First	1088.83		85.51
	Second	89.7671		
	Third	5.93044		
5–Methyl–2–furoic acid, RT	Fourth	1.97113	1186.49867	
	First	321.305		64.33
	Second	187.645		
	Third	53.5035		
HMFA, 95 °C	Fourth	53.5035	661.4432	
	First	202.479		26.16
	Second	63.7367		
	Third	5.0078		
Furoic acid, 95 °C	Fourth	1.43668	272.66018	
	First	825.492		69
	Second	118.854		
	Third	10.8798		
5–Methyl–2–furoic acid, 95 °C	Fourth	1.65101	956.87681	

Table 4.2.2. Standard residue after alkaline hydrolysis treatment

Standard residues	Area
HMFA, RT	35.7512
Furoic acid, RT	0
5–Methyl–2–furoic acid, RT	0
HMFA, 95 °C	33.7351
Furoic acid, 95 °C	0
5–Methyl–2–furoic acid, 95 °C	0

Table 4.2.3. Recovery after Alkaline Hydrolysis Treatment at 95 °C in First Extraction

Slope / Treatment	HMFA	2–Furoic acid
Standard in water	3086.3	3162.9
Standard in water after alkaline hydrolysis	1720.7	2375
Recovery (%)	**55.73**	**75.1**
Standard in Urine after alkaline hydrolysis	1818.9	2471.4
Recovery (%)	**58.93**	**78.14**

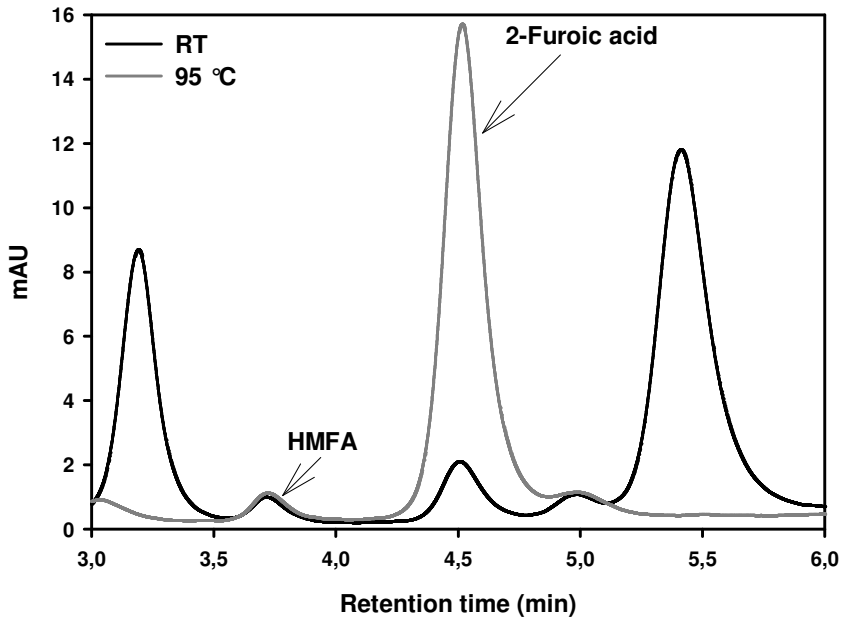


Figure 4.2.1. HPLC chromatogram of HMFA and 2–furoic acid after alkaline hydrolysis at RT and 95 °C in urine

Alkaline hydrolysis of furan derivative metabolites diluted in water at room temperature gives higher recovery than alkaline hydrolysis at 95 °C (Tab. 4.2.1). 5–Methyl–2–furoic acid has the highest recovery followed by HMFA and then the lowest recovery is 2–furoic acid (Tab. 4.2.1); although there are some residue of

HMFA (Tab. 4.2.2). However, alkaline hydrolysis treatment in urine at 95 °C produces higher amount of 2–furoic acid than treatment at room temperature; the HMFA level also slightly higher in 95 °C than treatment at room temperature (Fig. 4.2.1). Moreover, alkaline hydrolysis treatment at 95 °C to furan derivative metabolite standards diluted in water and furan derivative metabolite standards diluted in urine gives higher recovery in furan derivative metabolite standards diluted in urine than standard diluted in water. This means that the urine contain furan derivative metabolites that already separated from glycine conjugation (Tab. 4.2.3).

4.3. HMFA, 2–Furoic Acid, 5–Methyl–2–Furoic Acid in Urine using HPLC Analysis

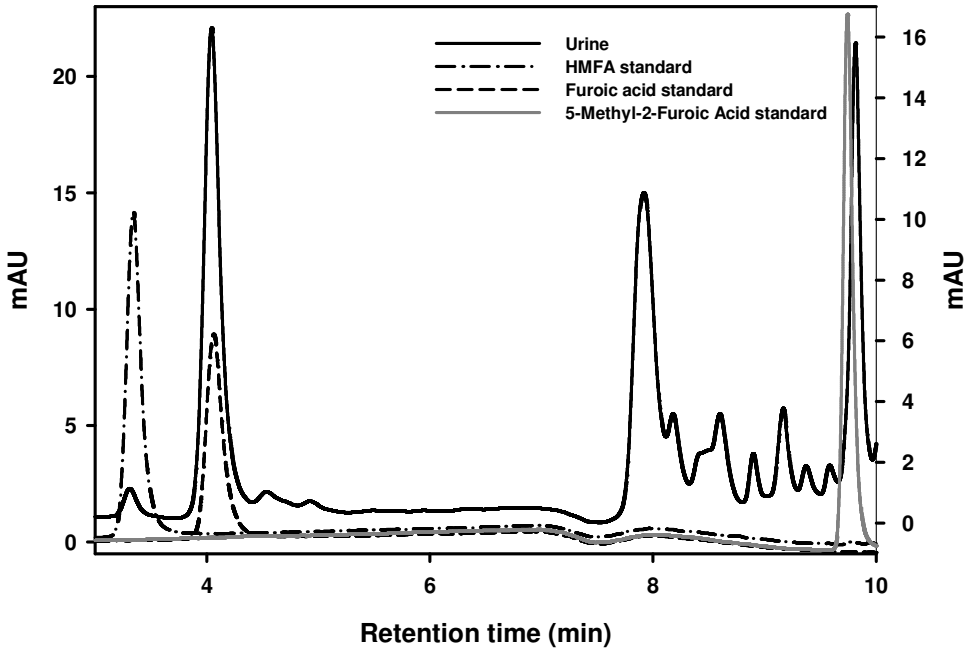


Figure 4.3.1. HPLC chromatogram of urine undergo alkaline hydrolysis treatment at 95 °C

Alkaline hydrolysis at 95 °C for 1 h it is needed to break the conjugation between furan derivatives metabolite and glycine. Furan derivative metabolites that were previously analysed were 5–hydroxymethyl–2–furoic acid (HMFA) which is a metabolite from HMF, 2–furoic acid as metabolite from furfuryl alcohol as well from furfural, and 5–methyl–2–furoic acid as metabolite from 5–methyl–2–furaldehyde as well as 5–methylfurfuryl alcohol. The furan derivatives metabolites from urine which already underwent alkaline hydrolysis could be separated from each other using gradient elution with a mobile phase that contains methanolic and small amount of acid solution (Fig. 4.3.1).

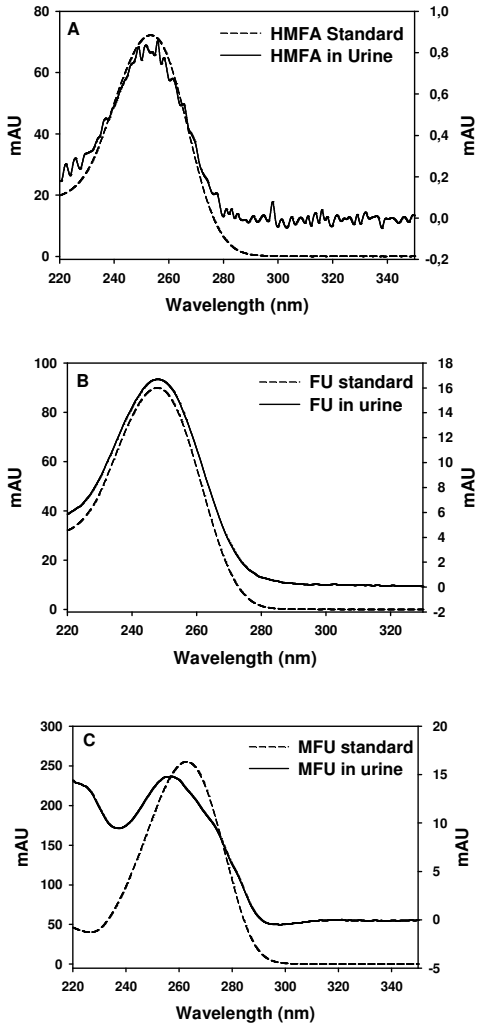


Figure 4.3.2. UV–spectra of HMFA (A), 2–furoic acid or FU (B), 5–methyl–2–furoic acid or MFU (C)

The spectra of HMFA and 2–furoic acid from urine have the same spectra as the standard (Fig. 4.3.2A and 4.3.2B, respectively). However, the spectra of 5–methyl–2–furoic acid from urine have different spectra from its standard spectra although it seemed to have same retention time (Fig. 43.2C). Hence, mass spectrometric analysis is needed to clarify the 5–methyl–2–furoic acid analysis.

4.4. Analysis of 5–Methyl–2–Furoic Acid with Isocratic Elution using AB Sciex LC/MS/MS

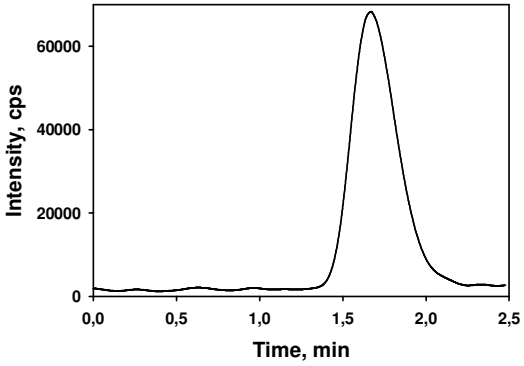


Figure 4.4.1. Product ion scan chromatogram of a 5–methyl–2–furoic acid standard

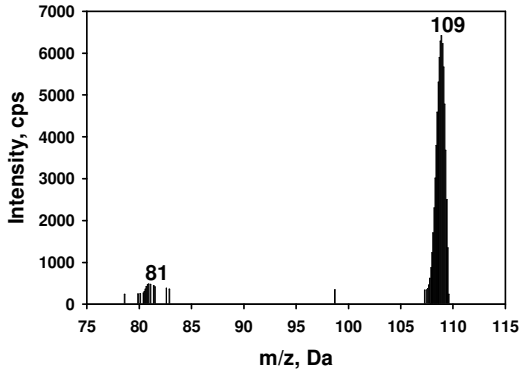


Figure 4.4.2. Fragmentation of a 5–methyl–2–furoic acid standard

Positive mode mass spectrometry of a 5–methyl–2–furoic acid standard gives an ion with m/z 127 $[M+H]^+$. The fragmentation of 5–methyl–2–furoic acid produces m/z 109 and m/z 81 (Fig. 4.4.2) and it spectra (Fig. 4.4.1). The precursor ion analysis showed that m/z 109 and m/z 81 are fragments of m/z 127. The fragmentation of 5–methyl–2–furoic acid occurs by loosing water first and then by loosing carbon monoxide is inline with the results found by (Mrochekand & Rainey, 1972). This fragmentation pattern of 5–methyl–furoic acid is nearly the same as the fragmentation pattern of 5–hydroxymethylfurfural; using the same measurement conditions. The difference between these two is the retention time and the abundance of the product ions (m/z 109 and m/z 81); the abundance of the product ion is in opposite way.

4.5. Analysis of 5–Methyl–2–Furoic Acid in Gradient Elution AB Sciex LC/MS/MS

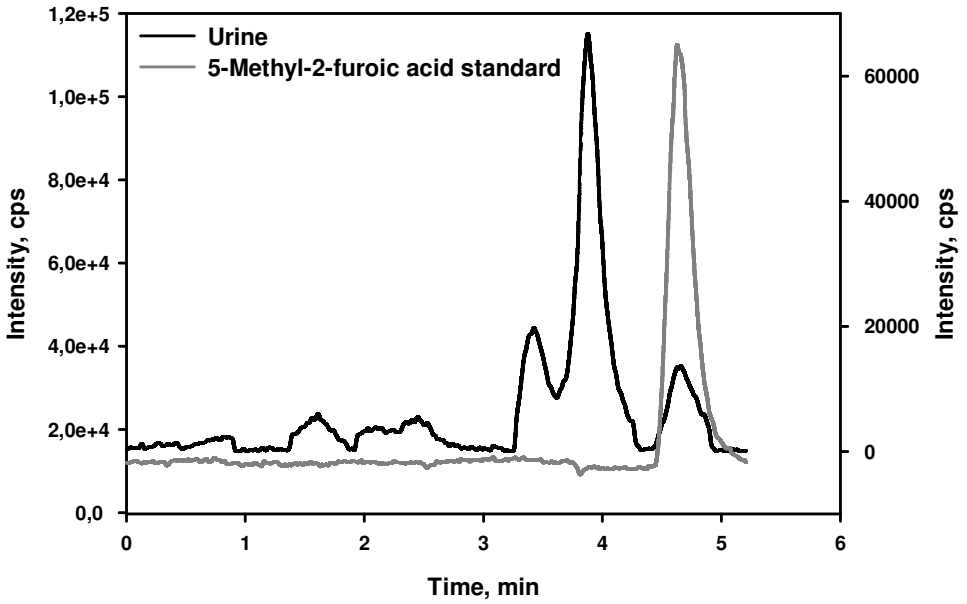


Figure 4.5.1. SIM chromatogram of 5–methyl–furoic acid and urine using gradient elution.

Gradient elution chromatography is needed to identify 5–methyl–2–furoic acid in urine because using the isocratic elution all the 3 furan derivative metabolites eluted at the same retention time. Selected ion monitoring (SIM) of m/z 127 analysis, 5–methyl–2–furoic acid could be analysed in urine (Fig. 4.5.1). The fragmentation of m/z 127 in the urine sample shows the same pattern as the standard (Fig. 4.5.2) and it has the same retention time of SIM m/z 127 standard (Fig. 4.5.3). The product ions also have the same precursor ion which is m/z 127. The multiple reaction monitoring (MRM) shows that m/z 109 and m/z 81 are fragments of m/z 127. Besides that, the chromatogram of the 2 MRMs are at the same retention time as SIM m/z 127 (Figs. 4.5.4, 4.5.5).

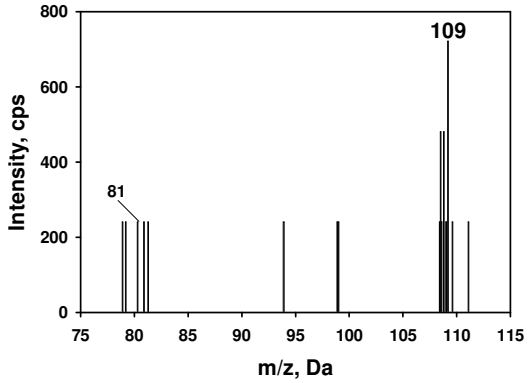


Figure 4.5.2. Mass spectrum of product ion scan of m/z 127 of urine

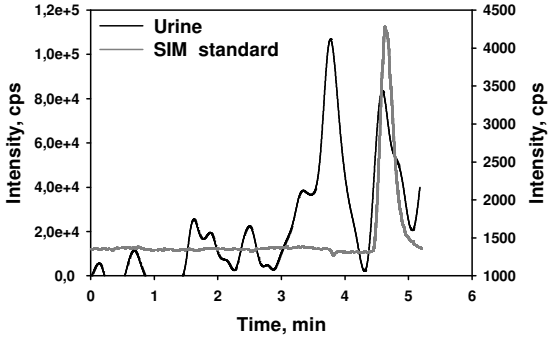


Figure 4.5.3. Chromatogram of the product ion m/z 127 → 109 of urine

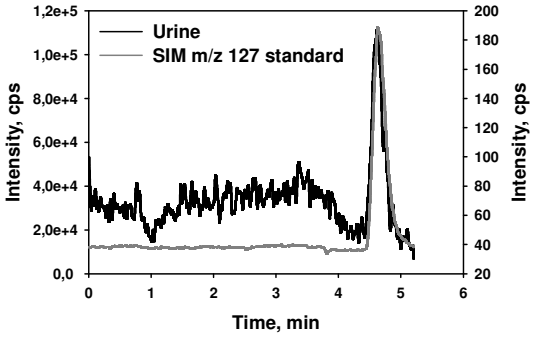


Figure 4.5.4. MRM chromatogram m/z 127 → 109 of urine

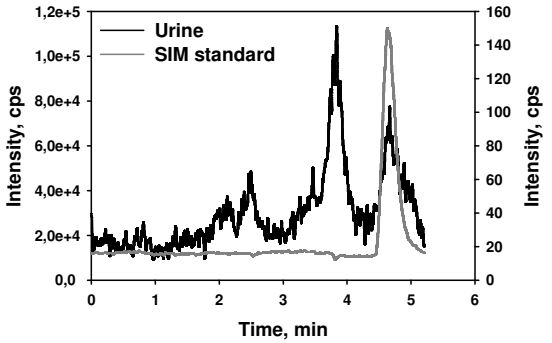


Figure 4.5.5. MRM chromatogram m/z 127 → 81 of urine

V. CONCLUSION

Urine samples should undergo alkaline hydrolysis reaction and then extracted 3 times to have a sufficient recovery before furan derivative metabolites can be analysed by HPLC or LC/MS/MS. 5–Hydroxymethyl–2–furoic acid and 2–furoic acid can be identified using HPLC Agilent 1100 with gradient elution of methanolic and low acid solution. 5–Methyl–2–furoic acid only can be analysed using the LC/MS/MS because the amount is low and it cannot be separated well in HLPC.

VI. REFERENCE

Kagami, K., Onda, K., Oka, K. & Hirano, T., (2008), Suppression of Blood Lipid Concentrations by Volatile Maillard Reaction Products, Nutrition, 24, 1159 – 1166.

Karpe, F. & Frayn, K.N., (2004), The Nicotinic Acid Receptor – A New Mechanism For an Old Drug, The Lancet, 363, 1892 – 94

Arribas–Lorenzo, G. & Morales, F.J., (2010), Estimation of Dietary Intake of 5–Hydroxymethylfurfural and Related Substances from Coffee to Spanish Population, Food Chem. Toxicol., 48, 644 – 649.

Mrochekand, J.E. & Rainey,Jr., W.T., (1972), Identification and Biochemical Significance of Substituted Furansin Human Urine. Clin. Chem., 18, 821 – 828.

Germond, J.E., Philippossian, G., Richli, U., Bracco, I, & Arnaud, M.J., (1987). Rapid and Complete Urinary Elimination of [^{14}C]–5–hydroxymethyl–2–Furaldehyde Administered Orally or Intravenously to Rats, J. Toxicol. Environ. Health, 22, 79 – 89.

Godfrey, V.B., Chen, L.J., Griffin, R.J., Lebetkin, E.H. & Burka. L.T., (1999), Distribution and Metabolism of (5–hydroxymethyl)furfural in Male F344 Rats and B6C3F1 Mice After Oral Administration, J. Toxicol. Environ. Health A, 57, 199 – 210.

Murkovic, M. & Pichler, N., (2006), Analysis of 5–Hydroxymethylfurfural in Coffee, Dried Fruits and Urine. Mol. Nutr. Food Res., 50, 842 – 846.

Gandini, A. & Belgacem, M.N., (1997), Furans in Polymer Chemistry, Prog. Polym. Sci., 22, 1203 – 1379.

American Conference of Government Industrial Hygienists (ACGIH), (1994), Documentation of Biological Exposure Indices. Cincinanti, Ohio.

Jöbstl, D., Husøy, T., Alexander, J., Bjellaas, T., Leitner, E. & Murkovic, M., (2010), Analysis of 5–Hydroxymethyl–2–furoic acid (HMFA) the Main Metabolite of Alimentary 5–Hydroxymethyl–2–furfural (HMF) with HPLC and GC in Urine. Food Chem., 123, 814 – 818.

Jellum, E., Børresen, H.C. & Eldjarn, L., (1973), The Presence of Furan Derivatives in Patients Receiving Fructose–Containing Solutions Intravenously. Clin. Chim. Acta, 47, 191 – 201.

Husøy, T., Haugen, M., Murkovic, M., Jöbstl, D., Stølen, L.H., Bjellaas, T., Rønningborg, C., Glatt, H. & Alexander, J., (2008), Dietary Exposure to 5–Hydroxymethylfurfural from Norwegian Food and Correlations with Urine Metabolites of Short–term Exposure, Food Chem. Toxicol., 46, 3697 – 3702.

Bähre, F. & Meier, G., (2009), New Nonvolatile Acids in Coffee, Dtsch. Lebensmitt. 95, 399 – 402.

Murkovic, M. & Bornik, M., (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

Savolainen, H. & Pfäffli, P., (1983), Neurotoxicity of Furfuryl Alcohol Vapor In Prolonged Inhalation Exposure. Environmental Research, 31, 420 – 427.

Pfäffli, P., Tossavainen, A. & Savolainen, H, (1985), Comparison of Acid Excretion Techniques Inhaled Furfuryl Alcohol Vapour with Urinary Furoic in Exposed Foundry Workers by Chromatographic, Analyst, 110, 377 – 379.

Tan, Z.B., Tonks, C.E., O´Donnell, G.E. & Geyer, R., (2003), An Improved HPLC Analysis of the Metabolic Furoic Acid in the Urine of Workers Occupationally Exposed to Furfural. J. Anal. Toxicol. 27, 43 – 46.

Yaylayan, V., (2006), Precursors, Formation and Determination of Furan in Food. J. Verbr. Lebensm. 1, 5 – 9.

Parkash, M.K. & Caldwell, J., (1994), Metabolism and Excretion of [^{14}C] Furfural in The Rat and Mouse. Food Chem. Toxicol., 32, 887 – 895.

Nomeir, A.A., Silveira, D.M., McCornish, M.F. & Chadwick, M., (1992), Comparative Metabolism and Disposition of Furfural and Furfuryl Alcohol in Rats. Drug. Metab. Dispos., 20, 198 – 204.

Jellum, E., Stokke, O. & Eldjarn, L., (1973), Application of Gas Chromatography, Mass Spectrometry, and Computer Methods in Clinical Biochemistry. Analytical Chemistry, 45, 1099 – 1106.

Pettersen, J. E. & Jellum, E., (1972), The identification and Metabolic Origin of 2 Furoylglycine and 2.5–Furandicarboxylic Acid in Human Urine. Clin. Chim. Acta 41, 199.

Mardens, Y., Kumps, A., Planchon, C. & Wurth, C., (1992), Comparison of Two Extraction Procedures for Urinary Organic Acids Prior To Gas Chromatography–Mass Spectrometry. J. Chromatogr, 577, 341 – 346.

Eldjarn, L., Jellum, E. & Stokke, O., (1974), Application of Gas Chromatography–Mass Spectrometry in Routine and Research in Clinical Chemistry. J. Chromtogr, 91, 353 – 366.

Crabb, D. W., (1995). Ethanol Oxidizing Enzymes: Roles in Alcohol Metabolism and Alcoholic Liver Disease. Prog. Liver Dis., 13, 151 – 172.

Cederbaum, A. I., (1996). Metabolism of Ethanol, Acetaldehyde, and Condensation Products. In: H. Begleiter, & B. Kissin (Eds.), The Pharmacology of Alcohol and Alcohol Dependence. Oxford: Oxford University Press. pp. 59 – 109.

Cornell, N.W., Crow, K. E., Leadbetter, M. G. & Veech, R. L., (1979), Rate Limiting Factors for Ethanol Oxidation In Vivo and In Isolated Hepatocytes. In T. K. Li, S. Schenker,&L. Lumeng (Eds.), Alcohol and Nutrition. Washington, DC: U.S. Government Printing Office, pp. 315 – 330.

Havre, P., Abrams, M. A., Corrall, R. J., Yu, L. C., Szczepanik, P. A., Feldman, H. B., Klein, P., Kong, M.S., Margolis, J.M. & Landau, B.R., (1977). Quantitation of Pathways of Ethanol Metabolism. Arch. Biochem. Biophys. 182, 14 – 23.

Seitz, H.K., Gärtner, U., Egerer, G. & Simanowski, U.A., (1994), Ethanol Metabolism in The Gastrointestinal Tract and Its Possible Consequences. Alcohol Alcohol Suppl., 2, 157 – 162.

Crabb, D.W, Bosron, W.F. & Li T.K., (1987), Ethanol Metabolism. Pharmacol. Ther., 34, 59 – 73.

Bosron, W.F., Crabb, D.W. & Li, T.K., (1983), Relationship between kinetics of liver alcohol dehydrogenase and alcohol metabolism. Pharmacol. Biochem. Behav. 18 Suppl., 1, 223 – 227.

Lieber, C.S., (1976), The Metabolism of Alcohol. Sci. Am., 234, 25.

Koopmana, F., Wierckxa, N., Winde, J.H. & Ruijssenaars, H.J., (2010), Identification and characterization of the furfural and 5–(hydroxymethyl)furfural degradation pathways of Cupriavidus basilensis HMF 14, PNAS, 107, 4919 – 4924.

Prior, R.L., Wu, X. & Gu, L., (2006), Identification and Urinary Excretion of Metabolites of 5–(Hydroxymethyl)–2–furfural in Human Subjects following Consumption of Dried Plums or Dried Plum Juice., J. Agric. Food Chem., 54, 3744 – 3749.

Appendix

1. Recovery extraction furan derivatives metabolite

Standard	Area	Standard	Area	Standard	Area
HMFA		Furoic acid		5–Methyl–2–furoic acid	
Standard in water					
0	0	0	0	0	0
0.1	370.593	0.1	3296.73	0.1	378.754
0.2	619.783	0.2	6405.82	0.2	721.51
0.3	932.731	0.3	9539.4	0.3	1083.53
0.4	1212.63	0.4	12539.9	0.4	1448.82
Standard in water with cleaning treatment					
0	0	0	1.66	0	0
0.1	166.799	0.1	1619.8	0.1	233.648
0.2	325.218	0.2	4208.52	0.2	361.356
0.3	510.008	0.3	7703.22		
0.4	703.733	0.4	9525.63	0.4	835.979
Recovery (%)	55.73		75.1		56.51
Standard in Urine with cleaning treatment					
0	8.34	0	210.038	0	432.573
0.1	178.443	0.1	1967.5	0.1	665.078
0.2	380.122	0.2	3630.61	0.2	999.427
0.3	540.783	0.3	7287.94	0.3	1369.81
0.4	723.95	0.4	10762.4	0.4	432.573
Recovery (%)	105.71		104		166.67

2. HMFA

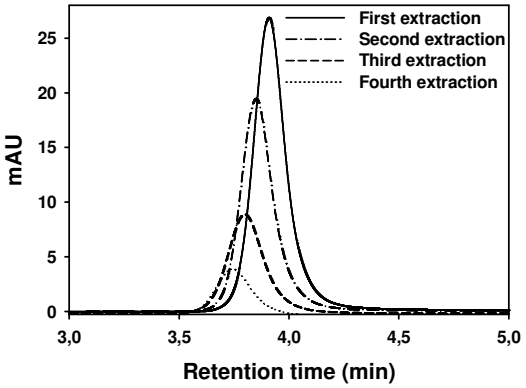


Figure 1. HPLC chromatogram of extraction HMFA in RT

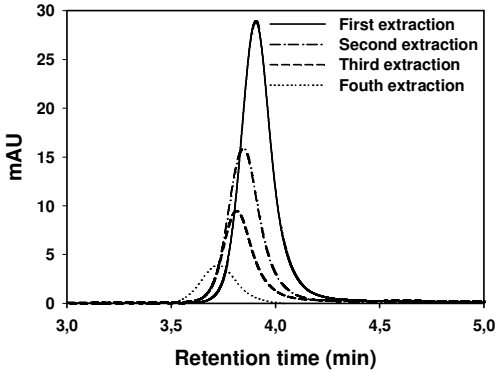


Figure 2. HPLC chromatogram of extraction HMFA in 95 °C

3. Furoic acid

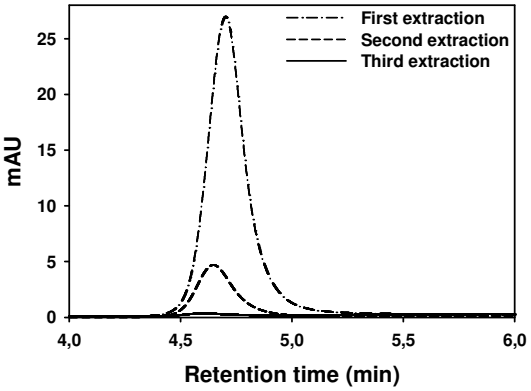


Figure 3. HPLC chromatogram of extraction furoic acid in RT

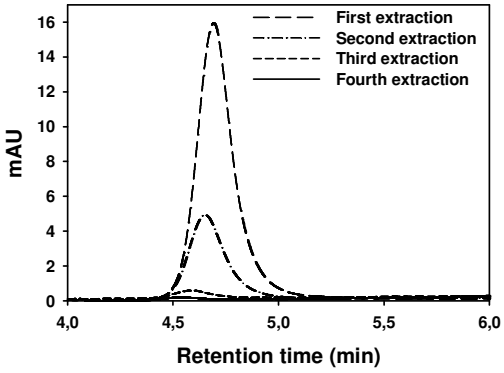


Figure 4. HPLC chromatogram of extraction furoic acid in 95 °C

4. 5–methyl–2–furoic acid

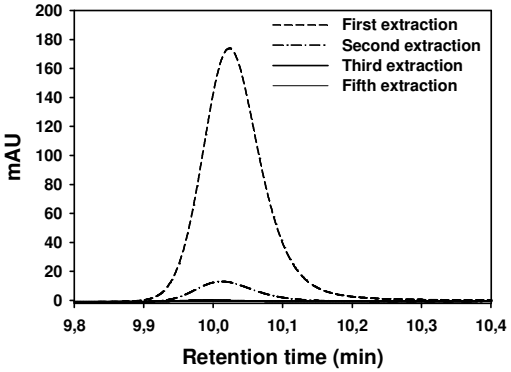


Figure 6. HPLC chromatogram of extraction 5–Methyl–2–furoic acid in RT

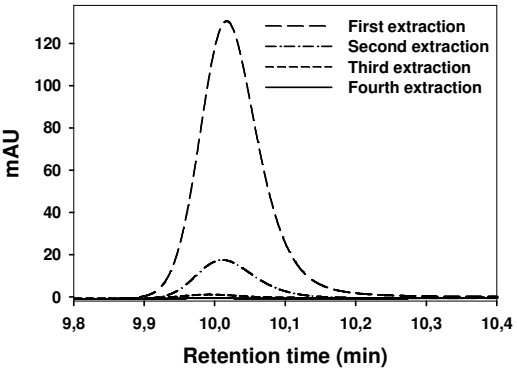


Figure 7. HPLC chromatogram of extraction 5–Methyl–2–furoic acid in 95 °C

5. 5–Methyl–2–furoic acid standard

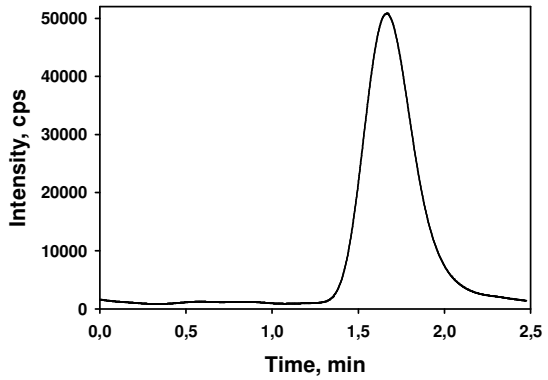


Figure 8. Chromatogram of precursor ion m/z 109

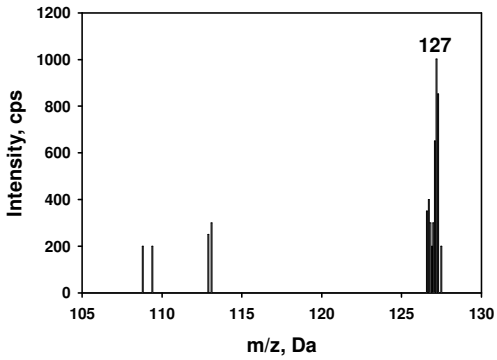


Figure 9. Fragmentation of precursor ion m/z 109

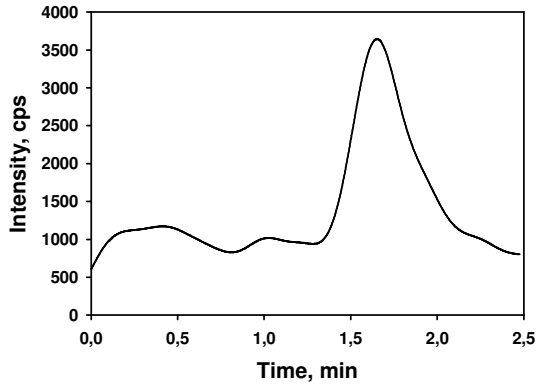


Figure 10. Chromatogram of precursor ion m/z 81

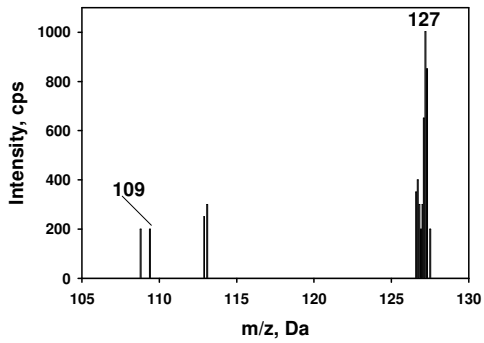


Figure 11. Fragmentation of precursor ion m/z 81

6. Selected Ion Monitoring (SIM) standard in gradient elution

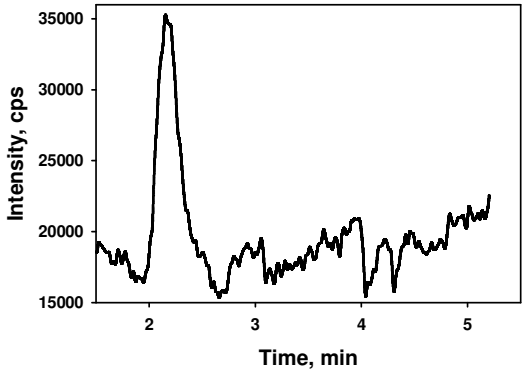


Figure 12. SIM Chromatogram HMFA in gradient elution

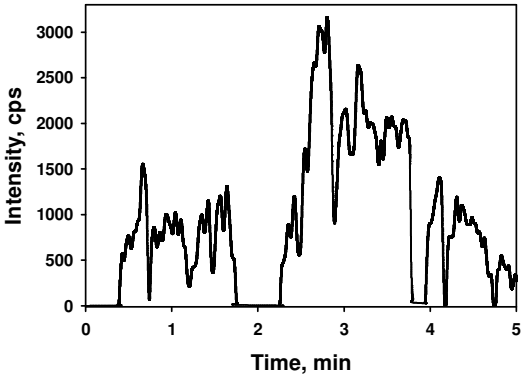


Figure 13. SIM furoic acid chromatogram in gradient elution

7. Urine

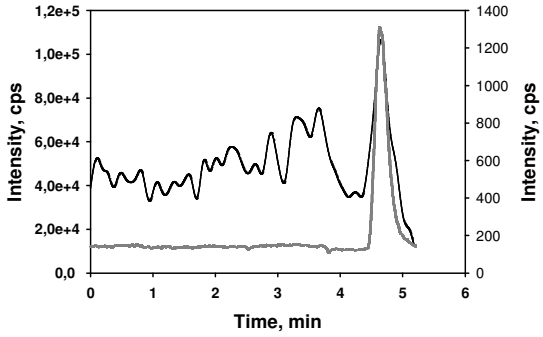


Figure 14. Chromatogram precursor ion m/z 109 in urine

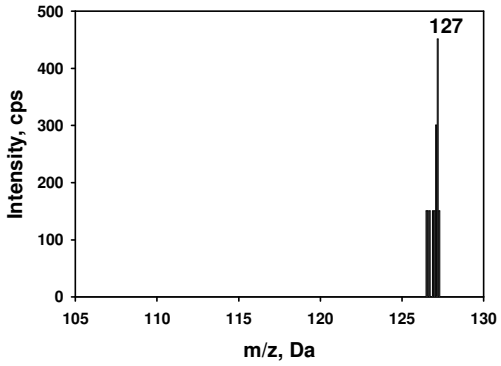


Figure 15. Mass spectra precursor ion m/z 109 in urine

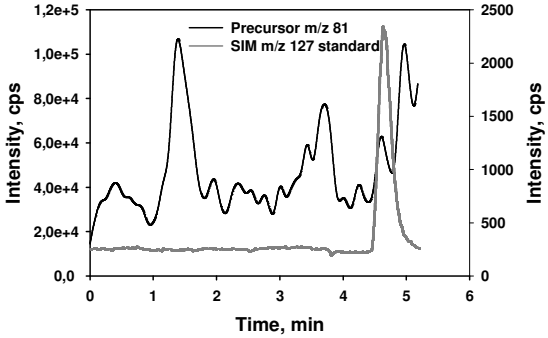


Figure 16. Chromatogram precursor ion m/z 81 in urine

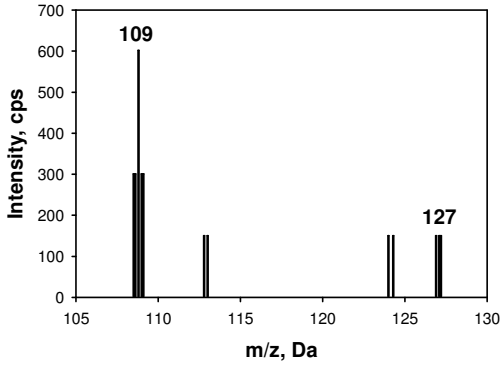


Figure 17. Mass spectra precursor ion m/z 81 in urine

SECTION 4

PUBLICATIONS

1. Characterization of the Polymerization of Furfuryl Alcohol during Roasting of Coffee

Food & Function

Dynamic Article Links

Cite this: DOI: 10.1039/c2fo30020f

www.rsc.org/foodfunction

PAPER

Characterization of the polymerization of furfuryl alcohol during roasting of coffee

Yuliana Reni Swasti* and Michael Murkovic

Received 3rd February 2012, Accepted 28th June 2012
DOI: 10.1039/c2fo30020f

The polymerization of furfuryl alcohol contributes to the formation of the brown colour in heated foods, in addition to the Maillard and caramelization reactions. During the heating of food, furfuryl alcohol is formed *via* the degradation of quinic acid or 1,2-enediols. Furfuryl alcohol is a mutagenic compound. In acidic conditions it is able to polymerize and form aliphatic polymers that show a brown colour. Herein we show that furfuryl alcohol polymerizes in a model system by incubating it in 1 M HCl at room temperature. Some of the reaction products are dimers, trimers, tetramers, and pentamers with methylene linkages. The degree of polymerization and the amount of those furfuryl alcohol oligomers increased with increasing reaction time. The results of this model system were used to characterize the polymerization of furfuryl alcohol which is produced during roasting of coffee. The coffee was roasted at 210 °C for 2, 3, 4, 5, and 6 min with a home coffee roaster. Furfuryl alcohol and its dimer were found in roasted coffee after 2 and 3 min of roasting respectively, reaching a maximum amount after 4 min. Perhaps due to further reactions, the dimeric furfuryl alcohol concentration starts to decrease after 4 min. We propose that the polymers of furfuryl alcohol contribute to the brown colour of roasted foods.

Introduction

The formation of the brown colour in heated foods is a result of caramelization and the Maillard reaction. In many foods, the brown colour is a positive quality trait, for example, in bread, coffee, or fried potatoes. In the course of these reactions not only the colour changes but also aroma active substances are formed giving the heated foods a typical appearance to the consumer. In addition to the desired products, some toxic compounds are also formed during heating such as heterocyclic amines,[1] acrylamide,[2] and furan.[3]

Recently, furfuryl alcohol has attracted safety research attention due to new biological activation reactions which have been identified that are associated with furfuryl alcohol activation. It has been found that furfuryl alcohol can become a DNA-reactive intermediate that has a mutagenic effect.[4] The potential mutagenic effect is reduced as the alcohol chain of the compound increases.[5] Although it undergoes polymerization, the concentration of the monomeric furfuryl alcohol is still high in heated food products. Furfuryl alcohol is responsible for the burnt,[6,7] cooked-sugar,[8] and rubber-like odors,[9] and produces the bitter taste of roasted coffee due to the interaction with dihydroxybenzene or trihydroxybenzene.[10] Nevertheless, furfuryl alcohol is used as a flavouring agent with an acceptable daily intake of 0–0.5 mg kg^{-1} (ref. 11).

Graz University of Technology, Institute of Biochemistry, Petersgasse 12/2, 8010 Graz, Austria. E-mail: yulianareni@yahoo.com

Furfuryl alcohol can be formed by the degradation of 1,2-enediols[12] and quinic acid[13] at high temperatures. Quantitatively, furfuryl alcohol, a furan derivative, is predominant in roasted coffee.[10] Thus, furfuryl alcohol is predicted to have an influence on the formation of the brown colour during the roasting of coffee. Furfuryl alcohol can polymerize in acidic conditions *via* the condensation of the hydroxyl group and the hydrogen atom of the heterocycle at carbon 5 to produce a polymer with methylene linkages that has a brown colour. Dimerization of furfuryl alcohol could also occur *via* the condensation of the two hydroxyl groups producing dimethylene ether linkages. However, in acidic conditions this type of condensation releases formaldehyde to form the methylene linkage.[14] The brown colour of the aliphatic furfuryl alcohol polymer could be induced by the loss of one hydrogen atom from a central carbon.[15] It is also possible that furfuryl alcohol and its oligomers are introduced into the melanoidins—high molecular weight products that are brown in colour. Other heterocyclic ring systems (pyridines pyrazines, pyrroles and imidazoles) also contribute to melanoidin formation.[16] Generally, melanoidin forms *via* the condensation of amino compounds and products from Amadori rearrangements which undergo sugar dehydration and sugar fragmentation.[17] In coffee beverages, melanoidin contributes up to 25% of the dry matter.[18]

In this research, the formation of intermediate furfuryl alcohol oligomers during the roasting of coffee will be described. This is important because this reaction could contribute to the browning of coffee during roasting and it is expected that with increased chain length the mutagenic potential of furfuryl alcohol is reduced.

Materials

Furfuryl alcohol (FA, ≥99%) was purchased from Fluka (Buchs, Switzerland), hydrochloric acid (HCl, 32%) was purchased from Merck (Darmstadt, Germany), methanol HPLC grade was purchased from Mallinckrodt Baker (The Netherlands), 100% acetic acid was purchased from Roth (Karlsruhe, Germany), and solid phase extraction (SPE) accucat (200 mg, 3 ml) was purchased from Varian (Agilent Technologies, USA). Additionally, robusta green coffee was used in the study.

Methods

Polymerization of furfuryl alcohol

Furfuryl alcohol (40 µl) was mixed with 40 µl aqueous acid (1 M HCl) and 40µl MeOH then incubated at 22 °C for 6 h. The reaction mixture was next diluted to 25 ml with methanol and then further to a 1 : 5 ratio of sample/methanol. The samples were then analyzed using a LC-MS Agilent 1100. LC-MS conditions: LiChrospher 100 RP-18 (125 × 3 mm, 5 µm) as column from Agilent Technologies; 5 µl injection volume (for model system); 10 µl injection volume (for roasted coffee extract); 0.6 ml min^{-1} solvent flow rate; gradient elution until 15 min: 25% MeOH, 73% water, 2% acetic acid in water pH 2.5 (constant during 15 min); diode array detector (DAD) at 228 nm; ESI, positive scan mode, 70 V; mass range 75–200 (dimer), 75–280 (trimer), 75–350 (tetramer), and 75–450 (pentamer).

Fractionation of furfuryl alcohol oligomer

The furfuryl alcohol polymerization reaction mixture was purified by taking fractions from the HPLC separation immediately after DAD. To obtain an adequate amount of substance this was repeated 5 times. The eluent was then evaporated with a flow of nitrogen and redissolved in 300 µl MeOH and analysed by LC-MS.

Determination of furfuryl alcohol and its oligomers in roasted coffee

Green coffee (40 g) was roasted in a household coffee roaster (i-Roaster 40211) at 210 °C for 2, 3, 4, 5 and 6 min. Ground coffee (1 g) was then extracted with 10 ml methanol by vortexing for 2 min. Of the filtered extract, 2 ml was purified by SPE accucat (conditioned with 5 ml MeOH and 3 ml water) and the purified extracts were analyzed by LC-MS.

Results and discussion

Polymerization of furfuryl alcohol

The incubation of furfuryl alcohol in an acidic medium at 22 °C for 6 h resulted in the formation of oligomers (Fig. 1). In experiments without the addition of acid, no significant polymerization was observed (Fig. 1). Over the course of the reaction, the concentration of furfuryl alcohol decreased and the concentration of the resulting oligomers increased. The polymerization of furfuryl alcohol in an acidic medium proceeds by attaching one molecule to another and eliminating water *via* the reaction of the hydroxyl group and the α-hydrogen of the heterocycle at carbon 5. These two positions are the most reactive in furfuryl alcohol.[20] In addition, the activation energy for the initiation of the furfuryl alcohol polymerization is lower compared to chain prolongation.[21] At the beginning of polymerization, the solution is green, then gradually becomes brown before finally becoming dark brown in a solid form.[19] The formation of the brown colour of the aliphatic furfuryl alcohol oligomers begins with hydride ion exchange[15] which leads to the formation of carbenium ions; thus, its colour arises from the formation of conjugated sequences, as a result of successive proton and hydride ion losses.[14] The polymer itself should not absorb in the visible range since the conjugated system is interrupted by methylene groups.

Fractionation of oligomers of furfuryl alcohol

The fractionated samples of the furfuryl alcohol oligomers also displayed the same retention time as in the direct analysis. The conditions indicate that the peaks are oligomers and non-volatile compounds (Fig. 2). Moreover, all the peaks, except the oligomers, that were present in the direct analysis were no longer present.

Structural determination of the oligomers of furfuryl alcohol

The major peaks 1, 2, 3, and 4 in the chromatogram obtained from the LC-MS analysis are identified as dimer, trimer, tetramer, and pentamer with the fragments of *m/z* 161 (Fig. 3), *m/z* 241 (Fig. 4), *m/z* 321 (Fig. 5), and *m/z* 401 (Fig. 6) respectively. The ion 161 is formed by elimination of water $[M - 18 + H]^+$. Elimination of water in vicinal alcohols is common and the molecular ion (*m/z* 179) cannot be observed in the mass spectrum.[22] After elimination of water from the dimers, an alkene that is attached to the O-bearing carbon is usually eliminated $[M - 46 + H]^+$ and then followed by elimination $[M - 74 + H]^+$ which could be seen in the mass spectrum as *m/z* 133 and *m/z* 105 with 70 V fragmentation voltage (Fig. 7). Further fragmentation $[M - 102 + H]^+$ can be seen as ion with *m/z* 77 which is only formed when the fragmentation voltage is as high as 130 V (Fig. 8); at lower fragmentation voltages (*e.g.* 100 V) this fragment cannot be observed (Fig. 9). The elemental formula of this dimer is $C_{10}H_8O_2$.

Trimeric furfuryl alcohol cannot be fragmented at a fragmentation voltage of 70 V. At least 100 V is needed to obtain

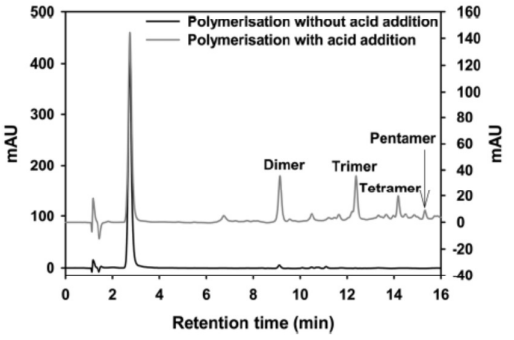


Fig. 1 Chromatogram DAD of furfuryl alcohol polymerization at 22 °C for 6 h.

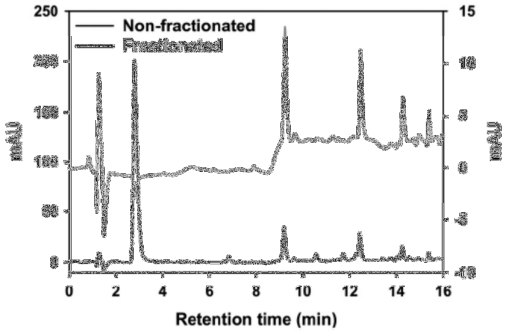


Fig. 2 Chromatogram fractionation of furfuryl alcohol oligomers.

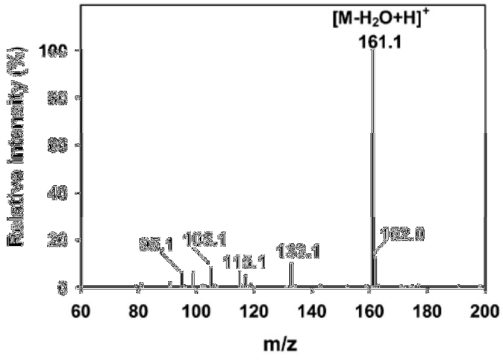


Fig. 3 Fragmentation of dimeric furfuryl alcohol at 70 V in ESI-MS.

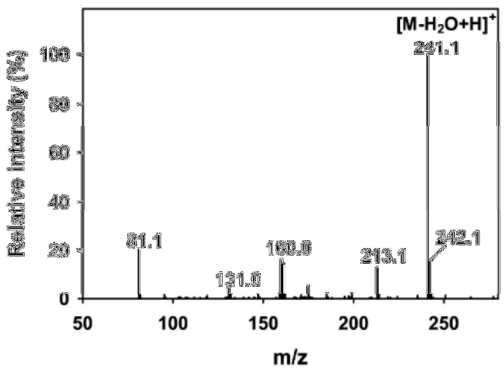


Fig. 4 Fragmentation of trimeric furfuryl alcohol at 100 V in ESI-MS.

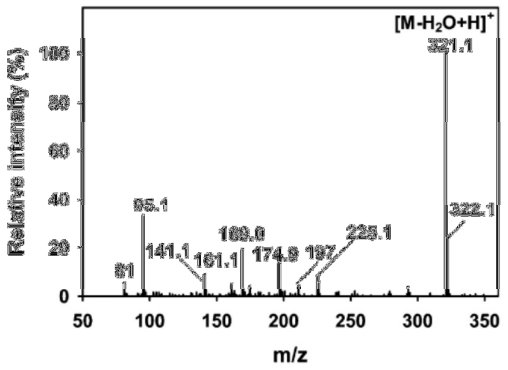


Fig. 5 Fragmentation of tetrameric furfuryl alcohol at 130 V in ESI-MS.

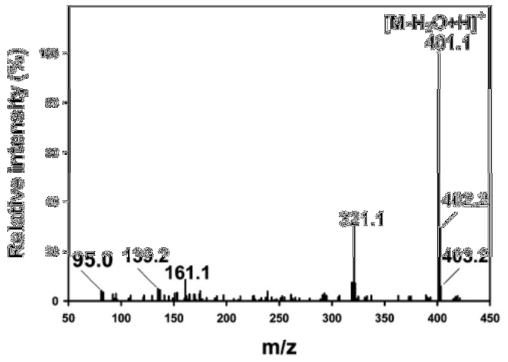


Fig. 6 Fragmentation of pentameric furfuryl alcohol at 130 V in ESI-MS.

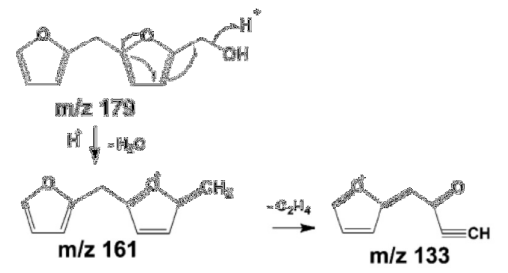


Fig. 7 The suggested mechanism fragmentation of dimeric furfuryl alcohol.

collision induced fragments (Fig. 4) with an increasing fragmentation at 130 V (Fig. 10). This oligomeric furfuryl alcohol also loses water during ionization. Analogous to the other oligomers the molecular ion (*m/z* 259) cannot be seen. Trimeric furfuryl alcohol fragments into *m/z* 29 + R_1 + R_2 (59, 73, 87,…)[22] and it can be seen in the mass spectrum with masses of *m/z* 241, *m/z* 213. The subsequent fragmentation *m/z* 199 is not formed extensively with 100 V fragmentation voltage, but it could be seen at higher voltages (*e.g.* 130 V) (Fig. 10). The elemental formula of this trimer is $C_{15}H_{12}O_3$.

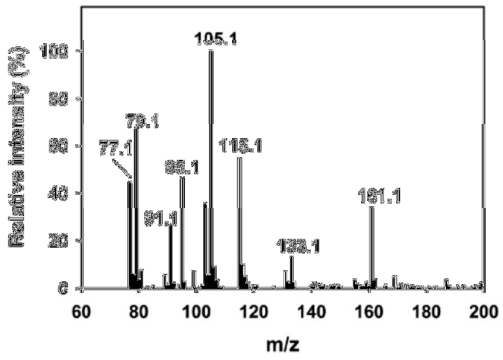


Fig. 8 Fragmentation of dimeric furfuryl alcohol at 130 V in ESI-MS.

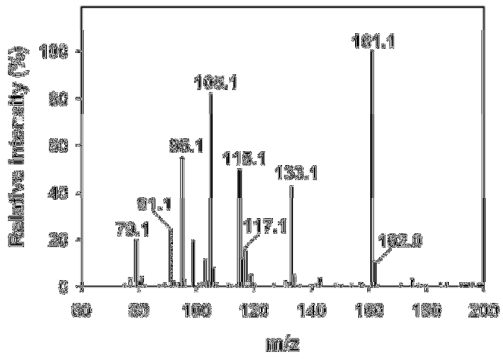


Fig. 9 Fragmentation of dimeric furfuryl alcohol at 100 V in ESI-MS.

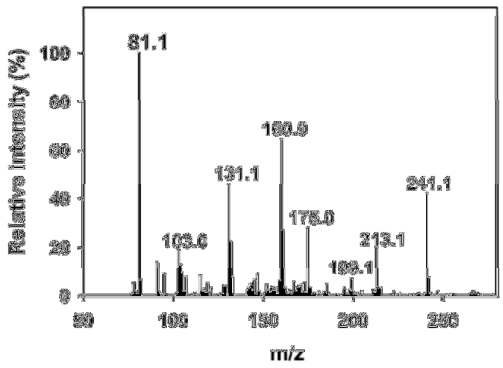


Fig. 10 Fragmentation of trimeric furfuryl alcohol at 130 V in ESI-MS.

Tetrameric and pentameric furfuryl alcohol need a fragmentation voltage of 130 V to induce fragmentation (Fig. 5 and 6). The tetramer and the pentamer also loose water from its molecule ion during ionization.

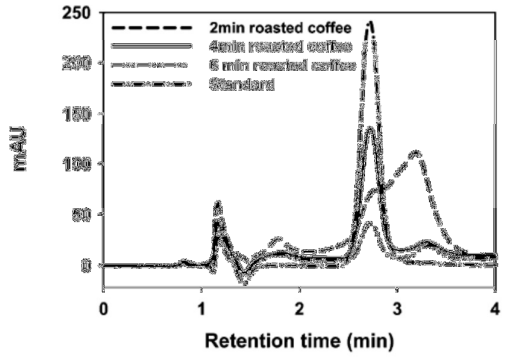


Fig. 11 Chromatogram of furfuryl alcohol during roasting coffee at 210 °C.

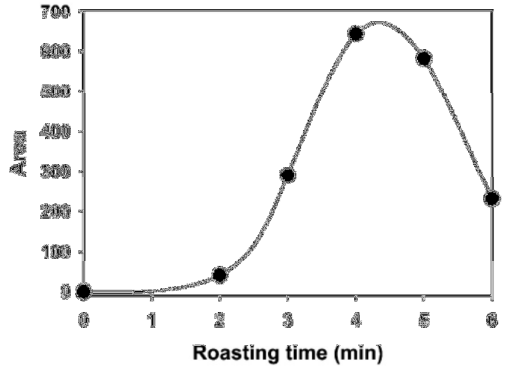


Fig. 12 The kinetic of furfuryl alcohol production during roasting coffee at 210 °C.

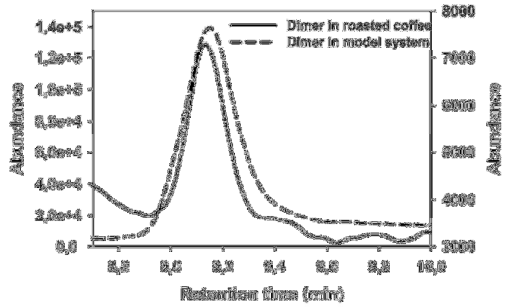


Fig. 13 Chromatogram of dimeric furfuryl alcohol during roasting coffee at 210 °C.

The type of linkage formed during the polymerization of furfuryl alcohol in 1 M HCl was identified by interpretation of the fragment ions. A dimer of furfuryl alcohol with a methylene

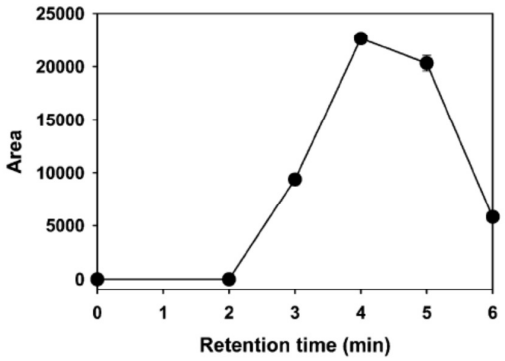


Fig. 14 The kinetic of dimeric furfuryl alcohol production during roasting coffee at 210 °C.

linkage will eliminate water during mass spectrometric analysis. In contrast, the dimer with a dimethylene ether linkage will not eliminate water[23] and fragments in a different way. The formation of furfuryl alcohol oligomers with methylene linkages is significantly higher and it seems to be favourable in the furfuryl alcohol polymerization. This condition is in line with earlier results that found that the methylene linkage is more abundant than the dimethylene ether linkage in furfuryl alcohol oligomers.[24]

Furfuryl alcohol and its oligomers in roasted coffee beans

Green coffee does not contain any furfuryl alcohol. However, furfuryl alcohol is formed during roasting. After 2 min of roasting at 210 °C, the formation of furfuryl alcohol begins, with a significant increase during continued roasting, and then reduces after 5 min of roasting (Fig. 11 and 12). This means that a degradation of the 1,2-enediol (key intermediate in the isomerisation reaction of fructose and glucose)[12] and the degradation of quinic acid[13] may occur. The formation of the dimer begins after 3 min of roasting and reaches a maximum at 5 min. Longer roasting times result in a decrease of the dimer (Fig. 13 and 14). This means that furfuryl alcohol polymerizes during roasting under acidic conditions. The acidic conditions may be formed *via* the degradation of 1,2-enediols which produces formic acid, and also *via* the degradation of 2,3-enediols that produce acetic acid during the high heat treatment of sugar.[12] Therefore, we conclude that furfuryl alcohol is able to polymerize under these conditions and contributes to the formation of the brown colour of roasted coffee.

Conclusions

The polymerization of furfuryl alcohol in acidic conditions leads to the formation of oligomers. The structures that were identified contained 2 to 5 units of furfuryl alcohol with methylene linkages. Higher degrees of oligomerization could not be identified. The concentration of the oligomers increased during the course of the reaction. The dimer of furfuryl alcohol and furfuryl alcohol itself are both found in roasted coffee. The polymerization of furfuryl alcohol in roasted coffee can also contribute to the brown colour.

Acknowledgements

The authors are grateful to Asea Uninet Scholarship for financial support.

References

1 M. T. Galceran and L. Puignou, in *Acrylamide and Other Hazardous Compounds in Heat-Treated Foods*, ed. K. Skog and J. Alexander, Woodhead Publishing, Cambridge, 2006, pp. 68–116.
2 E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson and M. Törnqvist, *J. Agric. Food Chem.*, 2002, **50**(17), 4998–5006.
3 C. Perez Locas and V. A. Yaylayan, *J. Agric. Food Chem.*, 2004, **52**(22), 6830–6836.
4 H. Glatt and Y. Sommer, in *Acrylamide and Other Hazardous Compounds in Heat-Treated Foods*, ed. K. Skog and J. Alexander, Woodhead Publishing, Cambridge, 2006, pp. 328–357.
5 H. Glatt, *Chem. Biol. Interact.*, 2000, **129**, 141–170.
6 S. M. Lee, B. C. Seo and Y. Kim, *J. Food Sci.*, 2006, **71**(3), 146–156.
7 Y. Wang and S. J. Kays, *J. Amer. Soc. Hort. Sci.*, 2000, **125**(5), 638–643.
8 J. S. Bonvehí, *Eur. Food Res. Technol.*, 2005, **221**, 19–29.
9 Y. Karagül-Yüceer, K. R. Cadwallader and M. Drake, *J. Agric. Food Chem.*, 2002, **50**, 305–312.
10 S. Kreppenhofer, O. Frank and T. Hofmann, *Food Chem.*, 2011, **126**, 441–449.
11 R. Walker and J. L. Herrman in *55th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*, FAO and WHO, Geneva, 2000.
12 C. M. J. Brands and M. A. J. S. Boekel, *J. Agric. Food Chem.*, 2001, **49**, 4667–4675.
13 J. Moon and T. Shibamoto, *J. Agric. Food Chem.*, 2010, **58**, 5465–5470.
14 M. Choura, N. M. Belgacem and A. Gandini, *Macromolecules*, 1996, **29**, 3839–3850.
15 A. Gandini and M. A. Belgacem, *Prog. Polym. Sci.*, 1997, **22**, 1203–1379.
16 H. Nursten, *The Maillard Reaction Chemistry, Biochemistry and Implications*, The Royal Society of Chemistry, United Kingdom, 2005, p. 20.
17 J. E. Hodge, *J. Agric. Food Chem.*, 1953, **1**(15), 928–943.
18 R. C. Borrelli, A. Visconti, C. Mennella, M. Anese and V. Fogliano, *J. Agric. Food Chem.*, 2002, **50**, 6527–6533.
19 P. S. Batista and M. F. Souza, *Polymer*, 2000, **41**, 8263–8269.
20 S. B. Wallon, J. B. Barr and B. A. Petro, *J. Chromatogr.*, 1971, **54**, 33–41.
21 N. Guigo, A. Mija, L. Vincent and N. Sbirrazzuoli, *Phys. Chem. Chem. Phys.*, 2007, **9**, 5359–5366.
22 R. G. Cooks, in *The Chemistry of the Hydroxyl Group, Part 2*, ed. S. Patai, Interscience, London, 1971, p. 1045.
23 E. D. Loughran, E. M. Wewerka and G. J. Hammons, *J. Heterocycl. Chem.*, 1972, **9**, 57–65.
24 A. H. Fawcett and W. Dadamba, *Makromol. Chem.*, 1982, **183**, 2799–2809.

2. 5-Hydroxymethyl-furfural and furfuryl alcohol: Occurrence, exposure, and detection

5-Hydroxymethyl-furfural and furfuryl alcohol: Occurrence, exposure, and detection

Michael Murkovic, Yuliana Reni Swasti
Graz University of Technology, Institute of Biochemistry, Petersgasse 12/2, 8010 Graz

Abstract

During the recent years a new class of food borne heat generated compounds became the focus of risk evaluation. The identification of a new mechanism of activation of vinylogous alcohols by sulphotransferases has given insight into a possible contribution of these types of substances to carcinogenesis. In this chapter two o of these compounds – 5-hydroxymethyl-furfural (HMF) and furfuryl alcohol (FA) – are described. These two compounds occur in high amounts in some selected foods and even if the mutagenic/carcinogenic activity is low, there might be a risk due to the high exposure. Both compounds are formed by heating of carbohydrate rich foods. HMF could be formed via the Maillard reaction or via direct dehydration of fructose and FA from hexoses or pentoses. The concentrations of FA can reach several hundred µg/g and of HMF several mg/g.

Introduction

HMF is one of the compounds that are formed during the Maillard reaction or by direct dehydration of hexoses. Higher temperatures, an acidic environment, and a low water activity favour the formation of HMF. However, even at lower temperatures (e.g. storage at room temperature) – which means under practically any conditions of food processing – HMF is formed; sometimes rather high concentrations are occurring. Estimates of the mean daily intake are in the range of 30 to 150 mg/capita. Various studies conducted in the 1960's and 1970's consistently showed a low acute and chronic toxicity of HMF in mice and rats. It was therefore concluded that HMF, which is formed in foods during processing or as a result of sterilisation of parenteral solutions, does not seem to pose any significant toxicological problem. Because of the reactive chemical structural moieties of HMF that include a furan ring, an α,β-unsaturated carbonyl, and an allylic hydroxyl group there might be a genotoxic and carcinogenic hazard [Glatt and Sommer, 2006]. Indeed, HMF initiated and promoted preneoplastic lesions, aberrant crypt foci (ACFs), in the rat colon [Zhang et al., 1993]. The initiation of these lesions implies the induction of

gene mutations in the colon mucosa. It is not only HMF but also furfuryl alcohol that can be activated to highly reactive metabolites [Glatt et al., 2011].

Although HMF showed a very low chronic and acute toxicity the exceptionally high human exposure, the induction of ACFs and the genotoxicity prompted the National Toxicological Program [NTP] of the USA to conduct long-term carcinogenicity studies with HMF in mice and rats. The NTP reported that ...There were increased incidences of lesions (degeneration and metaplasia) of the olfactory and respiratory epithelium of the nose in male and female rats and male and female mice that received 5-(hydroxymethyl)-2-furfural. Many of the male and female mice receiving 750 mg/kg died before the end of the study, and some exhibited seizures or other signs of neurological response. In the other two groups of female mice receiving 5-(hydroxymethyl)-2-furfural, there were increased incidences of hepatocellular adenoma of the liver... In this report it was concluded that ...5-(hydroxymethyl)-2-furfural caused liver cancer in female mice but did not cause cancer in male or female rats or male mice. In addition, 5-(hydroxymethyl)-2-furfural was associated with increased lesions of the olfactory and respiratory epithelium of the nose in male and female rats and mice... [National Toxicological Program, 2010]

Analysis of 5-hydroxymethyl-furfural

Normally the analysis of HMF is carried out by HPLC. Older methods for analysis of HMF in honey use photometry after reaction with e.g. bisulfite [e.g. AOAC 980.23, White, 1979] or after derivatization with p-toluidine [Winkler method] and barbituric acid [e.g. DIN 10751-1]. However, the routine analysis is normally carried out by HPLC with UV detection at 280 nm. For the separation a reversed phase (RP-18) column can be used and the HMF is eluted with 5 % methanol in water [Murkovic and Pichler, 2006]. Another method was published earlier using a RP-8 column with 5 % acetonitril in water [Mijares et al., 1985]. When a better selectivity or even a higher sensitivity is needed HMF can be derivatized with dinitrophenyl-hydrazine (DNPH) to give a stable hydrazone that can be detected at 380 to 400 nm or with a good response by ESI-MS. Since the product occurs in both *Z*- and *E*-configuration the limit of detection is reduced but both peaks can be used for quantification.

Due to the good solubility of HMF in water this can be used for extraction of the analyte. The extract can be clarified by the use of Carrez I and Carrez II instead of acids (trichloroacetic TCA, m-phosphoric, sulfosalycilic). TCA was evaluated in detail by Ameur and co-workers [2006] which gave the best recovery without the formation of

artefactual HMF during sample preparation. The addition of TCA to the extraction solvent was also used in other matrices such as milk [Morales and Jimenez-Perez, 2001; van Boekel and Rehman, 1987] and fruit preparations [Ibarz et al., 2000; Rada-Mendoza et al., 2002].

Other possibilities of separation include the use of flow injection analysis [de la Inglesia et al., 1997] or micellar electrokinetic chromatography (MEKC) using sodium dodecyl sulphate (SDS) as background electrolyte buffered at pH 8.5 [Teixido et al., 2011] or with sodium tetraborate at pH 9.3 [Rizelio, 2011]. With this method a limit of quantification of 2.5 mg/kg could be reached. This method was applied for breakfast cereals, toasts, honey, orange juice, apple juice, jam, coffee, chocolate, and biscuits. After derivatization with e.g. BSTFA it is also possible to analyse HMF by GC-MS. In this method a clean-up including a solid phase extraction was necessary resulting in a limit of quantification of 6 mg/kg [Teixido et al., 2006].

Zappala and co-workers [2005] published a comparison of the routine methods for measuring the HMF content in honey. They concluded that the HPLC method and the photometric method according to White gave similar results, whereas the photometric method according to Winkler generally resulted in higher values.

Formation of 5-hydroxymethyl furfural

Antal and co-workers [1990] proved experimentally that the mechanism of the HMF formation goes through cyclic intermediates as is shown in Fig. 1. Their interpretation was supported by the experimental results that 1) HMF is easily formed from fructose or sucrose, 2) 2,5-anhydro-D-mannose converts easily into HMF (this compound is a parent aldehyde to the enol), 3) when the reaction was carried out in D_2O starting from fructose, deuterium was absent in HMF.

Similar results were obtained by Perez-Locas and Yaylayan [2008] who showed by pyrolysis GC-MS that especially in dry and high temperature systems at temperatures above 250 °C, 90 % of HMF originated from the fructose moiety and only 10 % originated from the glucose. Alternatively, when sucrose was refluxed in acidic methanol at 65 °C, 100 % of HMF was generated from the glucose moiety. When comparing the conversion efficiency of the well known HMF precursor 3-deoxyglucosone with glucose, fructose, and sucrose they could show that fructose and sucrose had a significantly higher conversion rate which indicated that glucose is not a major precursor of HMF in fructose and sucrose solutions. Based on the data generated, they proposed a mechanism of HMF

formation from sucrose in which sucrose degrades into glucose and a very reactive fructofuranosyl cation. Subsequently, in dry conditions this cation can be effectively converted directly into HMF.

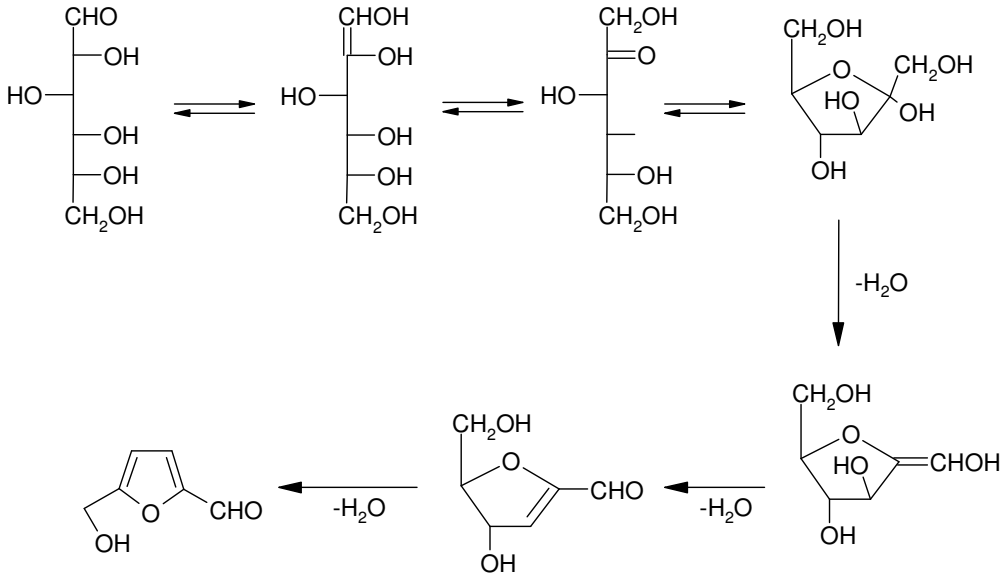


Fig. 1: Formation of HMF by dehydration reactions [Lewkowski, 2001]

Cämmerer and co-workers suggested a different mechanism of formation of HMF by isomerization of glyceraldehyde followed by dehydration to methylglyoxal and its subsequent condensation with another molecule of glyceraldehyde. They also concluded that HMF is formed by the two competing reactions presented here. [Cämmerer et al., 1999]

Looking at the concentration of HMF in similar products it can be seen that depending on the raw materials, variation in composition, and variation in heat load during processing a huge variation in the formation of HMF can occur. Ameur and co-workers [2006] observed a strong variation in the HMF concentration within the 17 commercial cookies, ranging from 0.5 to 74.6 mg/kg. These results are comparable to other reports e.g. between 0.4 and 65.5 mg/kg in infant cereals [Fernandez–Artigas et al., 1999; Ramirez-Jimenez et al., 2003], and between 3.7 and 193 mg/kg in breakfast cereals [Garcia–Villanova et al., 1993].

The processing of balsamic vinegar (especially traditional balsamic vinegar) includes a cooking step of the must. During cooking the must temperature is raised to the boiling

point followed by skimming to remove dispersed solids and denatured proteins. Then the temperature is kept at 80 – 90 °C for several hours to evaporate water and concentrate the soluble solids up to 35 – 60 °Brix.

During the heat treatment non-enzymatic browning reactions are occurring that are giving the product the typical dark brown colour. The high temperature, low pH value and reducing water activity enhance the formation of HMF. The high sugar concentrations – typically 236 g/L glucose and 211 g/L fructose – lead to such high concentrations as indicated in table 1 [Giudici et al., 2009]. In addition, traditional balsamic vinegar is stored for at least 12 years.

During the storage of a juice of peaches the content of HMF increases from ca. 0.3 up to 8 mg/kg. In these experiments the storage temperature was kept at 37 °C which is rather high but this should simulate the longer storage times which reflects a typical shelf life of 12 months of these products. [Lavelli et al., 2009]

The legal limit for HMF in honey was set due to restricted processing conditions to 40 mg/kg. This limit is not based on toxicological reasons [EC Directive 74/409/EEC]. In the fair trade standards a quality grading system is used to produce honey with a HMF content as low as possible suggesting values of below 20 mg/kg [Fair-trade Standards, 2005].

Comprehensive surveys of average honey composition have established that the major components are fructose (38.4 %), glucose (30.3 %), and water (17.2 %). In addition to the two major sugars are an array of more than 20 higher sugars, which are formed by linking the fructose and glucose in various combinations. Honey is therefore primarily a carbohydrate material, and sugars comprise over 95 % of its solids [Doner, 2003]. The average pH of honey was determined by White [1962] as 3.91 which ranges from 3.4 to 6.1. Because of this special composition honey is extremely sensitive to HMF formation and especially if heated the HMF concentration rises significantly. During processing of honey heating is not allowed ...to such an extent that its natural enzymes are destroyed or made inactive... [Council Directives 74/409/EEC, 2001/110/EEC] and HMF is analysed as an indicator for heat treatment.

Table 1: Occurrence of HMF (mg/kg; mg/L) in different foods

Food item	HMF	References
Honey	0.1 – 140	Nozal et al., 2001; Spano et al., 2006; Teixido et al., 2011; Rizelio et al., 2011; Teixido et al., 2006
Breakfast cereals	4 – 193	Garcia-Villanova et al., 1993; Rufian-Henares et al., 2009; Teixido et al., 2011; Teixido et al., 2006
Infant cereals	0.4 – 66	Fernandez–Artigas et al., 1999; Ramirez-Jimenez et al., 2003
Orange juice	< LOD – 22	Yuan and Chen, 1998; Teixido et al., 2011
Apple juice	< LOD – 3.5	Gaspar and Lucena, 2009; Mochizuki et al., 2009; Teixido et al., 2011
Jam	2.7 – 160	Rada-Mendoza et al., 2002; Rada-Mendoza et al., 2004; Vorlova et al., 2006; Teixido et al., 2011; Teixido et al., 2006
Caramel containing drinks	0.8 – 80	Brenna et al., 2009
Biscuits	< LOQ – 180	Delgado-Andrade, et al., 2009; Ramirez-Jimenez et al., 2000; Teixido et al., 2011
Cookies	0.5 – 75	Ameur et al., 2006
Bread	2.2 – 88	Cardenas Ruiz et al., 2004; Ramirez-Jimenez et al., 2001, 2000a
Toasted bread	1.7 – 2,030	Ramirez-Jimenez et al., 2001; Teixido et al., 2011
Pasta	0.08 – 7	Sensidoni et al., 1999
Coffee	110 – 1,900	Murkovic and Pichler, 2006; Murkovic and Bornik, 2007; Teixido et al., 2011
Coffee, instant	24 – 4,020	Arribas-Lorenzo and Morales, 2010; Murkovic and Pichler, 2006; Teixido et al., 2011
Chocolate	42 – 99	Teixido et al., 2011

Wines, fortified	20 – 170	Ho et al., 1999
Ketchup	0.8 – 190	Vorlova et al., 2006
Tomato purée	2.8 – 84	Vorlova et al., 2006
Dried fruits	1 – 2,200	Murkovic and Pichler, 2006
Syrup	1.3 – 27	Vorlova et al., 2006
Fruit baby food	2.1 – 9.8	Vorlova et al., 2006
Special regional products		
Abbamele (honey decoction)	880 – 4,800	Spano et al., 2008
Bread with dried fruits	450	Murkovic and Pichler, 2006
Aged sugar cane spirits	0.8 – 3.1	De Aquino et al., 2006
Pekmez (concentrated juice from grapes or mulberries)	< 14,000	Bozkurt et al., 1999
Balsamic vinegar	246 – 4,040	Masino et al., 2005; Masino et al., 2008
Churros (deep fried dough pastry)	74 ± 48	Morales and Arribas-Lorenzo, 2008
Treacle (black honey)	66 – 180	Edris et al., 2007

Exposure

The exposure to HMF was evaluated by Husoy and co-workers recently [2008]. In this study a group of 47 non-smokers was evaluated using a 24 hour dietary recall. The food list obtained from this dietary recall was collected and analysed for HMF and 5-hydroxymethyl-furanoic acid (HMFA). Since most of the HMF is metabolised to HMFA in the kidneys the urine was collected during the testing period. From this study the daily mean exposure was calculated being 5.6 mg. The 95th percentile of the estimated daily dietary intake of HMF was 27.6 mg. In the tested group the most important source of HMF was coffee (63 %), both because of the high levels of HMF in coffee and because of the high consumption of coffee among the participants. The second most important food sources of HMF were milk products (11 %) followed by juice (9 %), bread (7 %), and beer (4 %). The calculated uptake correlated well with the urinary excretion of HMFA [Jöbstl et al., 2010].

Furfuryl alcohol

Although furfuryl alcohol can polymerize in acid conditions the concentration of the monomer is still high in heated foods. Furfuryl alcohol gives a burnt, cooked-sugar, or rubber-like odour to sugar; when furfuryl alcohol interacts with dihydroxy benzene or trihydroxy benzene as it is occurring during roasting of coffee it will produce a bitter taste [Kreppenhofer et al., 2011]. Nevertheless, furfuryl alcohol is used as a flavouring agent with an acceptable daily intake of up to 0.5 mg/kg BW [FAO/WHO, 2000].

Analysis of furfuryl alcohol

The analysis of furfuryl alcohol can be done either by liquid or gas chromatography. Due to the better separation and more sensitive detection the analysis by GC-MS is preferred. In most of the products the furfuryl alcohol concentration is comparably low and these foods do not contribute significantly to the exposure. A method using head space solid phase microextraction (SPME) coupled to GC-MS was introduced by Yand and Peppard [1994]. They analysed roasted coffee and fruit juice beverages using a fused silica fibre coated with poly(dimethylsiloxane) (100 µm). The extraction of the furfuryl alcohol could be done by solvent extraction, simultaneous distillation extraction and nitrogen purge and steam distillation [Jerkovic et al., 2007]. The applicability of headspace GC-MS for the furfuryl alcohol analysis was shown by Kumazawa and Masuda [2003].

Another method using liquid chromatography with UV detection was published by Yuan and Chen in 1999. Different furanic compounds were separated on an Aminex HPX-87H column (300 x 7.8 mm) with a mobile phase consisting of acetonitrile and 5 mM sulfuric acid (16:84, v/v). With the diode array detector measuring at 254 nm they were able to analyse furfuryl alcohol in fruit juices.

Formation of furfuryl alcohol

Glucose or fructose can undergo isomerization reactions at high temperatures. The key intermediate in this isomerization reaction, 1,2-enediol, is also considered as the starting intermediate in the degradation reactions by β-elimination producing an unstable compound 3-deoxyaldoketose which then undergoes a cleavage reaction producing formic acid and a C_5-compound [de Bruijn et al., 1986]. The C_5-compound (2-deoxypentose) will react further by cyclization and aromatization forming furfuryl alcohol (Fig. 3) [Brands and van Boekel, 2001]. Besides that, heating of quinic acid at

250 °C for 30 min under a stream of nitrogen produces furfuryl alcohol (250 µg/g quinic acid), Fig. 2 [Moon and Shibamoto, 2010]. Quantitatively, furfural alcohol as a furan derivative is predominating in roasted coffee [Kreppenhofer et al., 2011].

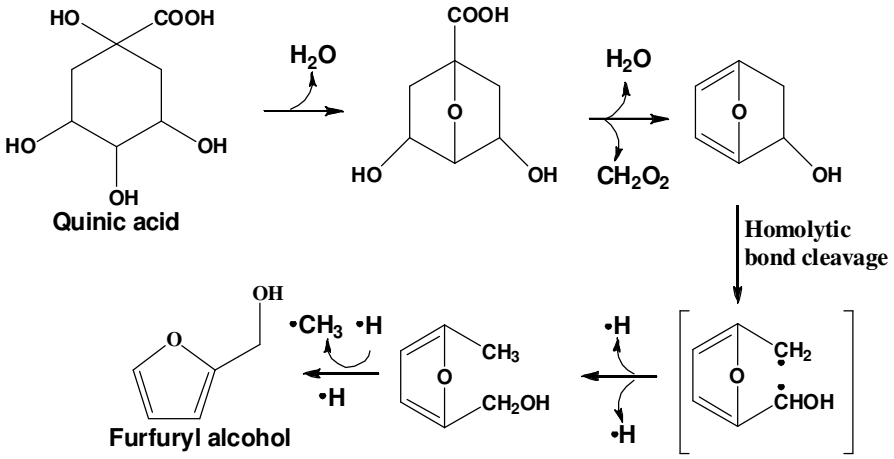


Fig. 2: Formation of furfuryl alcohol from degradation of quinic acid [Moon and Shibamoto, 2010]

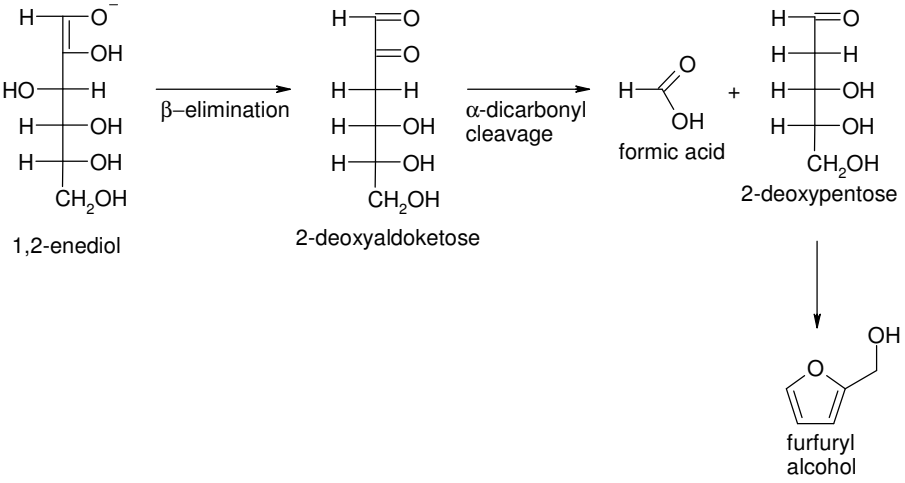


Fig. 3: Formation of furfuryl alcohol from degradation of reducing sugars [Brands and van Boekel, 2001]

Furfuryl alcohol in foods

Although the polymerization proceeds during the roasting of coffee the concentration of the monomeric furfuryl alcohol is still high in the finished products. The concentration of furfuryl alcohol is 267 µg/g in instant coffee and in 564 µg/g coffee roasted at 210 °C for

3 min [Golubkova, 2011]. Coffee of a medium roast contains more furfuryl alcohol compared to a light roast. [Moon and Shibamoto, 2009]. Furthermore, furfuryl alcohol is also found in rice cakes 2 – 2.3 µg/g [Buttery et al., 1999], bread 187 µg/g [Jensen et al., 2011], honey 1.55 µg/g [Vazques et al., 2007], toasted almond cv. Marcona 5.97 ± 1.09 µg/g, toasted almond cv. Comuna 8.88 ± 1.39 µg/g, toasted almond cv. California 4.40 ± 1.23 µg/g [Vázquez-Araújo et al., 2008], non fat dried milk stored for 3 months at room temperature 14.5 µg/g [Karagu-Yüceer et al., 2002], popcorn 0.0382 – 0.0821 µg/g [Park and Maga, 2006], corn tortilla chips 0.54 µg/g [Buttery and Ling, 1998], roasted cocoa powder 0.021 µg/g [Bonvehi, 2005], palm sugar made by a traditional heating process at 210 °C 0.139 µg/g, palm sugar which was made by an increased temperature of 240 °C contains significantly more furfuryl alcohol (0.518 µg/g) [Ho et al., 2007], baked "Jewel" sweet potato 0.014 µg/g fresh weight [Wang and Kays, 2000], and citrus honey 0.011 µg/g [Castro-Vázques et al., 2007]. In addition, furfuryl alcohol was found in oil that was used for frying of beef, veal, and chicken [Takeoka et al., 1996].

Health issues of furfuryl alcohol

Estimated furfuryl alcohol intake is 130 µg/kg BW [Munro and Danielewska-Nikiel, 2006]. Furfuryl alcohol is mutagenic to *Salmonella typhimurium* strains TA100 engineered for the expression of human SULT1A1 because sulphotransferases can activate furfuryl alcohol into a mutagenic compound, 2-sulfooxymethylfuran. The 2-sulfooxymethylfuran is generated intracellularly in proximity to the bacterial DNA leading to the formation of 2-methylfuranyl adduct. The covalent 2-methylfuranyl adduct causes the mutagenic effect. The mutagenicity of furfuryl alcohol is dose dependent and increases its mutagenicity when the amount of furfuryl alcohol is increased from 3 to 200 nmol per plate [Monien et al., 2011]. In mice which received furfuryl alcohol with the drinking water the DNA samples of liver, kidney, and lung contain 2-methylfuranyl adducts. In rodents which were exposed to furfuryl alcohol tumours that contained 2-methylfuranyl adducts were formed [NTP, 1999].

Conclusions

Furfuryl alcohol and HMF can occur in foods at very high concentrations. Although the acute toxicity of these compounds is not relevant in the foods they could be activated with sulphotransferases to highly reactive compounds which are then mutagenic/carcinogenic.

The detailed risks of these compounds and other compounds that could be metabolized to similar substrates for these enzymes is not yet known and additional work is pending.

References

Ameur L.A., Trystram G., Birlouez-Aragon I. (2006) Accumulation of 5-hydroxymethyl-2-furfural in cookies during the backing process: Validation of an extraction method. Food Chem. 98, 790-796.

Antal M. J., Mok W. S. L.. Richards, G. N. (1990) Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from D-fructose and sucrose. Carbohydr. Res. 199, 91-109.

Arribas-Lorenzo G., Morales F.J. (2010) Estimation of dietary intake of 5-hydroxymethylfurfural and related substances from coffee to Spanish population. Food Chem. Toxicol. 48, 644-649.

Bonvehì J.S. (2005) Investigation of aromatic compounds in roasted cocoa powder. European Food Research and Technololgy. 221, 19-29.

Bozkurt H., Gogus F., Eren S. (1999) Non-enzymic browning reactions in boiled grape juice and its models during storage. Food Chem. 64, 89-93.

Brands C.M.J., Boekel M.A.J.S. (2001) Reactions of monosaccharides during heating of sugar-casein systems: building of a reaction network model. J. Agric. Food Chem. 49, 4667-4675.

Brenna O.V., Ceppi E.L.M., Giovanelli G. (2009) Antioxidant capacity of some caramel-containing soft drinks. Food Chem. 115, 119-123.

Buttery R.G., Orts W.J., Takeoka G.R., Nam Y. (1999) Volatile flavour components of rice cakes. J. Agric Food Chem. 47, 4353-4356.

Buttery R.G., Ling L.C. (1998) Additional studies on flavor components of corn tortilla chips. J. Agric. Food Chem. 46, 2764-2769.

Cämmerer B., Wedzicha B.L., Kroh L.W. (1999) Nonenzymatic browning reactions of retro-aldol degradation products of carbohydrates. Eur. Food Res. Technol. 209, 261-265.

Cardenas Ruiz J., Guerra-Hernandez E., Garcia-Villanova B. (2004) Furosine is a useful indicator in pre-baked breads. J. Sci. Food Agric. 84, 366-370.

Castro-Vázquez L., Díaz-Maroto L.C., Pérez-Coello M.S. (2007) Aroma composition and new chemical markers of Spanish citrus honeys. Food Chem. 103, 601-606.

de Aquino F.W.B., Rodrigues S., do Nascimento R.F., Casimiro A.R.S. (2006) Simultaneous determination of aging markers in sugar cane spirits. Food Chem. 98, 569-574.

de Bruijn J. M., Kieboom A. P. G., Van Bekkum H., Van Der Poel P. W. (1986) Reactions of monosaccharides in aqueous alkaline solutions. Sugar Technol. Rev. 13, 21-52.

de la Inglesia F., Lazaro F., Puchades R., Maquieira A. (1997) Automatic determination of 5-hydroxymethylfurfural (5-HMF) by a flow injection method. Food Chem. 60, 245-250.

Delgado-Andrade C., Rufian-Henares J.A., Morales F.J. (2009) Hydroxymethylfurfural in commercial biscuits marketed in Spain. J. Food Nutr. Res. 48, 14-19.

DIN 10751-1, Norm, 2010-08, Untersuchung von Honig - Bestimmung des Gehaltes an Hydroxymethylfurfural - Teil 1: Photometrisches Verfahren nach Winkler.

DIN 10751-3 (2002-02), Determination of the hydroxymethylfurfural content of honey by high-performance liquid chromatographic, Determination of hydroxymethylfurfural (HMF) content.

Doner L.W. (2003) Honey, in Caballero B., Trugo L.C., Finglas P.M. (Eds) Encyclopedia of Food Sciences and Nutrition, 2nd Ed., Academic Press, Amsterdam, pp 3125-3130.

Edris A.E., 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.

Fernandez–Artigas P., Guerra-Hernandez E., Garcia-Villanova B. (1999) Browning in model systems and baby cereals. J. Agric. Food Chem. 47, 2872-2878.

FAO (2001) Revised Codex standard for honey, CODEX STAN 12-1981, Rev.1 (1987), Rev.2 (2001).

Gaspar E.M.S.M., Lucena A.F.F. (2009) Improved HPLC methodology for food control – Furfurals and patulin as markers of quality. Food Chem. 114, 1576-1582.

Giudici G., Gullo M., Solieri L. (2009) Traditional balsamic vinegar; in Solieri L., Giudici P. (Eds) Vinegars of the world. Springer, Heidelberg, Germany, pp 157-177.

Garcia-Villanova B., Guerra-Hernandez E., Martinez-Gomez E., Montilla J. (1993) Liquid chromatography for the determination of 5-(hydroxymethyl)-2-furaldehyde in breakfast cereals. J. Agric. Food Chem. 41, 1254-1255.

Glatt H.R., Sommer Y. (2006) Health risk of 5-hydroxymethylfurfural (HMF) and related compounds. Skog K., Alexander J. (Eds) Acrylamide and other hazardous compounds in heat-treated foods. CRC press, Boca Raton, USA, pp 328-357.

Glatt H.R., 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, 41-48.

Golubkova T. (2011) Bildung von potentiell toxischen Furanderivaten in Lebensmitteln. Diplomarbeit. Institut für Biochemie TU Graz. Austria, 38-40.

Harmonised methods of the European Honey Commission, Apidologie - special issue, 28, 1997.

Ho P., Hogg T.A., Silva M.C.M. (1999) Application of a liquid chromatographic method for the determination of phenolic compounds and furans in fortified wines. Food Chem. 64, 115-122.

Ho C.W., Wan Aida W.M., Maskat M.Y., Osman, H. (2007) Changes in volatile compounds of palm sap (Arenga pinnata) during the heating process for production of palm sugar. Food Chem. 102, 1156-1162.

Husoy T., Haugen M., Murkovic M., Jöbstl D., Stolen L.H., Bjellaas T., Ronningborg C., Grall H.R., Alexander J. (2008) Dietary exposure to 5-hydroxymethylfurfural from Norwegian food and correlations with urine metabolites of short-term exposure. Food Chem. Toxicol. 46, 3697-3702.

Ibarz A., Pagan J., Garza, S. (2000) Kinetic models of nonenzymatic browning in apple puree. J. Sci. Food Agric. 80, 1162-1168.

Jensen S., Ostdal H., Skibsted L.H., Thybo A.K. (2011) Antioxidants and shelf life of whole wheat bread. J. Cereal Sci. 1-7.

Jerkovic I., Mastelic J., Tartaglia S. (2007) A study of volatile flavour substances in Dalmatian traditional smoked ham: Impact of dry-curing and frying. Food Chem. 104, 1030-1039.

Jöbstl D., Husoy T., Alexander J., Bjellaas T., Leitner E., Murkovic M. (2010) Analysis of 5-hydroxymethyl-2-furoic acid (HMFA) the main metabolite of alimentary 5-hydroxymethyl-2-furfural (HMF) with HPLC and GC in urine. Food Chem. 123, 814-818.

Joint FAO/WHO expert committee on food additives. (2000). Fifty-fifth meeting. Geneva. FAO and WHO

Karagu-Yüceer Y, Cadwallader K.R., Drake M. (2002) Volatile flavor components of stored nonfat dry milk. J. Agric Food Chem. 50, 305-312.

Kreppenhofer S., Frank O., Hofmann T. (2011) Identification of (furan-2yl) methylated benzene diols and triols as a novel class of bitter compounds in roasted coffee. Food Chem. 126, 441-449.

Kumazawa K., Masuda H. (2003) Investigation of the change in the flavor of a coffee drink during heat processing. J. Agric. Food Chem. 51, 2674-2678.

Lavelli V., Pompei C., Casadei M.A. (2009) Quality of nectarine and peach nectars as affected by lye-peeling and storage. Food Chem. 115, 1291-1298.

Lewkowski J. (2001) Synthesis, chemistry and applications of 5-hydroxymethylfurfural and its derivatives. ARKIVOC 2001 (i) 17-54.

Masino F., Chinnici F., Franchini G.C., Ulrici A., Antonelli A. (2005) A study of the relationships among acidity, sugar and furanic compound concentrations in set of casks for Aceto Balsamico Tradizionale of Reggio Emilia by multivariate techniques. Food Chem. 92, 673-679.

Masino F., Chinnici F., Bendini A., Montevecchi G., Antonelli A. (2008) A study on relationships among chemical, physical, and qualitative assessment in traditional balsamic vinegar. Food Chem. 106, 90-95.

Mijares R.M., Park G.L., Nelson D.B., McIver R.C. (1985) HPLC analysis of HMF in orange juice. J. Food Sci. 51, 843-844.

Mochizuki N., Hoshino M., Suga K., Sugita-Konishi Y. (2009) Identification of an interfering substrate in apple juice and improvement for determination of patulin with high-performance liquid chromatography analyses. J. Food Prot. 72, 805-809.

Monien B.H., Hermann K., Florian S., Glatt H.R. (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 Advance Access.

Moon J., Shibamoto T.(2010) Formation of volatile chemicals from thermal degradation of less volatile coffee components: quinic acid, caffeic acid, and chlorogenic acid. J. Agric. Food Chem. 58, 5465-5470.

Moon J., Shibamoto T. (2009) Role of roasting conditions in the profile of volatile flavor chemicals Formed from Coffee Beans. J. Agric Food Chem. 57, 5823-5831.

Morales F.J., Jimenez-Perez S. (2001) Hydroxymethylfurfural determination in infant milk-based formulas by micellar electrokinetic capillary chromatography. Food Chem. 72, 525-531.

Morales F.J., Arribas-Lorenzo G. (2008) The formation of potentially harmful compounds in churros, a Spanish fried-dough pastry, as influenced by deep frying conditions. Food Chem. 109, 421-425.

Munro I.C., Danielewska-Nikiel B. (2006) Comparison of estimated daily intakes of flavouring substances with no-observed-effect levels. Food Chem. Toxicol. 44, 758-809.

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

Murkovic M., Bornik A.-M. (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.

National Toxicological Program (1999) Toxicology and carcinogenesis studies of furfuryl alcohol (CAS No.98-00-0) in F344/N rats and B6C3F1 mice (inhalation studies). Natl. Toxicol. Program Tech. Rep. Ser. NTP - Department of Health and Human Services, vol. 482.

National Toxicological Program (2010) http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/TR554.pdf

Nozal M.J., Bernal J.L., Toribio L., Jimenez J.J., Martin M.T. (2001) High-performance liquid chromatographic determination of methyl anthranilate, hydroxymethylfurfural and related compounds in honey. J. Chromatogr. A, 917, 95-103.

Park D., Maga J.A. (2006) Identification of key volatiles responsible for odour quality differences in popped popcorn of selected hybrids. Food Chem. 99, 538-545.

Perez-Locas C., Yaylayan V. (2008) Isotope labeling studies on the formation of 5-(hydroxymethyl)-2-furaldehyde (HMF) from sucrose by pyrolysis-GC/MS. J. Agric. Food Chem. 56, 6717-6723.

Rada-Mendoza M., Olano A., Villamiel M. (2002) Determination of hydroxymethylfurfural in commercial jams and in fruit-based infant foods. Food Chem. 79, 513-516.

Rada-Mendoza M., Sanz M.L., Olano A., Villamiel M. (2004) Formation of hydroxymethylfurfural and furosine during the storage of jams and fruit-based infant foods. Food Chem. 85, 605-609.

Ramirez-Jimenez A., Garcia-Villanova B., Guerra-Hernandez E. (2000) Hydroxymethylfurfural and methylfurfural content of selected bakery products. Food Res. Internatl. 33, 833-838.

Ramirez-Jimenez A., Guerra-Hernandez E., Garcia -Villanova B. (2000a) Browning indicators in bread. J. Agric. Food Chem. 48, 4176-4181.

Ramirez-Jimenez A., Garcia -Villanova B., Guerra-Hernandez E. (2001) Effect of toasting time on the browning of sliced bread. J. Sci. Food Agric. 81, 513-518.

Ramirez-Jimenez A., Guerra-Hernandez E., Garcia-Villanova B. (2003) Evolution of non enzymatic browning during storage of infant rice cereal. Food Chem. 83, 219-225.

Rizelio V.M., Gonzaga L.V., da Silva Campelo Borges G., Micke G.A., Fett R., Costa A.C.O. (2011) Development of a fast MEKC method for determination of 5-HMF in honey samples. Food Chem. DOI: 10.1016/j.foodchem.2011.11.058.

Rufian-Henares J.A., Delgado-Andrade C., Morales F.J. (2009) Assessing the Maillard reaction development during the toasting process of common flours employed by the cereal products industry. Food Chem. 114, 93-99.

Sensidoni A., Peressini D., Pollini, C.M. (1999) Study of the Maillard reaction in model systems under conditions related to the industrial process of pasta thermal VHT treatment. J Sci. Food Agric. 79, 317-322.

Spano N., Casula L., Panzanelli A., Pilo M.I., Piu P.C., Scanu R. (2006) An RP-HPLC determination of 5-hydroxymethylfurfural in honey: The case of strawberry tree honey. Talanta 68, 1390-1395.

Spano N., Ciulu M., Floris I., Panzanelli A., Pilo M.I., Piu P.C., Scanu R., Sanna G. (2008) Chemical characterization of a traditional honey-based Sardinian product: Abbamele. Food Chem. 108, 81-85.

Takeoka G., Perrino C. Jr., Buttery R. (1996) Volatile Constituents of used frying oils. J. Agric. Food Chem. 44, 654-660.

Teixido E., Santos F.J., Puignou L., Galceran M.T. (2006) Analysis of 5-hydroxymethylfurfural in foods by gas chromatography–mass spectrometry. J. Chromatogr. A 1135, 85-90.

van Boekel M.A.J.S., Rehman Z. (1987) Determination of hydroxymethylfurfural in heated milk by high-performance liquid chromatography. Netherlands Milk and Dairy Journal 41, 297-306.

Vázquez L., Verdú A., Miquel A., Burl F., Carbonell-Barrachina A.A. (2007) Changes in physico-chemical properties, hydroxymethylfurfural and volatile compounds during concentration of honey and sugars in Alicante and Jijona turrón. Europ. Food Res. Technol. 225, 757-767.

Vázquez-Araújo L., Enguix L., Verdú A., Garcıá-Garcıá E., Carbonell-Barrachina, A.A. (2008) Investigation of aromatic compounds in toasted almonds used for the manufacture of turrón. Europ. Food Res. Technol. 227, 243-254.

Vorlova L., Borkovcova I., Kalabova K., Vecerek V. (2006) Hydroxymethylfurfural contents in foodstuffs determined by HPLC method. J. Food Nutr. Res. 45, 34-38.

Wang Y., Kays S.J. (2000) Contribution of volatile compounds to the characteristic aroma of baked 'Jewel' sweet potatoes. J. Amer. Soc. Hort. Sci. 125, 638-643.

White J.W. Jr. (1962) Composition of American Honeys. Technical Bulletin No. 1261. US Department of Agriculture.

White J. (1979) Spectrophotometric method for hydroxymethylfurfural in honey. J. AOAC, 509

Yang X., Peppard T. (1994) Solid-phase microextraction for flavor analysis. J. Agric. Food Chem. 42, 1925-1930.

Yuan J.-P., Chen F. (1998) Separation and identification of furanic compounds in fruit juices and drinks by high-performance liquid chromatography photodiode array detection. J. Agric. Food Chem. 46, 1286-1291.

Yuan J.-P., Chen F. (1999) Simultaneous separation and determination of sugars, ascorbic acid and furanic compounds by HPLC-dual detection. Food Chem. 64, 423-427.

Zappala B., Fallico B., Arena E., Verzera A. (2005) Methods for the determination of HMF in honey: a comparison. Food Control 16, 273-277.

Zhang X.-M., Chan C.C., Stamp D., Minkon S., Archer M.C., Bruce W. Jr. (1993) Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethyl-2-furaldehyde in thermolyzed sucrose. Carcinogenesis 14, 773-775

SECTION 5

CURRICULUM VITAE

Personal Data

Name : Yuliana Reni Swasti
Place of Birth : Sleman, Indonesia
Date of Birth : 6th September 1980
Nationality : Indonesien
Address : Moserhofgasse 22/35, 8010 Graz, Austria
Email : yulianareni@yahoo.com

Education

2009 – 2012
PhD in Functional Food Group, Institute of Biochemistry, Graz University of Technology

2005 – 2007
Master Degree in Division of Food Science and Technology, Postgraduate School, Gadjah Mada University, Yogyakarta, Indonesia

1999 – 2004
Bachelor Degree in Division of Food and Agricultural Product Technology Faculty of Agricultural Technology Gadjah Mada University, Yogyakarta, Indonesia