AMERICAN UNIVERSITY OF BEIRUT

DETECTION OF 5-HYDROXYMETHYLFURFURAL AND FURFURAL IN AEROSOLS OF ELECTRONIC CIGARETTE

by
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AN ABSTRACT OF THE THESIS OF

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Title: Detection of 5-hydroxymethylfurfural and furfural in aerosols of electronic cigarette

The flavorant variety expansion in electronic cigarette market has heavily served in promoting youth vaping. Among various flavorants, sweetness has been identified as an important factor for the choice of e-liquids. Sugar compounds imparting the sweet flavoring might be intentionally added by manufactures or delivered from tobacco leaves during extraction processes. These compounds can thermally degrade into 5-hydroxymethylfurfural (HMF) and furfural (FA); a class of furnaic compounds that have raised potential health concerns.

In this work HMF and FA formation in electronic cigarettes aerosol was systematically studied by varying sugar type and concentration, battery input and puff duration. Specifically, PG/VG standard solutions containing sucrose, glucose and fructose were vaped using a customized puffing machine operating at (4.3 and 10.8) W power and (4 and 8) sec puff duration. Generated aerosols were collected on filter pads, extracted and then analyzed according to an optimized SPE-GC-MS method. It has been found that e-liquids with sucrose and glucose content > 0.02% (0.38 mg/mL) are considered potential sources of HMF and FA when vaped. Unlike puff duration, battery input and sugar concentration have significant influence on furanic yields.

Additionally, a LC-ESI-MS method with minimal sample handling was designed to assess sucrose, glucose and fructose compounds in e-liquid solutions. Sugar compounds were detected in e-liquids flavored with Carnival Cotton Candy, Creme Anglaise and Welsh Taffi.

It has been found that under certain conditions the presence of sugar compounds expose ECIG users to potentially harmful compounds.

CONTENTS

ILLUSTRATIONS

Figure Page

TABLES

Table Page

ABBREVIATIONS

%RSD	percent relative standard deviation
AP	acetyl propionyl
AUB	American University of Beirut
CIG	Cigarette
DA	diacetyl
DAD	Diode array detector
DHS	Dynamic Head Space
ECIG	Electronic Cigarette
EI	Electron impact ionization
ELSD	Evaporative light scattering detector
ENDs	Electronic nicotine delivery systems
ESI	Electrospray ionization
FA	Furfural
FEMA	Flavors extracts manufacturers association
FID	Flame Ionization Detector
GC	Gas chromatograph
HMF	5-hydroxymethylfurfural
HPLC	High performance liquid chromatography
IARC	International Agency for Research on Cancer
IS	Internal standard
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry

P	Power
PG	Propylene glycol
QA	Quality Assurance
QC	Quality control
R	Resistance
R^2	Correlation Coefficient
RI	Refractive index
RP-HPLC	Reversed phase high performance liquid chromatography
SPE	Solid phase extraction
TDU	Thermal Desorption Unit
TIC	Total ion chromatogram
TPM	Total particular matter
U.S. FDA	United States Food and drug agency
UV/Vis	Ultraviolet/Visible
V	Voltage
VG	Vegetable glycerin
WHO	World health organization
WPS	Water-pipes

CHAPTER I

INTRODUCTION

This work explores the potential impact of integrating sugar in e-liquid formulation. Chapter II describes the optimization and validation of SPE-GC-MS, chapter III ascertains FA and HMF generated from three different types of sugar (Glucose, sucrose and sorbitol) prepared in PG/VG .Solutions were vaped under two battery output (4.3 W and 10.8 W) and two puff durations (4 s and 8 s). In addition to that, the effect of sucrose concentration was studied under 4.3 W and 4s. Chapter IV presents the optimized LC-ESI-MS method and its implementation on sixteen flavored e-liquids

Over the past decades, tobacco industry has manipulated a vast array of strategies to achieve high volume sales.[1,2] The inclusion of variety of additives has served as a basic gateway towards emerging more competitive and profitable tobacco products.[1,3-6] Beside the prime addictive constituent nicotine, sugar and ammonia among many other compounds have been used for addiction and attractiveness enhancement.[7,8] Recently, manufactures have excessively exploited this tactic to advertise electronic nicotine delivery system (ENDs), a new emerging class of products.[9-11] After studying the adoption of additives in conventional tobacco products for years, the scientific community has started exploring the influence of additives in ENDs.

A. Additives in Tobacco Products

According to World Health Organization (WHO), additives are substances added to tobacco products during the course of manufacturing for wide range of purposes.[12] Typical additives include humectants to keep tobacco moist i.e. glycerol, propylene glycol and sorbitol, flavorants to impart unique aroma and taste i.e. sugar and menthol, casing materials

to improve smoking quality i.e. sugar and fruit extracts, in addition to nicotine delivery regulators to control nicotine delivery and harshness i.e. ammonia and lactic acid.[1,12-14] Tobacco industry in the United States (U.S.) has acknowledged the use of 599 cigarette additives and attributed about 10% by weight of U.S. style cigarettes to sugar, glycerol, propylene glycol and ammonia compounds.[1,15] Acting as flavorant, humectant and casing material, sugar has proved successful as one of the basic tobacco additives to promote and advertise cigarette smoking particularly among youth.[7] Concurrent with the considerable use of sugar by five major tobacco companies, the tobacco industry has not addressed the safety concerns associated with this additive.[7,15] In an effort to fill this gap, scientists have undertaken thorough studies to explore the contribution of cigarette sugar content to smoking behavior.[16-22]

A. Sugars in tobacco products: Amount, Function and Fate

Sugar including mono-di and polysaccharides are natural tobacco constituents present in levels up to 20% w/w (20 mg per g tobacco).[1] However; during processing and curing, tobacco leaves are prone to lose some of their sugar content. Manufactures have replenished this probable loss by adding sugar to a percent that can reach up to16% w/w.[8,14,23] Added in a variety of forms, sugar compounds can be divided into four main groups: monosaccharaides, disaccharides, sugar alcohol, and high intensify sweeteners as shown in Figure I 1.

The chemical analysis of several tobacco products has recognized glucose, fructose and sucrose as major sugar additives.[8,18,22,24] In 58 different brands of cigarette, Jansen et al. [25] have detected glucose, fructose and sucrose in average amounts of 52.3, 87.6 and 34.5 % w/w tobacco, respectively. Additionally, Clarke et al.[26] have reported the sum of the three sugar compounds in both combustible (cigarette and cigar) and smokeless (snuff and chewing

gum) class of products The range of total sugar content of around ten samples from each type was found to be: cigarette (6.667-12.285 % w/w), cigar (0.002 -1.023% w/w), snuff (0-0.19%w/w) and chewing gum (5.963 and 40.710% w/w). The discrepancy in sugar profile has been associated mainly with tobacco curing and treatment methods.[23] While some methods incorporate enzymes resulting in lower sugar content, others enrich the sugar profile by applying sugar containing additives i.e. honey, corn syrup and fruit juice.[26] This practice has been employed in the preparation of water-pipe tobacco where the main additive, molasses and sugarcane juice, are predominantly rich in sucrose.[27]

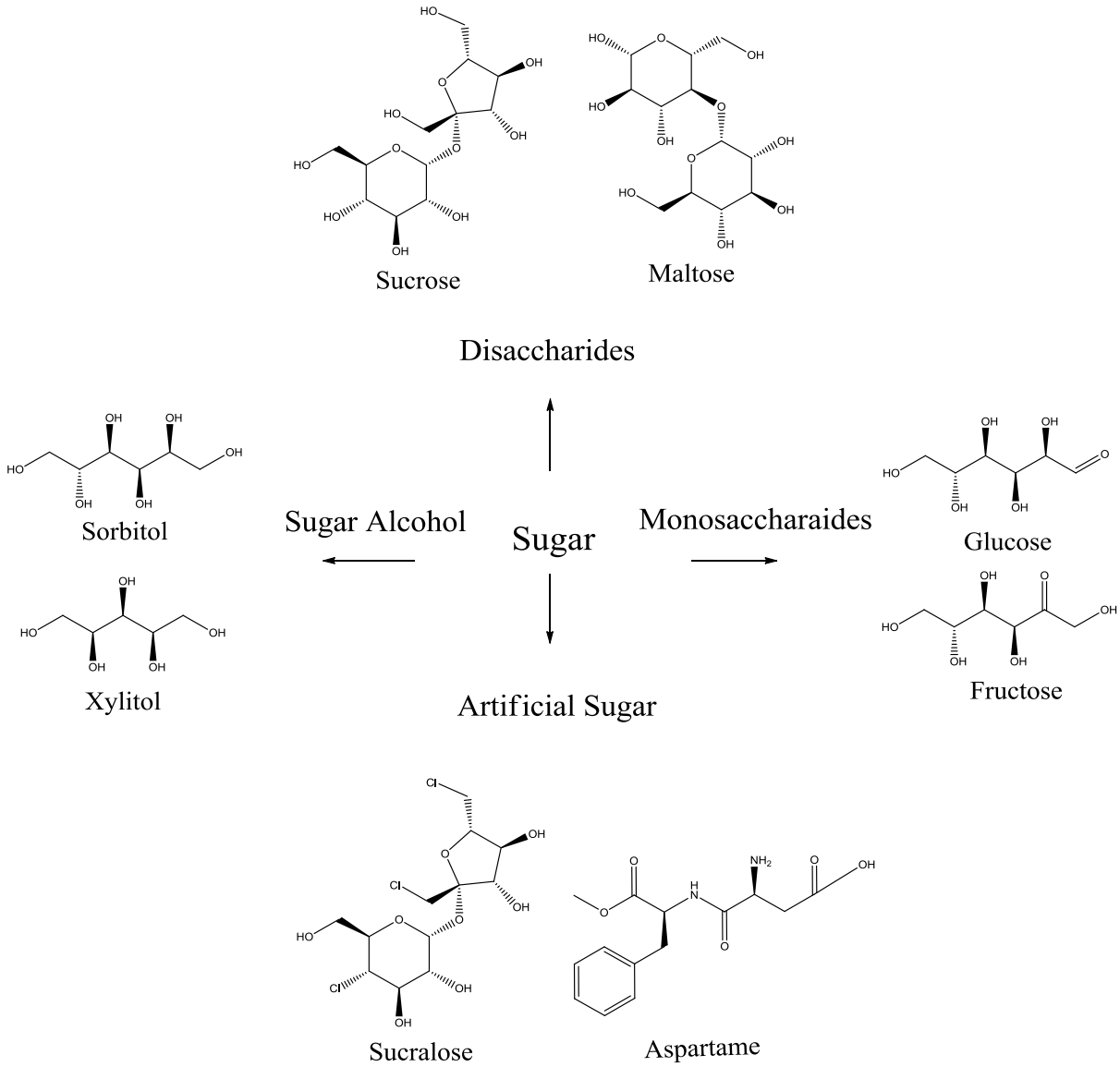

Figure I 1-Four main groups of sugar compounds used in tobacco processing

To understand the substantial interest of using sugar in cigarette products, scientists have studied the effect of sugar decomposition on smoke composition.[24,28-31] Using a smoking machine that mimics a burning cigarette, smoke from tobacco content was generated, collected and analyzed. Among various pyrolytic products, scientists have emphasized on the production of organic acids i.e. acetic acid and 3-methylbutanoic acid, furanic compounds i.e. furfural (FA) and 5-hydroxymethylfurfural (HMF) and aldehydes i.e.

formaldehyde and acetaldehyde. These byproducts have shown to contribute in masking smoke harshness, increasing tobacco attractiveness and promoting nicotine addiction.[32 33]

The smoothness of the smoke has been attributed to the acids, since at low pH there is a decrease in the level of free-base nicotine, the source of the bitter taste. The sweet aroma increasing the acceptability of smoking has been induced by furan derivatives having caramel flavoring. On another hand, the pro-addictive property has been associated with the formation of acetaldehyde. Animal research models have demonstrated that acetaldehyde can enhance addiction effects by inhibiting the act of monoamine oxidase, an enzyme responsible for degrading dopamine neutotransmitter.[1 ,15 ,34] More importantly, acetaldehyde along with other potential byproducts have exhibited toxicological concerns.

According to International Agency for Research on Cancer (IARC) formaldehyde is classified as a human carcinogen (Group 1)[35] while acetaldehyde as possibly carcinogenic (Group 2B).[36] FA has exhibited histopathological changes in the respiratory epithelium of mice in addition to pulmonary irritations in rat lungs.[37 ,38] In addition to that, HMF biotransformation into 5-sulfoxymethylfurfural has been associated with potential genotoxicity.[39-41]

All of these findings have raised questions regarding the safety of incorporating additives and called for urgent regulatory actions.

B. Regulations and Guidelines

Tobacco companies have never complied by the requirements of disclosing full description of the added ingredients nor provided inhalation toxicity studies to confirm the safety of the additives when burned.[42] Their justification was the use of food additives certified as safe by Flavors Extracts Manufacturers Association (FEMA).[43] However; food additives have been recognized as safe for oral administration without considering the effect

of their pyrolytic products.[44,45] Another essential point that has raised deeper concerns is the proliferation of flavored tobacco market especially among young people.[46-50] Studies have shown that youth between the ages of 12 and 24 have commenced smoking for the substantial interest in flavorants.[46,51-55]

In the absence of enough studies to evaluate the safety of additives and because their presence has begun to create new generations of smokers, a legislation to ban additives that can induce youth experimentation has been enacted by the parliament of Canada in 2009.[56] Similarly U.S. Food and Drug Administration (U.S. FDA) has prohibited the use of any natural or artificial additives other than tobacco and menthol.[57] Besides providing guidelines, the regulatory bodies join efforts with public health organizations to spread awareness about smoking health consequences.[12]

In a comprehensive report about smoking related diseases, U.S. Department of Health and Human Services has emphasized that smokers are at a greater risk to develop lung cancer, heart stroke and cardiovascular diseases.[58] Another public health warning has been issued by WHO revealing the annual death of nearly six million smokers.[59] In response to this alarming health threat pharmacological companies have proposed numerous cessation aids. The need for a novel method has led to the emerging of new class of products –electronic nicotine delivery systems-(ENDs) by most commonly called electronic cigarette (ECIG).

C. Electronic Cigarette (ECIG): Design and Operation

ECIG is a battery power device that uses a heating coil to aerosolize a solution (e-liquid) composed of varying ratios of propylene glycol (PG), vegetable glycerin (VG), water, nicotine and other additives, including flavorants.[60,61] The emerging of ECIG can be traced to Herbert A. Gilbert who has filed the first patent of smokeless non-tobacco product in 1963.[62]

Nevertheless, this invention has been ignored until 2003 when Hon Lik has introduced the modern version of ECIG to China market.[61]

The first ECIG model has resembled the conventional cigarette (Figure I 2). Upon activating ECIG either by air flow sensor or the use of control button, a power flow is prompted to the atomizer to start heating the e-liquid stored in the cartridge. The atomizer consists of a coil encircled around a wicking material that draws e-liquid content into it. When the user draws a puff, the generated vapors will be carried across the cylindrical cartridge into the mouthpiece. While flowing, the vapors begin to cool down and condense to form aerosols.[63-65] The act of inhaling and exhaling aerosols has been known as vaping.

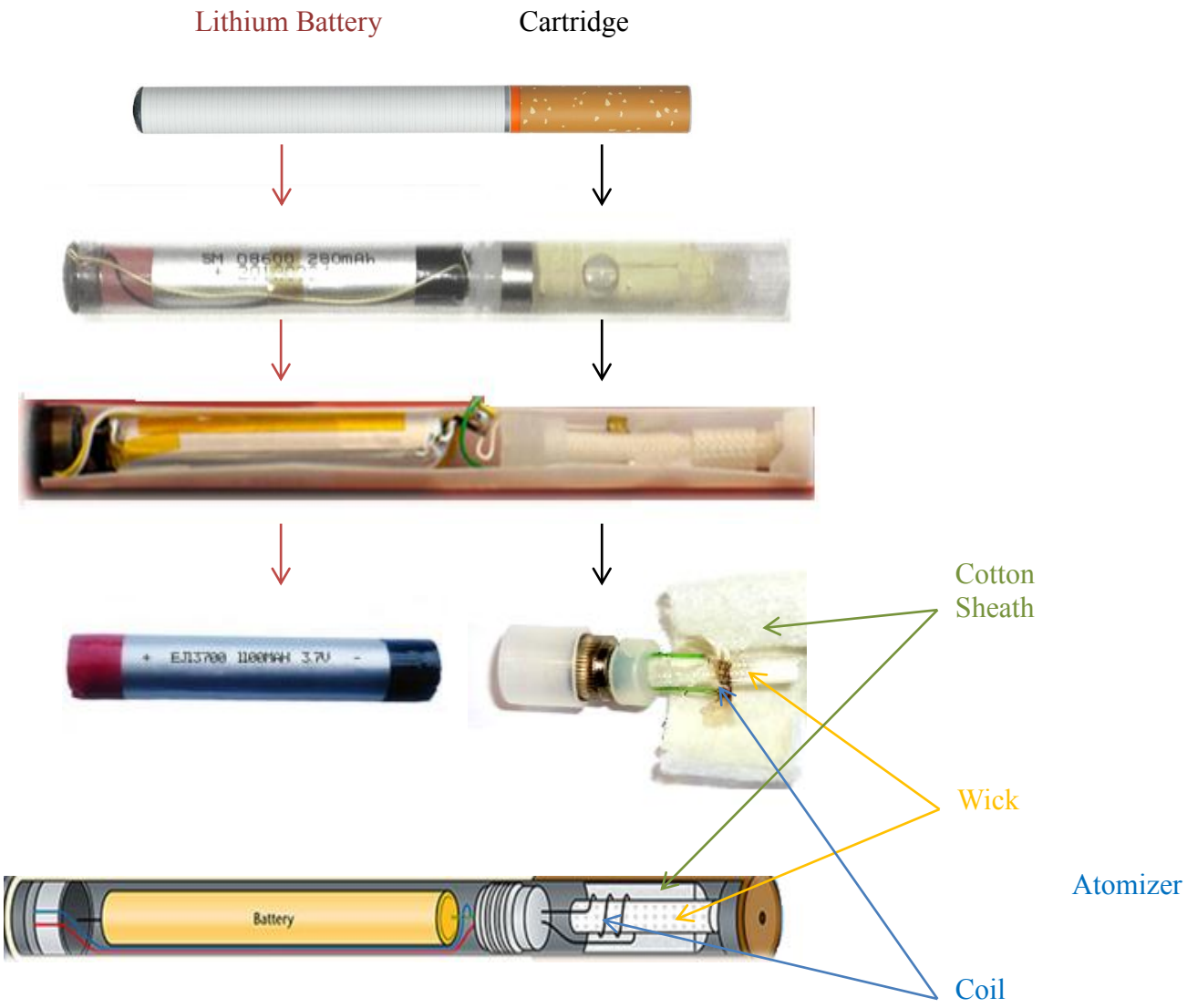

Figure I 2-Anatomy of ECIG first generation

After entering Europe and U.S. markets in 2007, ECIG has evolved in shape, design, and engineering characteristics while maintaining the three basic components: battery, heating element and e-liquid reservoir (Figure I 3). The new generations of ECIG have come with rechargeable batteries of higher capacity and newly designed atomizers combining wick/coil assembly with the cartridge. This combination has innovated two models of e-liquid reservoir: refillable (tank-clearomizer) and disposable (cartomizer). All of these features has allowed the user to customize e-liquid formulations, control battery output voltage (V= 3 V - 6 V) and heating element resistance (R= 1.0 Ω - 6.5 Ω).[66,67]

Figure I 3-Variety in ECIG configurations

The applied voltage (V) and resistance(R) determine the battery power input (P) according to $P=V^2/R$ which in turn is a critical indication for the temperature of the heating coil. Higher battery output voltage and lower resistance of heating coil have shown to achieve high temperatures that sometimes can reach 340 ºC.[60 60 ,68] Besides the impact of engineering characteristics, heating coil can experience a sudden increase in temperature when wick-

liquid supply is poor. This phenomenon is known as dry puff.[69] Scientists have reported that dry puff occurrence can potentially increase toxicant emission. Accordingly, they have associated the variances in some repeated measurements to the occasional occurrence of dry puffs.[70,71,60] An ECIG user cannot control the occurrence of dry puffs even by changing his puffing topographies. Nevertheless, by manipulating puffing topography he can adjust nicotine intake It has been indicated that users tend to take long puff duration to increase nicotine yield. This has been attributed to activating heating coil for longer time at a constant temperature dependent on device features.[65] Another important factor that can impact nicotine exposure is e-liquid composition.

E-liquids contain varying concentrations of nicotine (0-48 mg/ml) that has been proven to allow users self-titrate their nicotine intake.[65] Besides nicotine, additives have been extensively exploited to expand e-liquid market. Manufactures have released nicotine free solutions composed of flavorants in PG and VG solvents to promote ECIG to children.[72] In the light of the rapid growth of flavored ECIG especially among youth, the use of additives has raised considerable concerns.

1. Additives in CIG: Amount, function and fate

In the last two years much research has been devoted to study nicotine and few publications have addressed the issue of flavorants.[73] Comprehensive internet searches reported on 2014 more than 7000 unique flavorants.[74] For simplicity Zhu et al.[74] have categorized flavorants into eight main groups: Tobacco, Menthol, Tobacco-Menthol, Fruit, Dessert/Candy, Alcohol/Drinks, Snacks/Meals and Others. Since manufacturers do not disclose the chemical compositions and levels of flavorants, the classification is based on flavorant entitled names for example sugar-tooth, honey and chocolate are categorized under sweet group.[74]

The main investigation into chemical composition of e-liquids has been carried out by Hutzler et al.[75] By scanning 28 different e-liquids, they have identified 141 chemicals among which flavorants i.e. linalool, ethyl maltol, vanillin have been detected. This qualitative identification has been followed with quantitative measurement conducted by Tierney et al.[76]. In their study they have revealed that the level of flavorants in ECIG ranges between 1 and 4% (10–40 mg/mL). On another hand, more attention has been devoted to explore the toxicological effect of flavorants. Bahl et al.[77] and Sherwood et al.[78] have shown that flavored e-liquids have induced cytotoxicity in both human embryonic stem cells and airway epithelial cells. Similarly, Leigh et al.[11] have reported that aerosol generated from menthol, coffee and strawberry flavored e-liquids have reduced the viability and metabolic activity of human bronchial epithelial cells. In addition to this, e-liquids containing benzaldehyde, diacetyl (DA), acetyl propionyl (AP) and cinnamaldehyde have caused respiratory problems when inhaled. Kosmider et al.[79] have detected benzaldehyde in the aerosols of 108 flavored e-liquids with the highest yield from cherry flavored products. Farsalino et al.[80] have found that 47% of sweet flavored e-liquids produce DA and AP at levels higher than safety limits. Similar concern was raised by Allen et al.[81] who detected DA and AP in the aerosols of flavored e-liquids (Fruit, Candy and Cocktail). The Detection of benzaldehyde, DA, AP and cinnamaldehyde has highlighted the influence of using food additives in tobacco products. Benzaldehyde, DA and AP compounds are recognized as safe by FEMA for ingestion use but have exhibited health problems when inhaled.[79 ,82-85]

The availability and variability of flavorants have promoted the dual use of ECIG and cigarette and initiated vaping among nonsmokers. This reflects a major gap in the utility of ECIG as cessation aid and questions the presence of countless number of flavorants.[9 ,10 ,86-89] A survey conducted among middle and high school students have showed that 70% of students have preferred the sweet-flavored e-liquids.[90] Sweetness has shown to be a vital

factor to enhance sensation and increase the satisfaction of the user. Kim et al.[10] have adopted a psychophysical method in which participants are asked to use two measurement scales: The labeled hedonic scale to measure liking/disliking of ECIG and the labeled magnitude scale to measure the perceived intensity of flavorant (sweetness, bitterness and coolness). The results have reinforced that liking of ECIG has been associated with the sweet flavorants. In response, Kubica et al.[91] have scanned e-liquids for their sugar content. Sucrose has been detected in different flavored e-liquids (i.e. menthol, cherry and chocolate) with concentrations ranging from 0.56 to 72.93 (μg/g).

2. Regulations and Guidelines

In the absence of enough scientific evidences to prove its efficiency as cessation aid device, ECIG has been recognized as a tobacco product and was placed under the regulatory authority of U.S. FDA.[92,60] Nowadays much work has been carried out on the potential effect of additives to present critical implications for future regulations. Because the sweet perception has a critical role in attracting users, this work aims to evaluate systematically the impact of sugar in ECIG.

3. Sugar in ECIG

Scientists have established precursor-product relationships based on kinetic and thermodynamic studies to analyze the fate of sugar in cigarette.[93-97] A similar approach should be employed for ECIG. Nevertheless cigarette and ECIG differ in composition, design, and operation therefore any extrapolation between the two systems might not be valid. A cigarette typically consists of shredded tobacco, a plant material composed of 3800 constituents including hydrocarbons, amino acids, ketones, phenols and sugar.[98] When burned, cigarette is divided into two regions (1) exothermic combustion zone: located at the

tip of the cigarette lit, produces carbon monoxide, carbon dioxide and water and reaches a temperature up to 950ºC. (2) endothermic pyrolysis/distillation zone: allocated immediately downstream the combustion zone, generates most of smoke products and the temperature ranges between 200 and 600 ºC.[95,98] Researches have described two main pathways for sugar decomposition inside burning cigarette: (1) Maillard reaction: sugar interacts with amino acid to decompose into aldehydes (2) Caramelisation: sugar breaks down into mixture of acids, aldehydes and furan derivatives in the absence of amino acids.[7,99]

Alternatively, e-liquid is composed of relatively simple matrix (nicotine, PG,VG, water and additives).This mixture is electrically heated to produce aerosol.[60] The temperature of the heating coil depends on many factors particularly the electronic features.[60] Talih et al.[68] have reported a heating temperature ranging from 130 to 350ºC. In the absence of amino acids, caramelisation is the plausible pathway for sugar decomposition inside ECIG.

D. Glucose thermal transformations

Sugar transformation has gained a significant attention for its potential use in food applications, biofuel production and cigarette smoke formulation.[100-104] One of the basic transformation route is caramelisation: a non-enzymatic browning reaction occurring when sugar is subjected to heat (> 120 ºC) in the absence of amino acids.[99] Scientists have selected glucose, the simplest unit of carbohydrates, to elucidate the transformation pathways. This is of considerate interest because glucose is one of the important food additives that tobacco manufactures claim to use.

The most abundant form of glucose (99%) is the cyclic six-member glucopyranose designated as β or α according to the OH direction at the anomeric carbon. Other minor conformations include the cyclic five-membered glucofuranose and open chain forms.[105,106] Glucose possesses six oxygenated groups as presented in Figure I 4. These activation sites

initiate multiple decomposition pathways when subjected to heat. Studies have identified many products including anhydrous glucose molecules, acids and aldehydes and furan derivatives.[107-110] Because these products arise from many parallel side reactions, the comprehensive degradation mechanism remains unclear.

1,6-anhydroglucose compound, also known as levoglucosan, has shown to be obtained from the intra-molecular dehydration of one water molecule between C1 and C2.[100] There has been a number of studies pertaining to the production of levoglucosan.[111-115] Sasaki et al.[116] have reported good yields of levoglucosan (up to 32%) when glucose solution is exposed to high temperature steam (300-400 ºC) under 7MPa. Alternatively, Kabyemela et al. [112] have detected negligible amounts of levoglucosan when glucose is dehydrated in sub- and supercritical water (300-400 °C ,25-40 MPa). This discrepancy is attributed to the dominant production of other products including erythrose (A) and glycolaldehyde (B). These compounds are found to be obtained from retro-aldol condensation of glucose.[112 ,117-119] Their further degradation will produce organic acids.[119 ,120] Saito et al.[121] have identified formic acid (C) and acetic acid (D) from glucose decomposition at 200-240 ºC and 15-20 MPa. In addition to these routes, sugar degradation into furan derivatives including HMF (E) and FA (F) has come under intense focus.[104 ,122-129] Scientists have conducted kinetic and theoretical studies to examine this degradation pathway under variety of conditions: aqueous/organic/ionic solutions, with/without catalysts, under different temperature and pressure.[109 ,112 ,122 ,130-133] A medium similar to e-liquid content was studied by Kuster et al.[134] who reported fructose decomposition into HMF in propylene glycol/water mixture (v:v = 70:30) at 95 ºC.

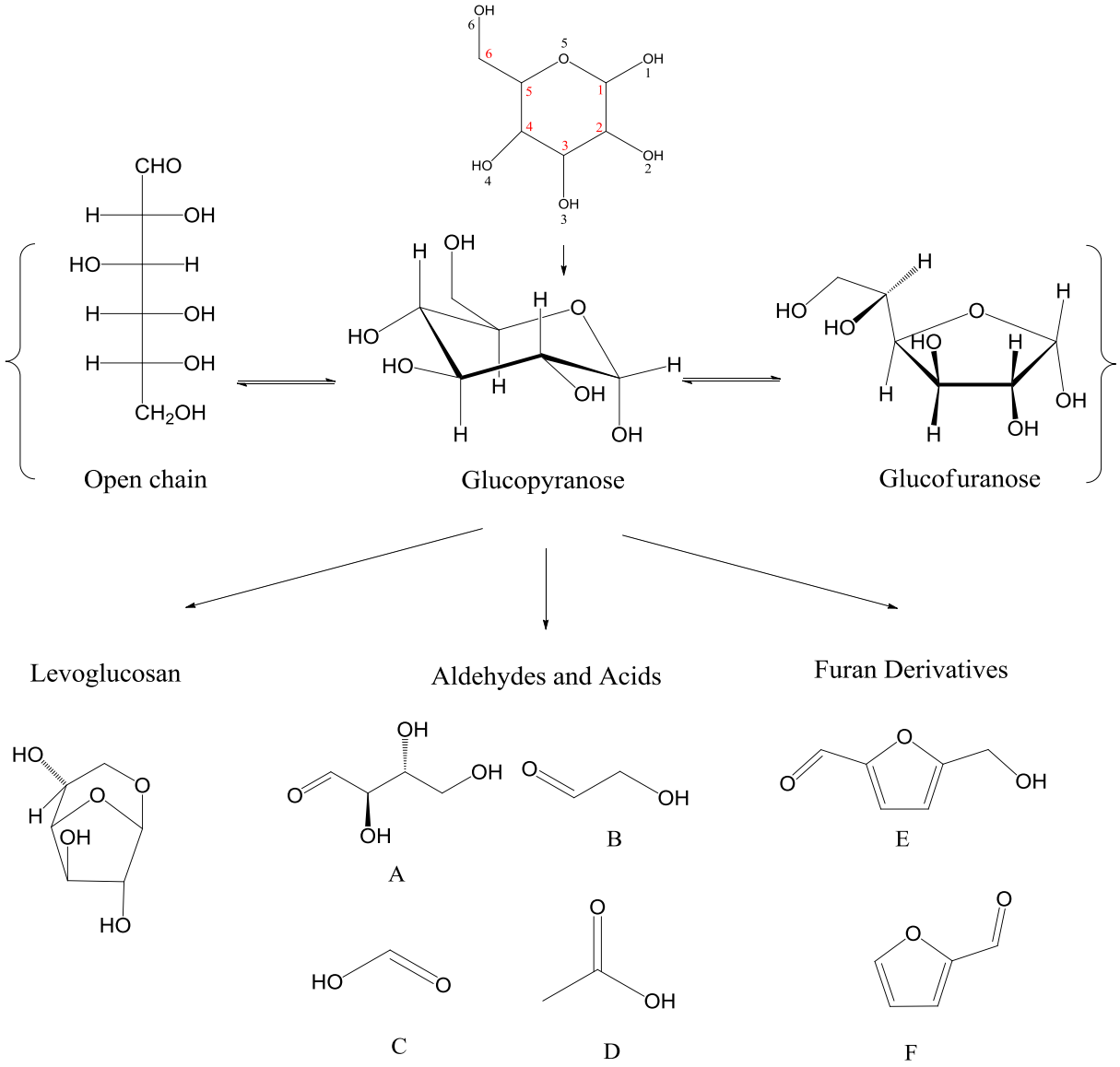

Figure I 4-Glucose potential thermal decomposition pathways. A=Erythrose, B=Glycolaldehyde, C= Formic acid, D= Acetic acid

The formation of furan derivatives and aldehydes is of particular concern due to their potential health risk. Aldehyde formation has been the center of studies throughout the past years.[70 ,135-138] Scientists have shown that thermal degradation and oxidation of PG and VG generate carbonyl compounds.[70] PG and VG are the carrier solvents so they represent around 70 to 80% of e-liquid composition.[137] The production of aldehydes from PG and VG is expected to surpass that from sugar. Therefore, it is interesting to study the formation of new family of group which is furan and particularly HMF and FA for their potential health effects.

E. Analytical method for HMF and FA assessment

This work presents a systematic study to assess the effect of sugar on aerosol composition and highlight its potential toxicity. A principal requirement for the analysis of HMF and FA is reviewing existing methods to define sample preparation strategies and measurement techniques. Table I 1represents the most utilized analytical techniques to study HMF and FA with brief explanation on sample preparation methods. Besides water-pipe and cigarette smoke, the majority of methods specific for HMF and FA detection have been reported notably in food. Historically, scientists have been interested in measuring HMF and FA to evaluate the quality of food processing and storage. However; over the past years the significant attention towards assessing HMF and FA has been attributed to their potential health concern.

Table I 1-The most applicable methods for HMF and FA analysis

Matrix	Preparation Method	Analytical Method[1]	Reference
Water-pipe smoke	Glass fiber filter, on which the smoke was collected, was spiked with 5-Chloro-2 furaldehyde as internal standard, extracted by methanol/water (50:50, v/v) and agitated on a shaker	RP-HPLC-DAD	Schubert et al. [27]
Cigarette Smoke	Glass fiber filter, on which the smoke was collected, was spiked with ethyl laurate as internal standard, extracted by dichloromethane and agitated.	GC-FID	Matsushima et al.[139]
Honey Citrus Juice	Sample was diluted, homogenized, filtered, derivatized with 2,4-dinitrophenylhydrazine and concentrated using rotary evaporator.	HPLC-UV/Vis	Coco et al.[140]

Food samples (i.e. biscuits and jam)	Sample was ground, homogenized, purified via solid-phase extraction and spiked with acrylamide-d3 as internal standard	HPLC-MS/MS	Teixid´o et al.[127]
Food samples (i.e. biscuits and jam)	Sample was ground, homogenized, purified via solid-phase extraction, derivatised with N,O-bis-trimethylsilyltrifluoroacetamide and spiked with furan-3-etinil-trimethylsilane as internal standard.	GC-MS	Teixido et al.[141]
Vinegar	Sample was extracted via dynamic headspace purging, trapped on tenax tube, and thermally desorbed into column injector.	DHS–TDU–GC/MS	Manzini et al. [142]

[1] RP-HPLC = Reversed Phase High Performance Liquid Chromatography, DAD = Diode Array Detector, GC = Gas Chromatography, FID=Flame Ionization Detector, UV/Vis= ultraviolet/visible Spectroscopic, MS=Mass Spectrometry, DHS= Dynamic Head Space, TDU= Thermal Desorption Unit

Initially, scientists have based their work on classical spectrophotometric techniques in which HMF and FA absorbance were measured at 285 and 275 nm, respectively.[143,144] However, spectrophotometric procedures remain unspecific and time consuming. Instead scientists have adopted chromatographic techniques that allow more selective, reproducible and reliable analysis. Reversed phase-high performance liquid chromatography (RP-HPLC) coupled with ultraviolet/visible spectroscopic (UV/Vis) or diode array detector (DAD) has been commonly used.[145] This method was utilized to detect HMF and FA among other furanic compounds in water-pipe smoke. On the other hand; some researches have reported that HMF quantification especially at low concentrations has been problematic due to interferences from compounds absorbing at the same working wavelength.[141] Consequently, to improve the sensitivity and selectivity of RP-HPLC-DAD/UV method, a derivitization step with 2,4-dinitrophenylhydrazine was integrated to form FA and HMF derivative compounds with higher UV absorbance (375-365 nm).[140,146-148] Moreover, mass spectrometry (MS)

detector has been proposed for its sensitivity and most importantly structure identification potential.[141,149,150] Scientists have coupled MS detector with both HPLC and gas chromatography (GC).[142,151,152] The semi volatility and thermal stability of HMF and FA have permitted their analysis using GC. According to a comparison study conducted by Teixido et al.[127,141], GC-MS can attain lower detection limit (12 ng g^{-1}) than HPLC-MS/MS (133 ng g^{-1}). In addition to MS, flame ionization detector (FID) coupled with GC has been utilized by Matsushima et al.[139] to assess HMF and FA in cigarette smoke.

Based on this literature review we decided to adopt GC-MS owing to its specificity, selectivity and sensitivity. The analysis of HMF and FA has encountered interferences from PG and VG peaks necessitating the development of solid phase extraction procedure. To the best of our knowledge, this is the first work to present a clean-up method for PG/VG matrix. Only one study has reported challenges associated with a broad peak of VG in ECIG aerosol. However no purification method was adopted because PG and VG did not co-elute with the analytes of interest.[153]

F. Effects of e-liquid content and puffing topography on aerosol composition

Scientists have hypothesized that e-liquid content, product design, and puffing topography can influence toxicant emissions.[60,154,155] Talih et al.[65] have reported a positive relation between nicotine e-liquid concentration and nicotine yield. Moreover, Kosmider et al.[137] have shown that PG-based e-liquid and high battery input have generated higher yields of aldehydes compared to VG based e-liquid and low battery input.[137]

Consequently, understanding how e-liquid composition and ECIG operation affect the production of HMF and FA is necessary to evaluate their potential impact.

G. Sucrose, fructose and glucose assessment

The analysis of sugar has been carried out for years in a wide range of food items.[156-163] This has been extended to tobacco products to assess the impact of sugar compounds.[18,25,26] Because the presence of sugar has shown to affect the sensory and chemical characteristics of both food and tobacco matrices, numerous analytical methods including GC and HPLC have been developed for the separation and quantification of different sugar compounds.[16,18,25,156,164-167] The major drawback to adopting GC is the need to prepare volatile sugar derivatives because of the low volatility of sugar compounds. On another hand, HPLC generally entails simple sample preparation rendering it widely utilized.

The existing HPLC methods have incorporated several detection modes including refractive index (RI), evaporative light scattering detector (ELSD) and MS.[20,91,158,165,168,169] The use of RI and ELSD have revealed major limitations. RI demands control over chromatographic conditions because it is highly susceptible to changes in temperature, flow-rate and mobile-phase composition resulting in baseline instability.[170] On another hand, ELSD shows poor linearity response to analyte concentration.[171] Most importantly, both RI and ELSD are not selective and provide high minimum detectable limits.[172] As a result, scientists have adopted the use of MS detector. Owing to its structure identification capabilities, robustness and sensitivity, MS has succeeded in addressing RI and ELSD limitations.[166,172,173] Taking the advantage of MS, we introduce an HPLC method coupled with electrospray ionization mass spectrometry ESI-MS for the assessment of sucrose, glucose and fructose. The method is partially inspired by the work of Wan et al.[172] with major variations in the choice of column and mode of ionization. Having optimized the method a number of commercial e-liquids were scanned for their sugar content.

CHAPTER II

ANALYTICAL METHOD OPTIMIZATION AND VALIDATION

In this chapter we elaborate the optimization of analytical procedure for HMF and FA assessment. When studying analytes in aerosols, a considerable attention is paid to three crucial stages: Aerosol generation and collection, sample extraction and purification and instrumental analysis. Aerosol generation and sampling were performed using customized puffing machine, sample preparation involved filter extraction, solid phase extraction procedure (SPE) and sample concentration and finally analysis was carried out via GC-MS method.

A. Sampling

A customized puffing machine at the American University of Beirut (AUB) was employed (Figure II 1). The machine is equipped with DC power supply and connected to a computer. The software program is used to control topography conditions. ECIG with a tank system is adopted because in this model the wick is short and the fluid easily accessible, and most importantly all the parts are easily disassembled which facilitated replacing liquids and cleaning the device between experimental conditions. The heating coil assembly of this ECIG model is typical of those found on the market, with overall coil dimensions of 1.8 mm diameter, 3.2 mm length, and 6 coil windings. Downstream the mouthpiece of the ECIG a quartz filter is placed in a filter holder to trap generated aerosols.

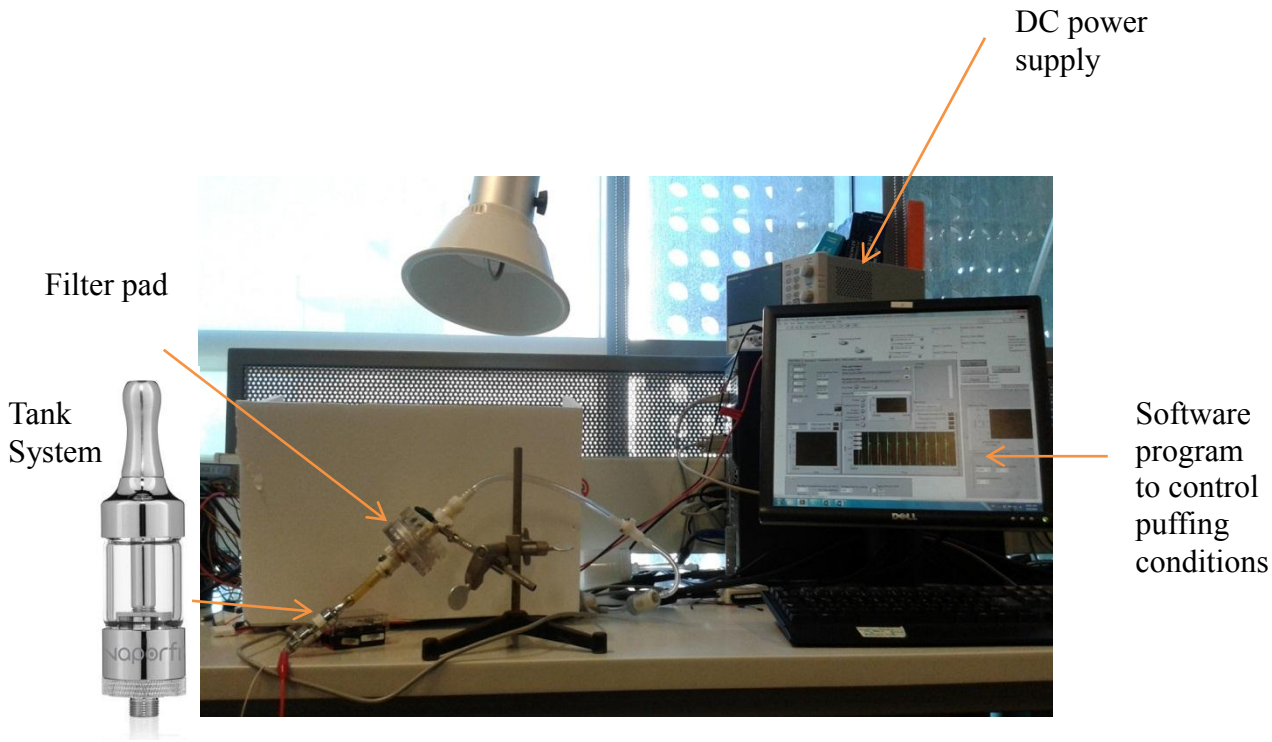

Figure II 1-A custom-designed digital puff production machine at AUB

B. Sample Preparation

1. Materials

SPE cartridges (1000 mg/6 mL HyperSep SI) and quartz filters (Advantec, QR-100, 47 mm) were procured from Thermo Scientific and Whatman International, respectively. HPLC-grade ethyl acetate, hexane, chloroform and acetonitrile, PG (99.5%), VG (99 - 101%), and HMF analytical standard were obtained from Sigma Aldrich. FA and internal standard (5-chloro-2-furfural) were obtained from Absolute Standards. Glucose, sorbitol and sucrose were food grade products provided by the Faculty of Agricultural and Food Sciences at AUB.

Method optimization is based on a systematic evaluation of all experimental conditions that can affect the recovery of the analyte. In every step, multiple variables were examined and optimum condition was determined based on recovery yields. The recovery was assessed using standard solutions of concentrations covering the lower limit of the

calibration curve (0.6-12 µg/mL) and by adding a constant amount of internal standard (4 µg/mL). It was found that the recovery of IS was lower than that of HMF and FA, and as such it was excluded from the sample preparation procedure and added before GC-MS runs. For the quantification analysis, the correction for analytes loss during sample extraction and purification was counted for by adopting an extracted calibration curve.

2. Filter Extraction

An extraction procedure was developed by optimizing three parameters: choice of solvent, extraction volume and extraction time. HMF and FA are semi polar compounds and soluble in many solvents among which ethyl acetate and chloroform are common, have similar polarity to HMF and FA and are compatible with GC-MS.[174,175] To evaluate the extraction efficiency, a quartz filter was placed in a glass vial (4 mL), spiked with HMF and FA standard solution (2 µg/mL) and extracted with 2 mL ethyl acetate or chloroform. The solution was sonicated for 30 min and finally injected into GC-MS. The recoveries of ethyl acetate and chloroform were 91% for HMF and 85 % for FA and 70 % for HMF and 50 % for FA, respectively. Ethyl acetate was chosen for its higher recovery yields. A volume of 2 mL was found to be sufficient to extract HMF and FA without causing an excessive dilution. Moreover, 30 min sonication was implemented because the recoveries of HMF and FA were comparable at three tested durations (30, 60 and 90 min).

3. Sample Concentration

Sample concentration is a critical requirement to increase HMF and FA concentrations. Our aim is to concentrate the sample down to 0.5 mL. Nitrogen evaporator was employed and both flow rate and temperature of evaporation were investigated. The vapor pressures at 25 ºC of HMF, FA and ethyl acetate are 5.28×10^{-3}, 2.21 and 93.2 mm Hg,

respectively. Consequently, sample concentration was carried out at atmospheric room temperature where HMF and FA are relatively less volatile than ethyl acetate.

To determine the optimal flow rates, standard solutions (0.5 µg/mL) were prepared in 2 mL ethyl acetate and concentrated down to 0.5 mL at 15 and 5 L/min. The recovery at 15 L/min was found to be less than 60% for both compounds compared to 100 and 90 % for HMF and FA, respectively, at 5 L/min. Consequently; the slowest flow rate of 5 L/min was selected.

4. Solid phase extraction procedure

In the initial stage of the sample preparation, we evaluated the severity of the matrix and tested for interferences from PG and VG. Aerosol generated from glucose standard solution prepared in PG/VG matrix were collected on a filter, extracted and injected into GC-MS. The chromatogram was saturated with two broad peaks of PG and VG overlapping with HMF and FA. Therefore minimizing the matrix interference necessitates developing a novel sample purification procedure. There are different types of clean-up techniques among which solid-phase extraction is the most applicable.

The general procedure starts by conditioning the SPE cartridge, loading the sample and then eluting analytes of interest. The conditioning step was performed by washing the cartridge with 10 mL hexane. This step is recommended by manufacturer's instructions to activate the functional groups on silica surfaces. Subsequently sample (0.5 mL) was loaded onto the SPE cartridge. Elution was performed using a suitable mobile phase composition that was investigated starting with ethyl acetate, the solvent used for filter extraction.

Ethyl acetate (10 mL) was tested on an SPE cartridge loaded with HMF and FA standard solutions which were prepared in PG/VG (70/30 v/v) solution. This ratio is selected for its prevalence in ECIG e-liquids. Subsequently, the collected sample was injected into

GC-MS. A major defect in using ethyl acetate is its tendency to dissolve the silica material. Other solvents were tested and only chloroform was found to be suitable. Nevertheless, a recovery in a range of 50-70 % was obtained by chloroform in filter extraction procedure. Thus to improve the recovery, a mixture of chloroform and acetonitrile was assessed.

It is important to ensure that acetonitrile volume is properly configured to enhance the elution recovery of HMF and HA without eluting PG and VG. Three different mixtures were prepared (chloroform: acetonitrile): 9:1, 8.5:1.5, and 8:2 mL. Standard solutions were transferred into conditioned SPEs and eluted using assigned mixtures. The recovery yields for (9:1), (8.5:1.5), (8:2) mixtures were 69, 92 and 87 % for HMF and 30, 70 and 65 % for FA, respectively. The highest yield was obtained by chloroform: acetonitrile mixture (8.5:1.5).

Further, the capacity of SPE cartridge was tested. The capacity of SPE refers to the amount of interfering compounds (PG and VG in this case) that can be retained by the SPE without breakthrough. To mimic experimental conditions, the capacity of the SPE was tested in relation to the amount of aerosols trapped on quartz filters which is in turn proportional to the PG/VG content of the aerosol. As such, glucose solution prepared in PG/VG was vaped and filters with different aerosol loadings (gravimetrically measured) were produced. The filters were extracted, purified and injected into GC-MS. It was noticed that PG/VG interference was removed by the SPE cartridges when the filter load was equal or below 100 mg. At higher filter loads, PG/VG interference started to appear.

Taken together, conditioning was performed using 10 mL hexane, elution was carried out by chloroform: acetonitrile mixture (8.5:1.5) and filter load was up to 100 mg.

5. Sample concentration

After completing SPE procedure a nitrogen evaporation step is essential to concentrate the sample down to 0.5 mL. This step is crucial because HMF and FA are

expected to be present at low amounts in the aerosol of ECIG. The evaporation was set at room temperature under a nitrogen flow of 5 L/min. Standard solutions were prepared in 10 mL chloroform: acetonitrile (8.5:1.5) and concentrated down to 0.5 mL. The recovery yields were 98 and 90% for HMF and FA, respectively.

Having optimized all the sample preparation procedure, the sample is ready for GC-MS analysis.

C. Instrumental analysis

The analysis of HMF and FA was accomplished by Thermo-Finnigan Trace GC-Ultra Polaris ITQ 900 MS coupled with AS 3000 II autosampler. Chromatographic separation occurred on a TG-5MS column (30m × 0.25 mm, 0.25 μm film thicknesses). Electron impact ionization (EI) with nominal electron energy of 70 eV was used. The carrier gas was helium of 99.999% purity with 1 mL/min flow rate. Injection mode was splitless and set at 200 ˚C. The oven program was as follows: initial temperature of 40 ˚C was held for 1 min, ramped at 30 ˚C/min to 80 ˚C, ramped at15 ˚C/min to 150 ˚C, and then ramped at 20 ˚C/min to 250 ˚C. The analytes were identified by their mass spectrum in which HMF, FA and IS have a relatively intense molecular ion with mass-to-charge ratio (m/z) of 97, 96 and 129, respectively. The linearity was evaluated by building an 8-point calibration curve in the range from 0.6 to 12 μg/mL, good linearity was observed with correlation coefficients (R^2) >0.997 for both HMF and FA.

As the application of GC-MS will prone the sample to high temperatures, it is necessary to investigate the stability of sugar compounds under the optimized temperature profile. Glucose and sucrose solutions, the potential sources of HMF and FA, were injected. No detectable amounts of HMF and FA were observed ensuring the absence of interference and that these sugars will not produce HMF and FA inside the GC.

D. Method validation

Validation is an integral part of quality control (QC) and quality assurance (QA) practices to judge the reliability, consistency and accuracy of the method. The key criteria for this evaluation are: linearity, limit of detection, limit of quantification, recovery and repeatability. Because QC and QA measure the effectiveness of sample preparation, all the standard solutions are prepared in PG/VG (70/30) to mimic the actual sample.

1. Linearity

Linearity evaluation verifies that HMF and FA are found in a range where their response is linearly proportional to their concentration. It is commonly judged by examining the correlation coefficient of calibration curve. In this study an extracted calibration curve is used, as opposed to the direct calibration curve, to correct for the possible loss of analytes during extraction. This step is necessary because the IS used failed to have similar extraction recoveries to HMF and FA and was thus spiked into the sample just prior to GC analysis.

Practically, quartz filters spiked by different amounts of HMF and FA standard solutions were extracted, purified and blown down under nitrogen flow to yield an extract concentration ranging between 0.5 and 40µg/mL. These extracts were analyzed using GC-MS after addition of IS at a constant amount (4µg/mL).

The relationship between the ratio of the analytes signal to the IS signal and analyte standard concentrations was found to be linear for the whole examined range with correlation coefficient >0.99 for HMF and FA as shown in Figure II 2.

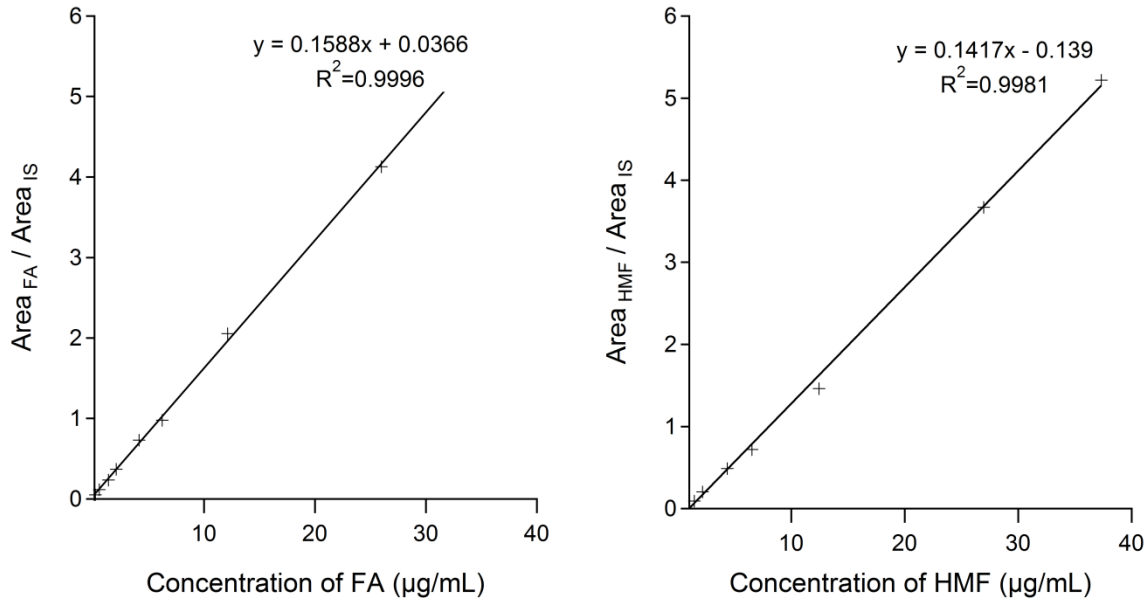

Figure II 2-Extracted calibration curves of FA and HMF

2. Limit of detection

Limit of detection (LOD) is the lowest concentration of analyte that can be detected but not necessary quantified. Based on the standard deviation response and slope, LOD is expressed as following:

$$\mathrm{LOD} = \frac{3\sigma}{s}$$

Where σ is the standard deviation of the ratio of the analytes signal to the IS signal of seven replicates of analytes prepared at a low concentration and s is the slope of the extracted calibration curve. LOD analysis was carried out using seven replicate extractions of 2 µg/mL HMF and FA. The results have shown that detection limits of HMF and FA are 0.05 and 0.2 µg/mL, respectively.

3. Limit of quantification

Limit of quantification (LOQ) is the lowest concentration of analyte that can be measured with an acceptable level of accuracy and precision. Based on the standard deviation response and slope, LOQ is expressed as following:

$$\mathrm{LOQ} = \frac{10\sigma}{s}$$

The quantification limits of HMF and FA were analyzed using seven replicate extractions (2 µg/mL). The results have shown that LOQ is equal to 0.1 for HMF and 0.7 µg/mL for FA.

4. Repeatability

Repeatability describes the closeness of agreement between a series of measurements obtained under the same operating conditions (one operator, same equipment and on the same day). It is expressed by the percent relative standard deviation (%RSD) of analytical results obtained from a minimum of five measurements at three different concentrations (low, medium and high). The acceptance criteria are based on type of analysis, complexity of matrix and the level of tested concentration.

The results of six replicate standards per three concentrations (2, 5 and 40 µg/mL) revealed %RSD <10 % for FA and <15% for HMF.

5. Recovery

High recovery of HMF and FA from PG/VG matrix is an important characteristic of sample preparation procedure. The recovery is the ratio of extracted concentration obtained from sample treated according to the whole extraction procedure to that of a sample of same concentration directly analyzed on GC-MS . It is assessed using six extraction measurements over two concentration levels covering the working range: 5 and 40 µg/mL. The recovery was found to be equal to 90% for HMF and 60% for FA.

Consequently, all the QC and QA requirements are fulfilled and the method is ready for measuring HMF and FA in the aerosol of ECIG.

CHAPTER III

DETECTION OF HMF AND FA IN AEROSOL OF ELECTRONIC CIGARETTE

Exposure to HMF and FA raises several health concerns. The potential mutagenic activity of HMF is attributed to one of its major toxin metabolite known as 5-sulfoxymethylfurfural.[40,65] As for FA, clear evidence for carcinogenic activity and histopathological changes in the respiratory epithelium of mice have been reported.[37,176] Because HMF and FA are pyrolytic products of sugar compounds we were interested in investigating their formation upon vaping flavored solutions.

Tobacco manufactures have never fully or accurately disclosed the name and concentration of each additive. To suppress regulatory legislation and public pressure, they claim the use of additives approved as safe by U.S. FDA and FEMA. However, this safety approval argument is misleading because the certification is provided for ingredients never intended to be burnt.[7,177] The rise of ECIG in an environment almost free of regulations has facilitated the rapid release of high number of flavored e-liquids. This has made the research difficult because by the time the work is complete, the studied product might become obsolete.[76,178] The situation has become more challenging in the presence of ECIG with an open system that allows the user to add any component to the e-liquid i.e. marijuana and fructose syrup.[179-182] Nevertheless, we have learned that major tobacco companies used to add glucose, fructose and sucrose in the production processes of conventional tobacco products.[7] In addition to that, kubica et al.[91] have reported the presence of sucrose in various flavored e-liquids including chocolate, tobacco, cherry and grape. They have suggested that sugar compounds can be intentionally added as additives or extracted along with nicotine as they

are naturally found in tobacco leaves. Accordingly, in this work we prepared standard solutions of glucose, sucrose and sorbitol in PG/VG to assess the formation HMF and FA under various battery input and puffing topographies.

A. Materials and methods

Glucose, sorbitol and sucrose used are food grade products provided by the Faculty of Agricultural and Food Sciences at AUB.

B. Liquid Preparation

Stock solutions of sucrose, glucose and sorbitol in distilled water were prepared with 345, 442 and 243 (mg/mL) concentrations, respectively. Subsequently, 0.5 mL of each stock solution was added to 10 mL of the PG/VG mixture (70/30). The percentage of sugar in the prepared liquids (1.01-1.91% by mass, equivalent to 11-21 mg/ml) was chosen to be in the range of reported concentrations found in commercial liquids (1-4% by mass).[76] Four e-liquids concentrations of 0.03, 0.25, 0.63, 1.23 % by mass were prepared. All the prepared liquids were sonicated for 2 h to ensure homogeneity before being used for aerosol generation.

C. Aerosol Generation and Sampling

Aerosols were generated from a custom-designed digital puff production machine at AUB.[65] A commercially available ECIG (VaporFi PLATINUM II Tank (VP)) was used.[183] To investigate potential effects of power level, aerosols were generated at 4.3 and 10.8 W, representing a typical and higher than average power input (the manufacturer lists an operating range of 3 to 15 W for this device).[184] In addition to the power, the effect of puff duration was assessed by applying two puff durations (4 or 8 s). Moreover, the effect of

sucrose concentration was studied at constant power of 4.3 W during two puff durations (4 or 8 s). The electrical resistances of the ECIG atomizers, which were measured before and after each use using a standard laboratory ohmmeter at 22 °C were found to be 2.3 ± 0.11 Ω.

The aerosols were generated at a constant puff velocity of 1 L/min and an inter puff interval of 10 s. The produced aerosols were drawn from the mouth end of the ECIG device, and collected on a quartz fiber filter (Advantec, QR-100, 47mm). To control for potential interactions with ECIG age and ECIG manufacturing variability, three ECIG devices of the same manufacturer and model were used in this study, and the experimental condition orders were randomized. Every experimental condition was conducted with each of the ECIG devices, and the results of the three atomizers were averaged for a given experimental condition.

D. Analytical Procedure

1. Filter Extraction

The quartz filter loaded with ECIG aerosols was transferred to a glass vial (4mL) and subsequently extracted with 2 mL of ethyl acetate after 30 min sonication. The filter was removed and the extract was concentrated at room temperature under nitrogen flow (5 L/min) to 0.5 mL.

2. SPE Clean-up Operating Procedure

The clean-up method was carried out using the optimized SPE procedure. The concentrated sample was loaded on the conditioned SPE cartridge. The elution was achieved using a mixture of chloroform /acetonitrile : 8.5/1.5 mL. Subsequently, the collected solution was concentrated down from 10 mL to 0.5 mL under nitrogen flow (5 L/min). Prior to GC-MS system injection, the sample was spiked with IS (4 µg/mL).

3. Gas Chromatography-Mass Spectrometry (GC-MS) conditions

The GC-MS analysis was achieved according to the optimized temperature profile. The initial temperature was 40 ˚C, held for 1 min, ramped at 30 ˚C/min to 80 ˚C, then at 15 ˚C/min to 150 ˚C, and finally at 20 ˚C/min to 220 ˚C. The quantitative analysis was done using selected ion mass (m/z = 97, 96 and 129 for HMF, FA, and IS, respectively).

E. Quality Control and Quality Assurance

Validating the analytical method is fully elaborated in chapter II. The maximum %RSD of each of the following concentrations: 5, 40 and 120 µg/mL was 15 and 3% for HMF and FA, respectively. In addition, the recovery of the method at 5 and 40 µg/mL was 90% for HMF and 60% for FA. LOD was 0.05 and 0.2 µg/mL for HMF and FA, respectively. LOQ was 0.1 and 0.7 µg/mL for HMF and FA, respectively. The quantitative analysis was carried out using extracted calibration curve. The linearity is obtained in the range of 0.1-100 µg/mL for HMF and 0.8-20 µg/mL for FA. The corresponding regression coefficients are higher than 0.995 every time the extracted calibration curve is prepared. In addition to that, to validate the method on a commercial ECIG matrix, a flavored Vapor Fi was selected and spiked with known concentration of sucrose (13 mg/mL equivalents to 1.23%) comparable to one of the prepared standard solution. Three replicates solutions were vaped at 5.0 W during 4 s puff duration.

F. Results

The effect of the power, puff duration, and sugar concentration on HMF and FA yields was assessed by a two tailed distribution and heteroscedastic t-test.

Both HMF and FA were reliably detected in the generated aerosols. Figure III 1 and Figure III 2 show the average levels of HMF and FA per mg of total particulate matter

(TPM). FA yields were considerably lower than HMF under all conditions. Prior to vaping, all liquids showed no detectable quantities of furans. Battery power output has revealed a significant effect on the TPM normalized yields of HMF generated from sucrose (p <0.01) and glucose (p <0.02). In particular, 4.3 W generated higher HMF concentrations than 10.8 W. FA concentrations appear to show an opposite trend, with greater power resulting in larger yields; however the large variances in repeated measures rendered the differences statistically insignificant, except for the glucose condition with 8 s puff duration at 10.8 W.

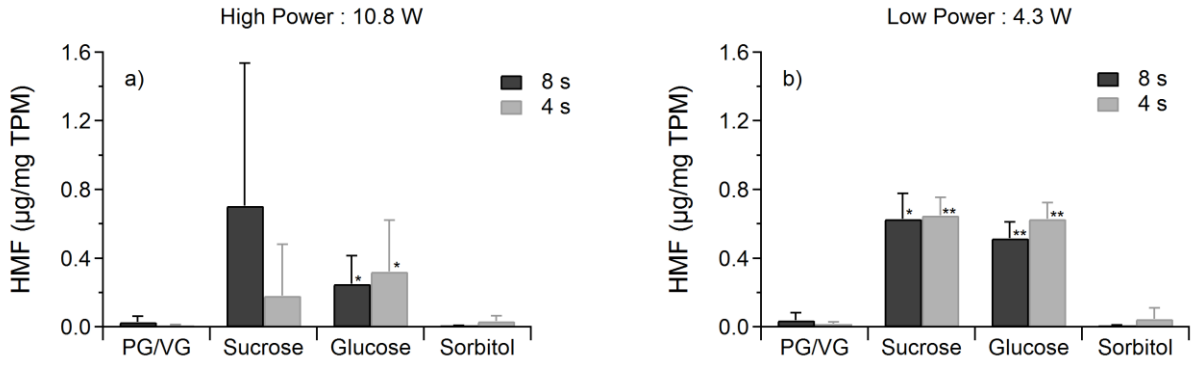

Figure III 1-Average HMF yield normalised by TPM (µg/mg) in aerosols generated from laboratory-prepared sucrose, glucose and sorbitol liquids vaped at 4.3 W (a) and 10.8 W (b) and at 8 and 4 s. * and ** indicate significant difference from the unflavoured liquid at p<0.05 and p<0.01, respectively. N=3 measurements for each condition. Error bars represent standard deviation of three different measurements. FA, furfural; HMF, 5-hydroxymethylfurfural; PG, propylene glycol; TPM, total particulate matter; VG, glycerin

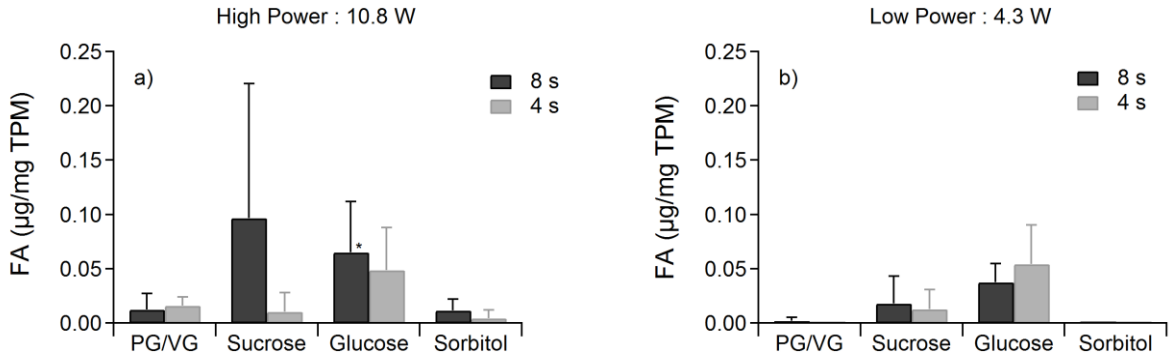

Figure III 2-Average FA yield normalised by TPM (µg/mg) in aerosols generated using sucrose, glucose and sorbitol-containing liquids vaped at 4.3 W (a) and 10.8 W (b). * indicates significant difference from theunflavoured liquid at $p<0.05$. N=3 measurements for each condition. Error bars represent standard deviation of three different measurements FA,furfural; HMF, 5-hydroxymethylfurfural; PG, propylene glycol; TPM, total particulate matter; VG, glycerin

Relative to the unflavored solution (PG/VG), sucrose and glucose solutions had greater HMF and FA yields, while the sorbitol solution showed similar yields to the unflavored solution. At 4.3 W (Figure III 1a), the differences in HMF of sucrose and glucose when compared to the unflavored solution are significant, while those for sorbitol are not. Similar trends were observed at 10.8 W (Figure III 1b), however HMF yields for the sucrose solution are not significantly different (*$p>0.05$)* than the unflavored condition due to the large variance in repeated measures. In a similar comparison, sorbitol has produced no significant change in FA (Figure III 2), while the sucrose and glucose conditions appear to have greater yields. Only the glucose solution vaped at 8 s puff duration and 10.8 W exhibited a statistically significant difference from the unflavored solution. Unlike power, vaping at two puff durations (4 s and 8 s) have generated similar levels of furans., Furthermore, HMF and FA yields in ECIG aerosol were tested in relation to varying sucrose content (0 – 1.49%) at the two puff durations (4 and 8 s) both HMF and FA yields show a significant correlation

with sucrose concentration at both puff durations ($p < 0.01$ and $p < 0.001$ at 4 s, and $p < 0.01$ and $p < 0.001$ at 8 s for HMF and FA, respectively) (Figure III 3).

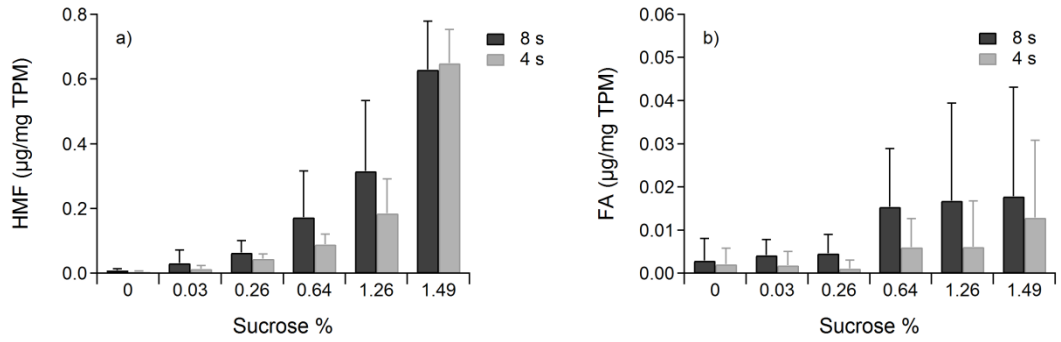

Figure III 3-Average level (µg/mg) of HMF (a) and FA (b) in the aerosol generated from different concentrations of sucrose in the e-liquid. Error bars represent standard deviation of three different measurements-

Chromatograms of the spiked commercial e-liquid showed no interferences preventing the detection and quantification of furan compounds. In addition to that, aerosols average concentrations (ng/mg) of HMF (4.26 ± 1.15) and FA (191.47 ± 62.55) were comparable to what was reported for the standard solutions.

G. Discussions

The thermal degradation of saccharide molecules has been the center of focus for years owing to its application in food science and renewable energy production.[125,185,186] To gain insights on reaction pathways, glucose molecule is chosen as a representative because sucrose hydrolyzes into glucose and fructofuranosyl cation which in turn thermally decompose into furanic derivatives.[186] The theories that have been proposed to explain the decomposition reactions can be grouped into two schools: (1) the acyclic conversions (Figure III 4)and (2) the ring system transformations (Figure III 5).[122,185,187,188]

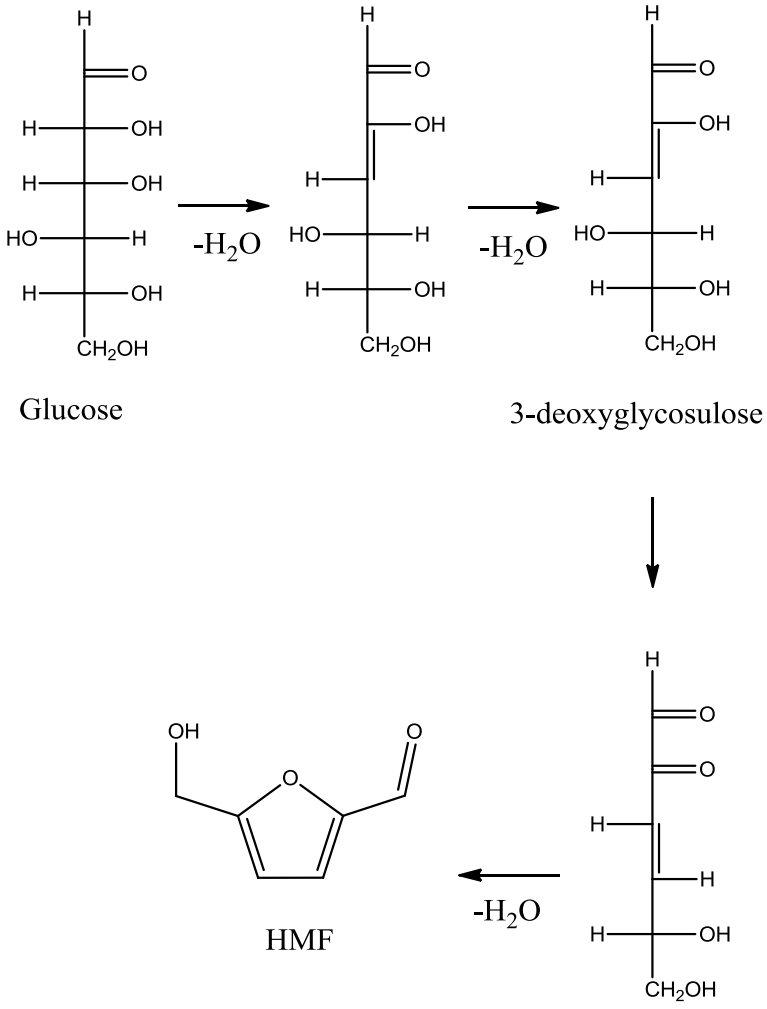

Figure III 4- Glucose thermal degradation into HMF via open chain transformation

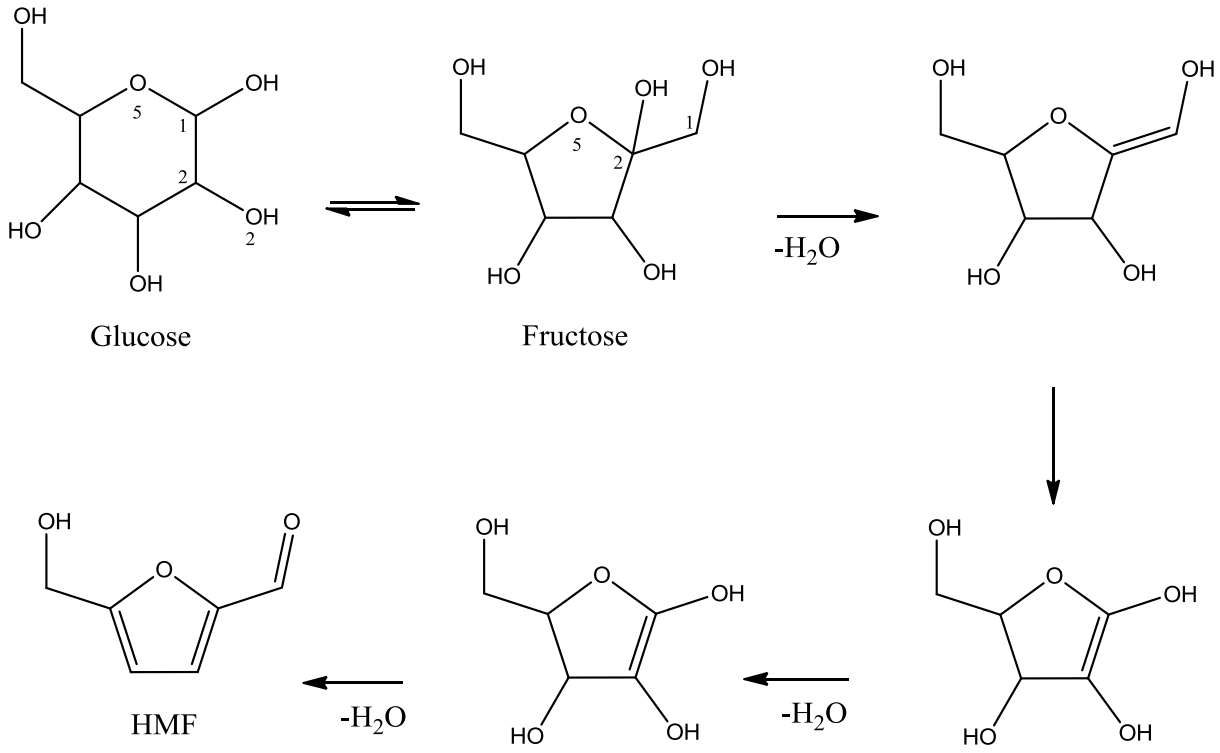

Figure III 5- Glucose thermal degradation into HMF via cyclic chain transformation

At first, the open chain mechanism has generally been adopted as presented in Figure III 4. The decomposition pathway includes the loss of three water molecules passing through 3-deoxyglycosulose intermediate.[123,124,189-191] Recent studies, however , have shown that glucose molecules are dominantly present in their cyclic conformations owing to the high stability of ring structures.[104,108] Figure III 5 shows dehydration of glucose molecule proceeding with intact ring structures through the formation of fructofuranose intermediate.[133,192,193] Besides open chain and cyclic presentation; the reaction pathways of HMF and FA have been thoroughly discussed. Saccharide molecules possess multiple hydroxyl groups that drive many degradation pathways.[107,109,194] It has shown that thermal decomposition of saccharides produce more than 37 analytes coming from many side reactions.[107,109,110,192-197] Consequently, driving a definite reaction pathway for HMF and FA formation is very complex.

Scientists have proposed that protonation of C2-OH favors the formation of stable 5-member ring intermediate that leads to HMF (Figure III 6).[198] Protonation of other activation sites has shown to form products of condensation (Figure III 7) and mutarotation (Figure III 8). Since fructose also thermally decomposes into HMF, there is another reaction pathway that includes glucose isomerization into fructose (Figure III 9).

FA formation has been reported to take place via two mechanisms. The degradation of HMF and the degradation of glucose (hexose) into arabinose (pentose) as rate determining step for FA formation (Figure III 10).[199 133] The two reaction pathways are proven to be temperature dependent.[125 ,199 ,200]

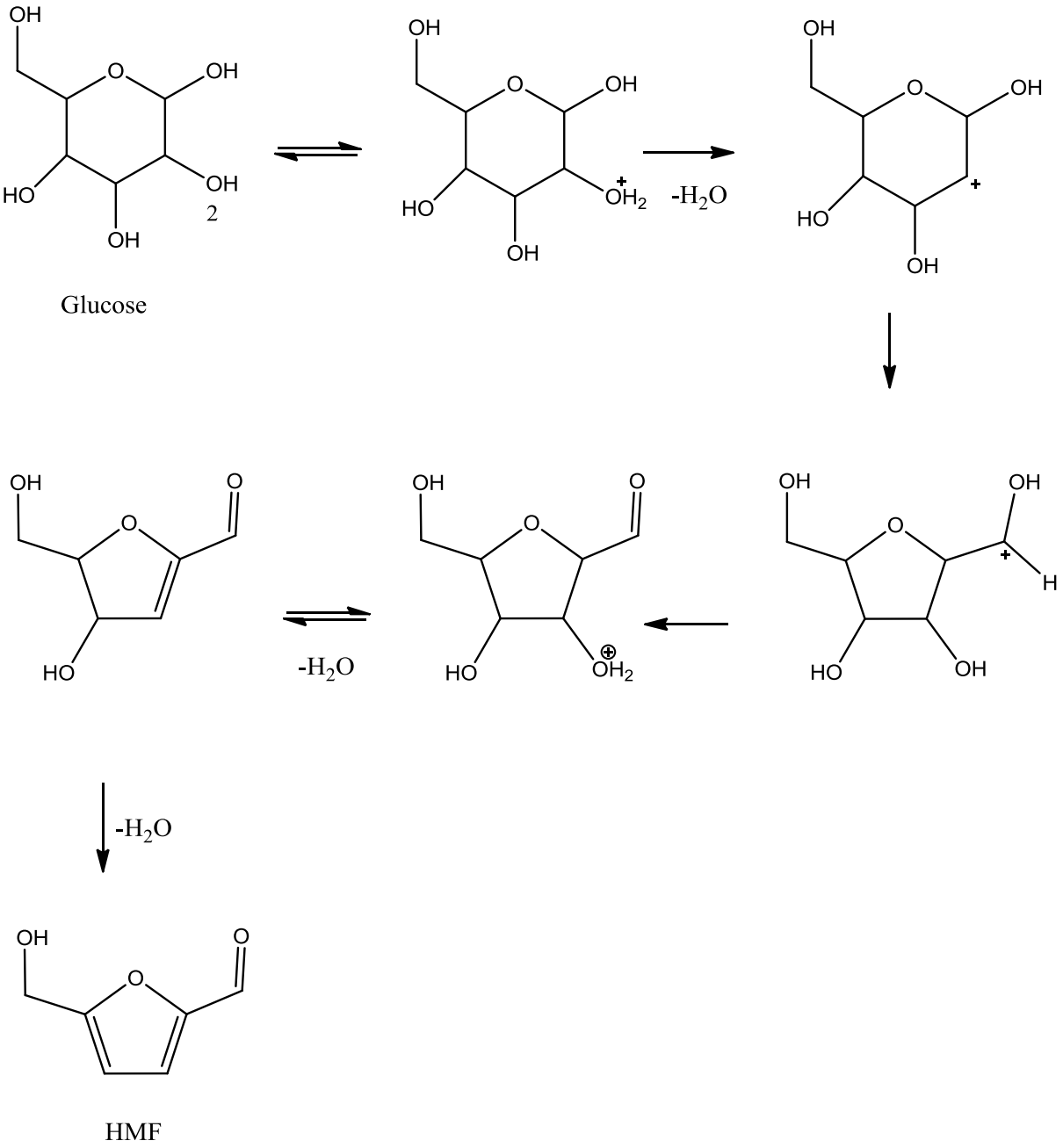

Figure III 6-Cyclic mechanism for glucose conversion into HMF

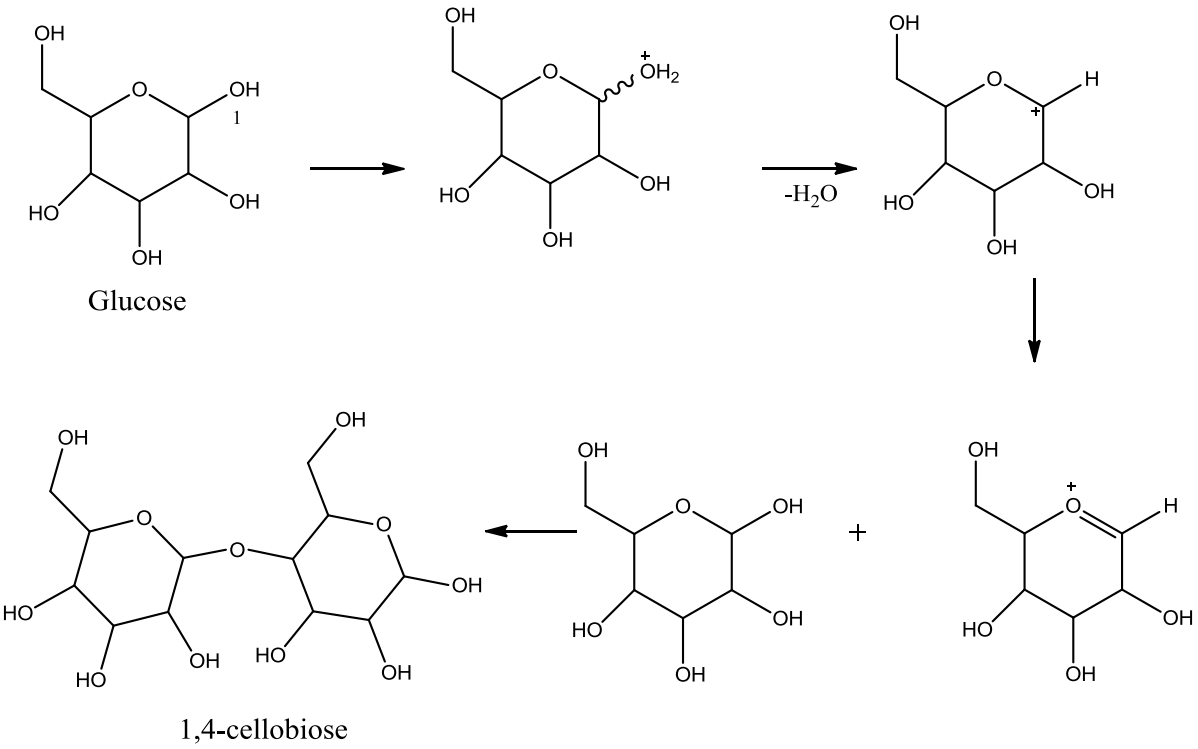

Figure III 7-Glucose condensation into cellobiose

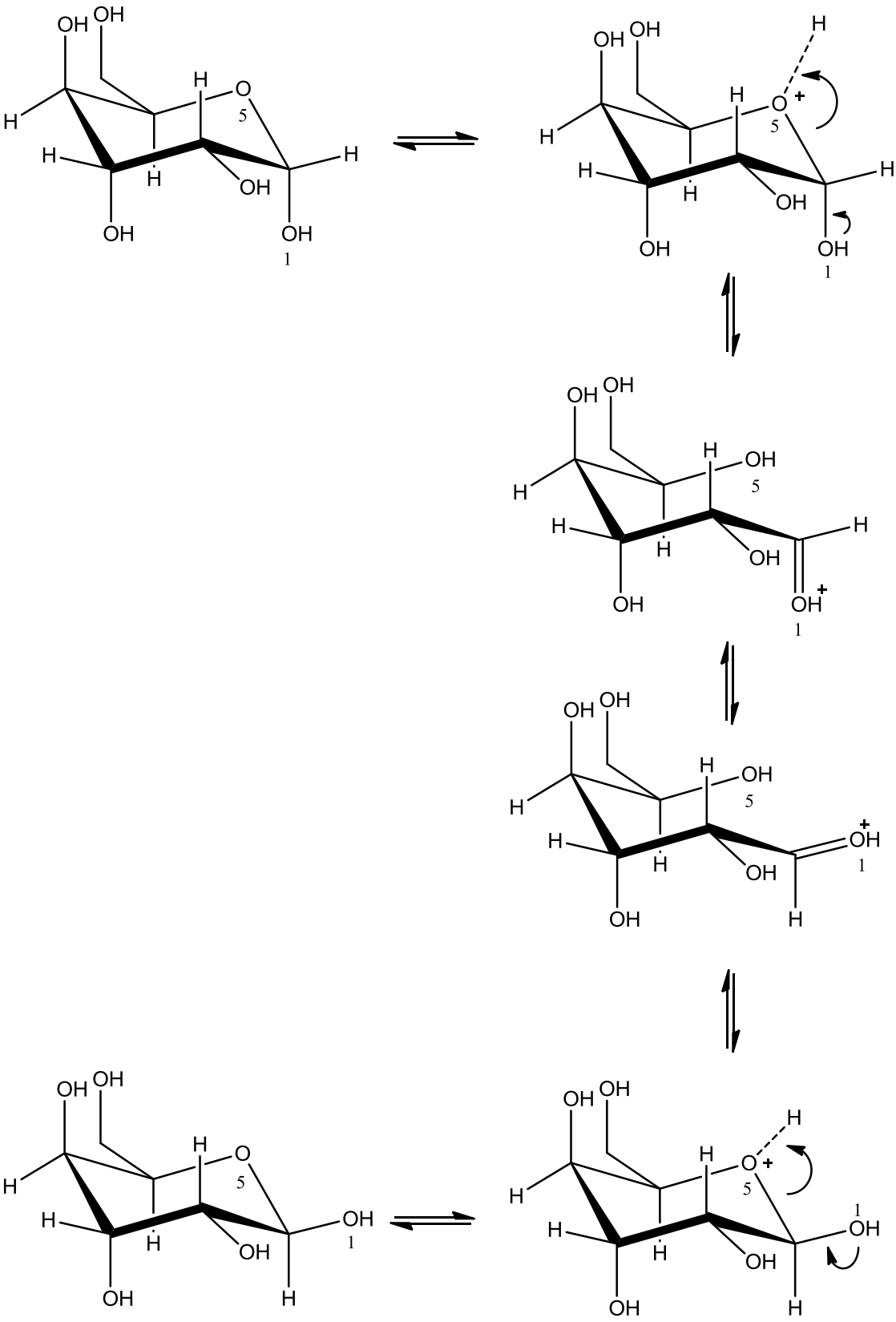

Figure III 8-Glucose mutarotation conversion

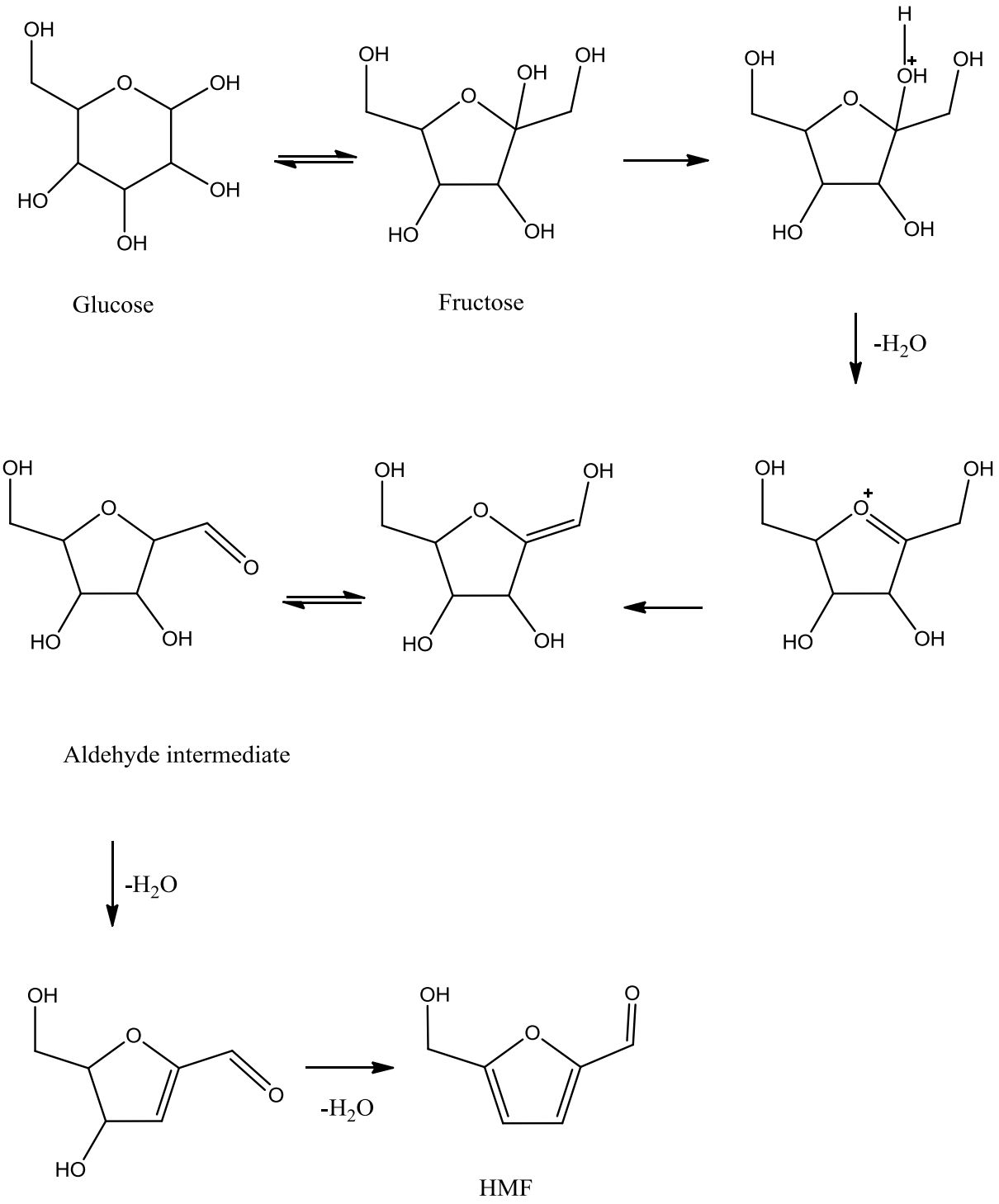

Figure III 9-Cyclic mechanism of glucose into HMF through fructose isomerization

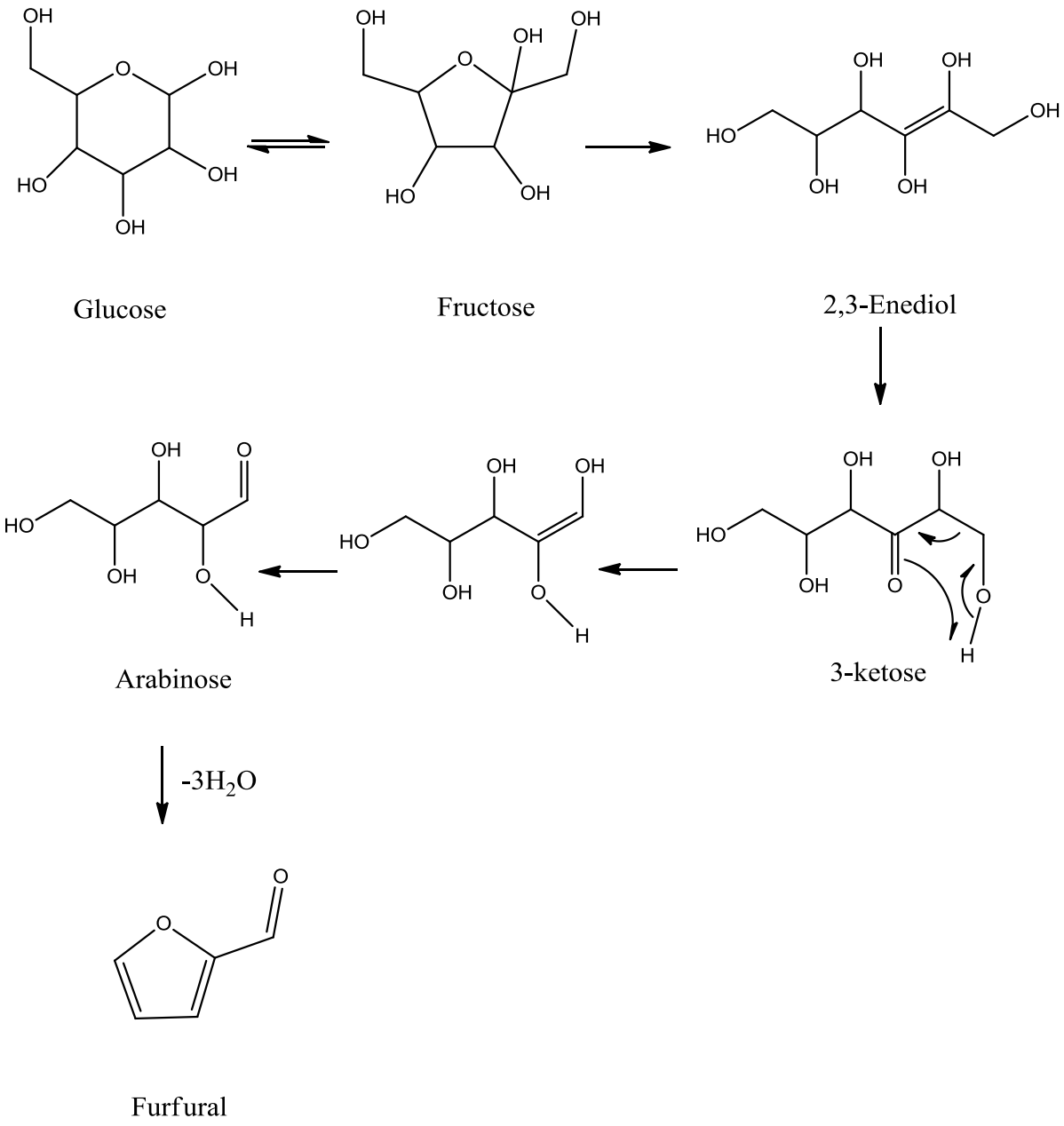

Figure III 10-Cyclic mechanism of glucose into FA

Our results show that HMF and FA are formed when sucrose and glucose containing e-liquids are vaped. Minimal amounts, however, were produced with sorbitol-containing liquid. Compared to glucose and sucrose, sorbitol is a sugar alcohol that lacks the carbonyl functional group therefore when thermally decomposed, sorbitol produces sorbitan.[201] (Figure III 11).

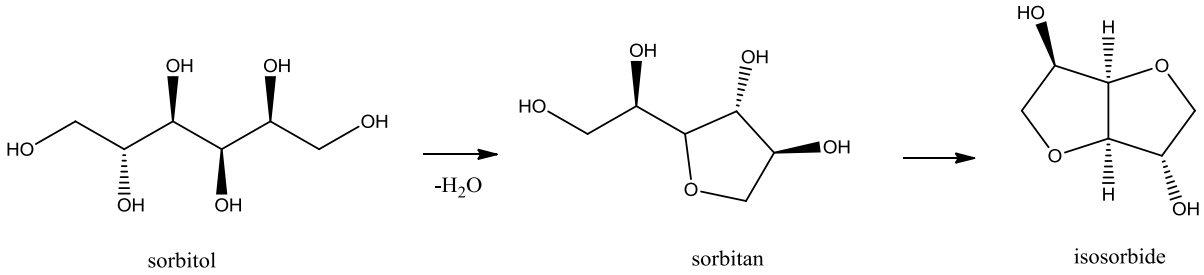

Figure III 11- Thermal decomposition of sorbitol

High concentrations of HMF were produced at lower battery power. Kinetic studies have shown that at temperature exceeding 200 ºC HMF yields decreases. Since high battery power is related to high temperature, glucose at 10.8 W is further decomposing into secondary products. Interestingly, this finding complicates the commonly held notion that lower power output leads to lower heating filament temperatures, and therefore lower emissions of toxicants which are formed by thermal degradation, such as volatile aldehydes. [65 ,137] Regarding FA, minimal amounts were produced at low power; this favors the formation of FA via HMF degradation.

Variances in repeated measures of HMF and FA were far greater at higher power conditions. Such variability may have been induced by occurrence of hot spots (dry puff) on the heating element where contact with the liquid-supplying wick was poor. These variations are comparable to those previously observed with aldehydes in ECIG aerosols.[70,71 ,202-204]

HMF and FA yields were also dependent on the initial sucrose concentration. However, the non-linear correlation between sucrose and the furan products (HMF and FA) indicates that the mechanism of formation of furans is complex.[200] So, in addition to the initial sucrose concentration, the degradation of sugar might be influenced by factors like the condition of the coil, the maximum temperature reached during its activation, and the multistep mechanism of furan formation.

Considering the full range of the aerosol furan content independent of the power, the puff duration, and the sugar type and concentration, the exposure level per puff of ECIG was

compared to the aerosol levels in tobacco cigarette and water pipe as shown in Table III 1. It is found that ECIG users are exposed to HMF and FA levels similar to the ones reported for combustible cigarette and to the lower limits of water-pipe smoke.

Table III 1-The range of HMF and FA aerosol concentrations per puff in ECIG, tobacco cigarette and water pipe

	ECIG	CIG	WPS	References
Sugar content (% by mass)	0.03-1.91	0.21-22.09	50-70	This study, [27]
HMF (µg/puff)	0.07-19.1	0.0-11	14.1-364.3	This study, [27,139]
FA (µg/puff)	0.01-2.6	0.0-2.9	0.2-2.3	This study, [27,139]

ECIG= electronic cigarette, CIG=cigarette, WPS=waterpipes

This chapter focuses on assessing the emission of toxic furans from sweet flavored ECIG solutions. Vaped under different conditions, the levels of furans have been found to be significantly different from the PG/VG base solutions and correlated with battery power output and sugar concentration. Surprisingly, no significant difference in yields was observed between the 4 s and 8 s puff durations. Per-puff emissions of HMF and FA from ECIGs using sweetened solutions were comparable to those found in cigarette and water-pipe smoke, suggesting that sugar based additives in ECIG solutions be regulated.

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CHAPTER IV

ASSESSMENT OF SUGAR IN E-LIQUIDS

Traditionally, sugar analysis has received significant attention in food industry to evaluate sensory and chemical characteristics of food products along with providing the customer with nutritional information of sugar intake.[205,156] Although numerous analytical approaches have been proposed, scientists have continued to design new methods that can be easily adapted for sugar routine analysis.[159,167,206] Most of the established methods are based on HPLC separation.[156] However, the main drawbacks of using HPLC reside in the low sensitivity of the generally adapted detectors i.e. RI and ELSD.[173] While MS is more powerful, reproducible and sensitive detector, few studies have found to couple HPLC with MS.[91,166] In this work we present an optimized LC-ESI-MS method for the rapid examination of sucrose, glucose and fructose. LC-ESI-MS is the first time to the best of our knowledge to be applied on commercial flavored e-liquids. Sixteen samples were tested for their sugar content and subsequently those containing sugar compounds were vaped to evaluate HMF and FA formation. The analysis of furanic compounds was completed according to SPE-GC-MS method.

A. Method Optimization and Validation

1. Chemicals

Sucrose, glucose and fructose are food grade provided by the Faculty of Agricultural and Food Sciences. Salicylic acid (internal standard) and ammonium acetate were obtained from chemistry department at AUB. HPLC grade Methanol was purchased from Sigma Aldrich.

2. Sample Preparation

This method entails minimal sample preparation. Because PG and VG eluted before sugar compounds we switched the effluent at the beginning of the run into the waste. Subsequently after discarding PG and VG, the effluent from the column was directed into MS .This has allowed the protection of detector from high concentrations of solvent.

3. HPLC-MS Conditions

The LC system consisted of a four-channel binary pump, autosampler and temperature-controlled column compartment from the Agilent 1100 series. Analysis was performed with MS (ESQUIRE 3000 PLUS, Bruker, Germany) equipped with ESI source operating in the negative mode. High purity nitrogen was employed for nebulization. Chromatographic separation was obtained on Aminex HPX-87H (300 × 7.8 mm) column operating at 40ºC and isocratically at 0.3 mL/min. The selection of mobile phase is critical to ionize the analytes. A combination of 20% ammonium acetate (10mM), 8 % methanol and 72% water was found to initiate the ionization of sugar compounds. The injection volume was 20 µl. The optimal MS settings were as follows: temperature; 350 °C, nebulizer nitrogen gas pressure; 30 psi and flow; 8 L/min. Under those conditions, well resolved peaks of IS, sucrose, glucose and fructose were obtained as shown in Figure IV 1. Because glucose and fructose are diastereomers and sucrose is a combination of the two, they have shared the same pseudo-molecular and fragmentation ions. Therefore peaks identification was based on the difference in retention time.

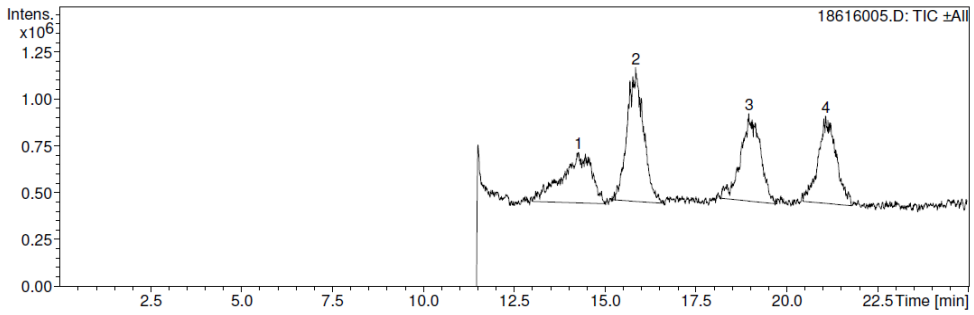

Figure IV 1-Total ion chromatogram of (1) internal standard, (2) sucrose, (3) glucose and (4) fructose

4. Standard Solutions

Stock solutions with 50 µg/mL concentrations were prepared by dissolving appropriate amounts of sucrose, glucose and fructose. Calibration solutions were established in PG/VG matrix to obtain concentrations within the range of 5-38 µg/mL. The internal standard was kept at 5 µg/mL. Solutions were sonicated for homogeneity and every week new solutions were prepared.

B. Quality Control and Quality Assurance

1. Linearity

Linearity evaluation verifies that sugar compounds are found in a range where their response is linearly proportional to their concentration. It is judged by examining the correlation coefficient of calibration curve. In this study a direct calibration curve is used where IS is added before injection to correct for the possible loss of analytes during HPLC run.

A six-point calibration curve was constructed using sugar standard solutions. The relationship between the ratio of the analytes signal to the IS signal and analyte standard concentrations was found to be linear for the whole examined range with correlation coefficient >0.996 for all analytes as seen in Figures IV 2, 3,4

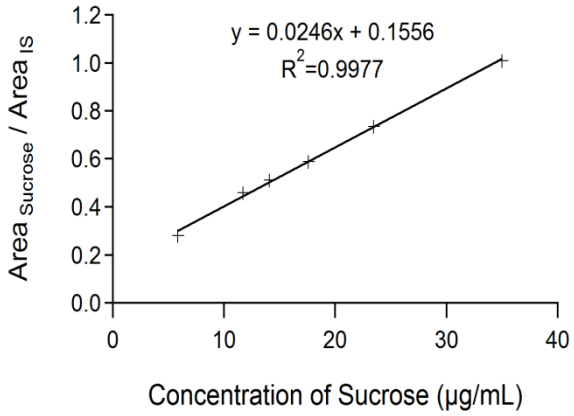

Figure IV 2-Calibration curve of sucrose

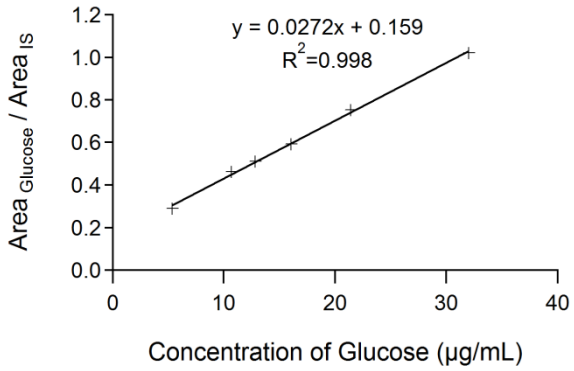

Figure IV 3-Calibration curve of glucose

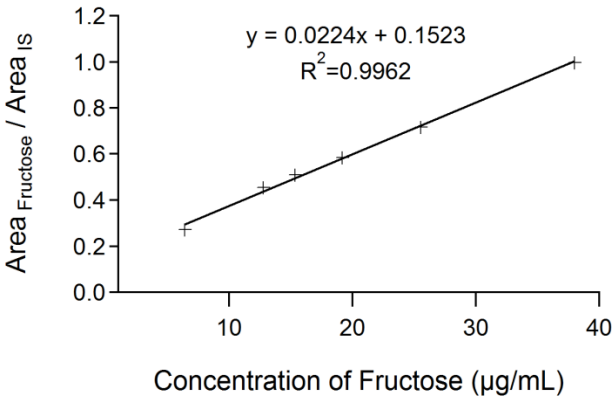

Figure IV 4-Calibration curve of fructose

2. Limit of detection

Limit of detection (LOD) is the lowest concentration of analyte that can be detected but not necessary quantified. Based on the standard deviation response and slope, LOD is expressed as following:

$$\mathrm{LOD} = \frac{3\sigma}{s}$$

Where σ is the standard deviation of the ratio of the analytes signal to the IS signal of seven replicates of analytes prepared at a low concentration and s is the slope of the extracted calibration curve. LOD analysis was carried out using seven replicate extractions of standard solutions in PG/VG matrix (10 µg/mL). The results have shown that a detection limit of sugar is 1.5 µg/mL.

3. Limit of quantification

Limit of quantification (LOQ) is the lowest concentration of analyte that can be measured with an acceptable level of accuracy and precision. Based on the standard deviation response and slope, LOQ is expressed as following:

$$\mathrm{LOQ} = \frac{10\sigma}{s}$$

The quantification limit of sugar was analyzed using seven replicate extractions (10 µg/mL). The results have shown that LOQ is equal to 5 µg/mL.

4. Repeatability

Repeatability describes the closeness of agreement between a series of measurements obtained under the same operating conditions (one operator, same equipment and on the same day). It is expressed by the percent relative standard deviation (%RSD) of analytical results

obtained from a minimum of five measurements at three different concentrations (low, medium and high). The acceptance criteria are based on type of analysis, complexity of matrix and the level of tested concentration.

Within-day precision was estimated by replicate (n=6) analysis of standards at three concentrations (5, 10 and 20 μg/mL). %RSD obtained was <4 % at all three concentrations.

C. Sample Analysis

Sixteen commercial e-liquids categorized under sweet flavorings were procured from different brands Table IV 1. No information regarding the sugar content was found. All e-liquids were analyzed at a 20-fold dilution in deionized water.

Table IV 1-List of commercial sweet flavored e-liquids analyzed for sugar content

Name	Brand
Welsh Taffi	Decadent Vapours
Charon Liquorice	MEDUSA Juice
Honey Bee E-Juice	Juishy
Swedish Fish	Emporium Vapour
Coffee with Cream & Sugar E-Liquid	the alchemist scupboard
Sweet Tooth	AVAIL
Fruit Sweetener	Vapor fi
Flavor enhancer	Vapor fi
Havana Rum	Vapor fi
Root Beer	Vapor fi
Carnival cotton candy	Vapor fi
Marshmallow	Vapor fi
Double Espresso	Vapor fi
Energy drink	Vapor fi
Creme Anglaise	Decadent Vapours
NomNom	CarpeDiemVapor

D. Result and Discussion

An HPLC-ESI-MS method was optimized to ascertain the sugar content of sixteen sweet flavored e-liquids. Ionization of sugar compounds was achieved on aminex HPX-87H column using a combination of 20% ammonium acetate (10mM), 8 % methanol and 72% water as an optimized mobile phase.

Results have revealed that out of the sixteen studied samples Carnival Cotton Candy flavorant has shown to contain 320 µg/mL glucose and 380 µg/mL fructose while Creme Anglaise and Welsh Taffi have shown detectable amounts of glucose.

Cotton candy flavored e-liquid was further analyzed for the formation of HMF and FA under variable battery input (4.3 and 10.8 W) and at constant puff duration (4 s). Filters loaded with collected aerosols were extracted, purified and analyzed according to the SPE-GC-MS method. HMF and FA were detected but not quantified. This further proves that e-liquids containing sugar can produce HMF and FA when vaped. The latter were only detected

and not quantified in the case of cotton candy because the percentage of sugar in cotton candy (0.02%) is at the lower limit of the investigated sugar range in this study (0.03-1.26 %).

This work has presented LC-ESI-MS method for the rapid assessment of sugar compounds in e-liquids. Sucrose, glucose and fructose were successfully separated without interferences from PG/VG matrix. The evaluation of limited number of samples has hypothesized that the addition of sugar compounds in e-liquids might be a common practice as has been in conventional tobacco products. It is important to note that a complete screening of all sweet flavors for sugar content and the latter analysis for HMF and FA production was not possible because of the huge number of flavor varieties available. Of the 16 analyzed flavors, sugar was present in three products. The latter emphasizes the need for tobacco companies to clearly label the ingredients of the additives used.

CHAPTER V

CONCLUSIONS

In this study we were concerned about the influence of sugar compounds on aerosol content and plausible toxicity. Sugar compounds might be intentionally added as additives or derived from the extraction process of nicotine. The thermal degradation of sugar into HMF and FA has gained our interest due to their potential toxicological impact. We have optimized and validated two analytical methods: SPE-GC-MS and LC-ESI-MS for furanic and sugar compounds analysis, respectively. The assessment of HMF and FA has been undertaken in a systematic approach to understand the effect of variable factors including various vaping topographies (battery input and puffing topography) and different type and concentration of sugar compounds. To isolate the effect of design features, three atomizers of the same type were used. Manipulating these parameters is critical to understand to what extent e-liquid ingredients contribute to the aerosol. Similar systematic approach has been so far applied only to nicotine and aldehydes. Unlike nicotine, HMF and FA yields were not influenced by puff duration. Additionally, studying the effect of battery input has revealed that the positive relation between power and toxicant yield is not always valid. Low level of HMF was observed at high power compared to that at low power. On another hand, this finding has supported the fact that at high battery input, more heat is produced favoring the further decomposition of HMF into FA. With respect to the effect of e-liquid precursor, it was found that the choice of sorbitol does not raise health concern compared to sucrose and glucose. Moreover, adding sugar compounds in a percentage $< 0.02\%$ is recommended where negligible amounts of HMF and FA are formed.

Inclusion of additives is part of an overall marketing strategy that has heavily served to promote youth initiation. The scientific community has acknowledged the potential health

risk associated with the presence of additives and has been working on providing regulatory bodies with enough scientific evidence.

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