IDENTIFICATION OF HYDROXYMETHYL FURFURAL (HMF) IN HONEY AND PEKMEZ BY USING PENCIL GRAPHITE ELECTRODE

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY

By
FATMA AMCA

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering

NICOSIA, 2020

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Approval of the Graduate School of Applied Science

Prof.Dr. Nadire ÇAVUŞ

We certify this thesis is satisfactory for the award of degree of Master of Science in Food Engineering

Examining Committee in Charge:

Assist. Prof. Dr. Süleyman AŞIR — Committee Chairman, Department of Materials Science and Nanotechnology Engineering, NEU

Assist. Prof. Dr. Perihan ADUN — Department of Food Engineering, NEU

Assist.Prof.Dr. V. Hazal ÖZYURT — Supervisor, Department of Food Engineering, NEU

I hereby declare that, all information in this document has been obtained and present in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Fatma Amca

Signature:

Date: 24.09.2020

To my parents…

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ABSTRACT

The aim of this research is to adapt a fast, easy, cost-effective and environmentally friendly method for the determination of hydroxymethylfurfural (HMF) in honey and pekmez. HMF is formed by the reaction of reducing sugars and amino acids in foods subjected to heat treatment, and in many foods their amounts are limited to reduce the heat treatment. HMF adversely affects food safety and human health, if the highest maximum limits set for each food are exceeded. For this reason, HMF amounts of different types of heat treated foods should be determined and the increase in HMF amount during their shelf life should also be observed separately.

In this study, 2 honey samples that were examined in the Government Laboratory and HMF amount exceeded the allowed limits, and 2 randomly selected honey samples from other brands; 1 homemade pekmez were analyzed by electrochemical methods. In the study an Autolab Potentiostat was used containing, pencil graphite electrode as working electrode, Ag-AgCl reference electrode and a platinium wire as auxiliary electrode.

The results obtained from the calibration studies and the analysis of the samples revealed that the method can be used as a screening method rather than quantitative analysis. There was no difference in HMF results regarding different aplicatitions of HMF on PGE i.e. dipping or coating. The average correlation coefficient of two methods was 0.98 and Limit of Detection (LOD) of HMF was 45.54 ppm.

Keywords: electrochemical; HMF; pencil graphite; DPV; honey; pekmez.

ÖZET

Bu araştırmanın amacı bal ve pekmezde hydroxymethylfurfural (HMF) tespiti için hızlı, kolay, uygun maliyetli ve çevre dostu bir yöntemin bulunmasıdır. HMF ısıl işleme maruz kalan gıdalarda indirgen şekerler ve aminoasitlerin tepkimeye girmesiyle oluşurlar ve birçok gıdada, miktarları bu ürünlerin eldesinde ısı işlemi azaltmak için sınırlandırılmıştır. Her gıda için belirlenen en yüksek sınırların aşılması durumunda HMF, gıda güvenliği ve insan sağlığını olumsuz etkilemektedir. Bu nedenle değişik tiplerdeki ısıl işleme tabi tutulmuş gıdaların HMF miktarları belirlenmeli ve rafta kalma süreleri boyunca HMF miktarında oluşabilecek artışın da ayrıca belirlenmesi gerekmektedir.

Çalışmada Devlet Laboratuvarında incelenmiş ve HMF miktarı izin verilen sınırlar üzerinde bulunan ballardan 2 adet ve diğer marka ballardan rastgele seçilmiş 2 adet; yüksek derecelerde kaynatılarak yapılan 1 adet ev yapımı pekmez grafit elektrot (PGE) kullanılarak elektrokimyasal yöntemlerle analiz edilmiştir. Çalışmada grafit elektrot (çalışma elektrodu) dışında Ag-AgCl referans elektrodu ve platin karşıt elektrot içeren Autolab Potensiyometre kullanılmıştır.

Kalibrasyon çalışmalarından ve örneklerin analizi ile elde edilen sonuçlar metodun nicel analizden çok bir tarama yöntemi olarak kullanılabileceğini ortaya koymaktadır. Kalem ucunun HMF çözeltisine daldırılarak veya kalem ucunun HMF ile önceden dışarıda kaplanması ve asetat buffer içinde elektrokimyasal analizleri arasında sonuçlar bakımından bir fark bulunmamıştır. İki yöntemin ortalama korelasyon katsayısı 0.98 ve minumum dedekte edilebilir HMF miktarı 45.54 ppm olarak bulunmuştur.

Anahtar kelimeler: elektrokimyasal; HMF; kurşunkalemgrafit; DPV; bal; pekmez.

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LIST OF ABBREVIATIONS

ABS: Acetate Buffer Solution
ACF: crypt foci
APC: polyposis coli
BBB: blood–brain barrier
CAT: catalase
CE: Counter electrode
CPE: Carbon Paste Electrode
CV: Cyclic voltammetry
DC: Consistent potential detection
DMF: 2,5 dimethyl- furan
DPPH: 2,2-difenil-1-pikrilhidrazil
DPV: Differential pulse voltammetry
dRP: 5′-deoxyribose phosphate
EFSA: European Food Safety Authority
FAP: polyposis syndrome
FFA: furfuryl alcohol
GCE: glassy carbon electrode
HACE: high-altitude cerebral edema
HAPE: high-altitude pulmonary edema
HIF: hypoxia-inducible factors
HMF: Hydroxymethylfurfural
HPLC-DAD: liquid chromatography combined with a diode array detector
HPLC: High Performance Liquid Chromatography
IFNγ: Interferon gamma
IgE: Immunoglobulin E
IL-4: Interleukin 4
IL-8: Interleukin 8
KH_2PO_4: Monobasic potassium phosphate
K_2HPO_4: Dibasic potassium phosphate

LOD:	low detection limit
MDA:	indirect determinant of lipid peroxidation
MEKC:	Micellar electro kinetic capillary chromatography
MRP:	Maillard Reaction Products
NaCl:	Sodium Chloride
NADPH:	Nicotinamide adenine dinu- cleotide phosphate
NaOH:	Sodium Hydroxide
NOS2:	nitric oxide synthase 2
OVA:	ovalbumin
PAPS:	3′-phosphoadenosine-5′- phosphosulfate
PGE:	Pencil Graphite Electrode
PRG5:	Pedestal Tacussel Potentiostat model
PV:	peroxide value
RE:	Reference electrode
SHE:	Standard Hydrogen Electrode
SMF:	Sulfoksimetilfurfural
SOD:	superoxide dis- mutase
SPCE:	Screen Printed Carbon (Graphite) Electrodes
SULT:	sulfotrans- ferases
TDI:	Tolerable daily intake
TdT:	terminal deoxynucleotidyl transferase
TRNC:	Turkish Republic of Northern Cyprus
UHT:	Ultra High Temperature
WE:	Working electrode
XO:	Xanthine oxidase

CHAPTER 1

INTRODUCTION

HMF (Hydroxymethylfurfural) is an intermediate product produced by the decomposition of hexos in acidic medium or during the Maillard reaction. In simpler terms, it occurs due to the storage of sugary foods at unsuitable temperatures and the heat treatment applied during their production. Under normal conditions, honey and pekmez retain their quality for several years, but excessive moisture or prolonged exposure to heat can spoil it. As a result, HMF content is used by many countries as an indicator of quality (Bogdanov et al. 1997; Bogdanov 1999). In the Turkish Food Codex Communiqué, HMF amounts are permitted up to 75 mg / kg in liquid pekmez, 100 mg / kg in solid pekmez and up to 40 mg / kg in honey.

The sensitivity of spectrophotometric (colorimetric) methods, which are the most common methods for the determination of HMF in foods, is low because other chromophores in foods may absorb radiation in the same wavelength region and may be misleading for the results. Chromatographic methods (liquid or gas high resolution chromatography) are more accurate and sensitive for this purpose. One of the biggest advantages of using chromatographic methods is the individual determination of HMF and furfural. They cannot be obtained by spectrophotometric methods (Erbersdobler and Somoza, 2007).

In the study, which is aimed to reduce the amount of honey and pekmez produced in the TRNC, hmf, it is aimed to develop a different method. By developing this method, HMF determinations are developed more precisely and faster than other methods, and low concentration operation and wide linear sensing range are developed.

It accelerates the search for other HMF determination methods that are more direct and simpler to use than they are currently used.

A lot of research has been done on HMF chemistry; however, there are several electrochemical studies on HMF. The existing ones focus only on electrochemical oxidation.

CHAPTER 2

THEORETICAL FRAMEWORK

2.1 Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (HMF) is an intermediate compound formed by the breakdown of hexoses under acid conditions or at high temperatures during Maillard reaction (a non-enzymatic browning reaction) (Arribas and Morales, 2010). The concentration of HMF is widely recognized as a parameter affecting freshness because it is typically absent (or only very small is available in fresh products). Its concentration tends to increase during processing and / or due to its long shelf life. Previous studies have reported that products stored at low temperatures and / or under fresh conditions are low or minimal. It was reported in a study that the amount of HMF affects the formation of color, taste and aroma. Therefore, it is determined that the increase of HMF decreases the sensory quality (Gökmen et al., 2016)

2.1.1 Formation of hydroxymethylfurfural

The formation of HMF is directly dependent on the heat intensity applied to food. It is considered a thermal damage marker for products with high carbohydrate concentrations, as it is not usually present in raw and fresh foods. It can also be used to monitor the thermal treatment applied to several different food products. For example; breakfast cereals containing dried fruits; caramel and honey; pasta and bakery products (Rufian-Henares et al., 2008).

2.2 Maillard Reaction

Maillard Reactions got its name from the French scientist Louis Camille Maillard (1878-1936). Maillard explained that a yellow-brown color develops after heating sugar and amino acids in water lightly. The reaction that leads to the colored compounds, first described by a simple observation, is actually the result of a complex path of chemical

reactions. Maillard reaction is often described in food systems (Finot, 2005; Gerrard, 2002a). Maillard Reactions are the reason for the color browning and aroma formation that occurs during the gradual heat treatment or storage of foodstuffs (Yoo M.A. et al., 2004). Formation products that contribute to specific aroma and color characteristics are called Maillard Reaction Products (MRP). These reactions, also known as non-enzymatic color browning/blackening, create colored or colorless reaction products depending on factors such as pH, type of reactants, temperature and water activity (Hidalgo and Zamora, 2000).

The consumption of Maillard Reaction Products (MRP) has increased in recent years. There is evidence that these substances are absorbed and that they can accompany the pathological processes such as cataracts, diabetes, degenerative diseases, atherosclerosis and chronic kidney failure. It is reported, on the other hand, that it also has beneficial effects such as antioxidant, antiallergenic and antimicrobial properties besides its harmful effects. Reactions take place in all foods baked in the oven (bread, cookies, cake, etc.), fried (meat, potato chips, etc.), and heat-processed foods (honey, pekmez, coffee, jam, etc.) in production and / or afterwards. The basic foods, in which the reaction takes place, react slowly during the shelf life, and react rapidly in case of heat-processing, and their MRPs are formed.

2.2.1 Factors affecting maillard reaction

The composition of the products formed changes with factors such as pH, acidity, type of reactants, temperature, time, concentration of oxygen and water activity (a_w).

a) Type of reactants

The variety of amino acids and sugars present in the medium directly affects Maillard reaction. It is the most reactive amino acid among amino acids since it contains the lysine free ε-amino group (Saldamlı, 2007). The presence of reducing sugars in this reaction formation is inevitable since they provide a carbonyl group. Low molecular weight sugars are more reactive than high molecular weight sugars. The general order of sugars in Maillard reaction reactivity is as follows in 2.1:

D-xylose> L-arabinose> hexoses> disaccharides (2.1)

b) Temperature and time

The most important factors affecting Maillard reaction kinetics are temperature and time (Jaeger et al., 2010). The speed of Maillard reaction increases generally with the increase in temperature and time.

c) Ph

While Maillard reaction rate is low in acidic pH (pH <3) values, it increases with pH increase up to pH 10 (Ashoor and Zent, 1984). There are more H^+ ions around substances with low pH values. These H^+ ions react with negatively charged parts of amino acids, decreasing their reaction with reducing sugars. At high pH values (about pH = 10), maillard reaction rate slows down, due to the lack of sufficient H^+ ions in the medium to catalyze Amadori compounds.

d) Water Activity (a_w)

Another factor affecting Maillard reaction is water activity. Maillard reaction rate is maximum in values where the water activity is 0.6- 0.7 (Kroh, 1994).

2.2.2 Mechanism of maillard reactions

Maillard Reactions begin with the condensation of the carbonyl group of reducing sugars with the amino group of amino acids, and their early volatile products form medium and high molecular weight polymers (Yoo and Kim, 2004).

It is known to be generated in three steps (Çelebi, 2006.). In the first step, Schiff base is formed when water is removed from the structure as a result of the condensation reaction (Figure 2.1) of sugar and amino acids. After the acid-base catalyzed and alternating reaction, Schiff base turns into aldosylamine.

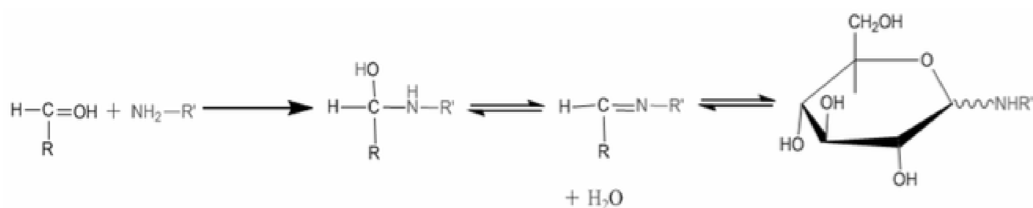


Figure 2. 1: Condensation reaction between free amino group and carbonyl group (Çelebi, 2006.)

At this stage, aldozylamine isomerized to ketosamine (1-amino-1-desoxycytosis) by Amadori regulatory reactions for aldoses (Figure 2.2). For ketoses, 2-amino-2-deoxialdose occurs as a result of Heyns rearrangement reactions.

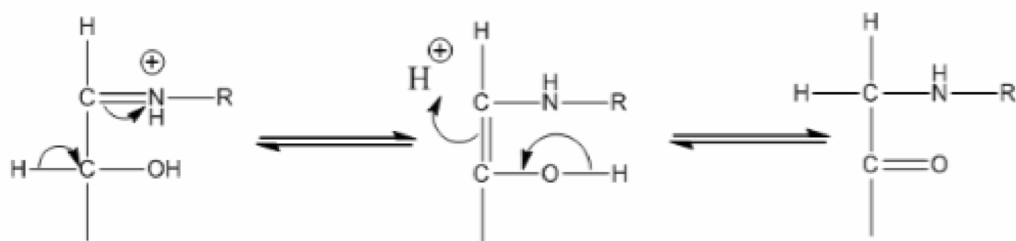


Figure 2. 2: Amadori rearrangement (Çelebi, 2006.)

The second step is the step where the color change starts as well. In this step, there are 3 different ways. In the first way, where the most important Maillard intermediates are formed, 1-amino-1-dexoxycetosis reacts with another aldose molecule and turns into less stable diketosamine. Diketosamine is broken down into many compounds, such as monofructosamine and 3-deoxyiosulosis (Çelebi, 2006). The second way of the second step is the enolization of Amadori substances (the phenomenon of the formation of an unsaturated alcohol, that is, the enol, with the migration of a proton from a carbon atom to the oxygen of the neighboring carbonyl group). When the pH is lower than 7, this way continues with the conversion of pentose sugars into furfural and hexose sugars into hydroxymethylfurfural while it enolizes highly reactive products in circumstances when pH is higher than 7 (Çelebi, 2006). The third way of the second step is called Strecker degradation (Figure 2.3). This step, where carbonyl groups are condensed with amino groups and can be characterized by CO_2 formation, is also the beginning of the aroma formation. The source of the aroma is aldehyde and aldehyde derivative compounds (Çelebi, 2006).

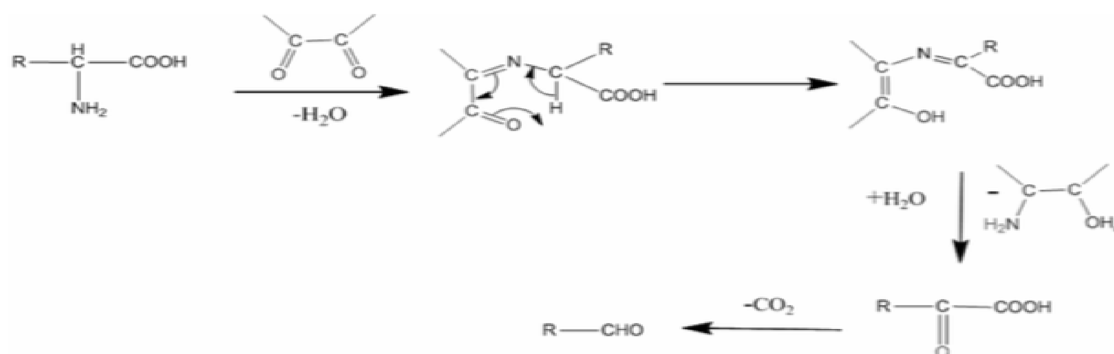


Figure 2. 3: Strecker degradation

The third step is the combination of the compounds formed in the previous step with the amines, the condensation of aldols, the polymerization of aldehydes and amines, and thus the formation of dark colored compounds called melanoid (Figure 2.4). The production of aromatic molecules occurs as a result of reaction sequences. These sequences are quite complex and are similar to chemical types such as hydroxymethylfurfural, dihydrofurans, furans, pyruvaldehyde or dimethylpyrazine (Edwards, 2000; Çelebi, 2006; Coca et al., 2004).

Figure 2. 4: The third step of Maillard reactions (Yıldız et al., 2010.)

2.2.3 The role of maillard reaction products in food acceptability

Maillard reaction is one of the most important reactions resulting from food processing. Maillard reaction products (MRPs) greatly affect basic food quality characteristics such as color, tissue, flavor and aroma. In fact, this reaction can be used to design foods that offer the sensory qualities demanded by the consumer (Ames, 1990; Yu and Zang, 2010).

a) Color

Color formation is the primary feature of Maillard reaction. In the last decade, efforts have been made to determine Maillard reaction kinetics and the rate of colored compounds

formation, especially with the use of model systems. Brown color development during processing and storage is desirable for many products such as baked foods, coffee and cookies, while it is not desirable in some foodstuffs such as orange juice, white chocolate, milk and powdered eggs. Estimating and controlling food color development is especially important for manufacturers to meet consumer preference. This is because of the fact that a complex sequence of melanoid produced by Maillard reaction depends on the food matrix composition as well as on the technological conditions of the reaction (Wang et al., 2011). Melanoidin might also be created by sugar caramelization without the participation of amino groups. The presence of melanoidins, high molecular weight pigments containing brown nitrogen, responds to the characteristic color of roasted foods such as coffee, cocoa, bread and malt.

b) Flavor and Aroma

The flavor and aroma development associated with Maillard reaction depends on the reaction temperature, duration, pH, water content, sugar and amino acid type (Yu and Zhang, 2010; Van Boekel, 2006). The intermediate and final stages of Maillard reaction are fragmented by dicarbonyls, especially by deamination and decarboxylation of amino acids (Ames, 1990; Rizzi, 2008). Sugar dehydration / fragmentation volatile products of Maillard reaction are furans, pyrones, cyclopentenes, carbonyls and acids. Amino acid decomposition products are aldehydes and sulfur compounds. Volatile substances produced by other interactions are pyrroles, pyridines, imidazoles, pyrazines, oxazoles, thiazoles and others. Pyrazines and alkylpyrazines are respectively associated with the flavor and aroma of cooked (roasted) and walnuts.

The characteristic aroma of food products and the compounds necessary for aroma are usually found at trace levels.

c) Texture

The definition of texture is complex and a general agreement has been reached, developing from the efforts of a number of researchers. According to Szczesniak (2002), the texture is the "sensory and functional manifestation of the structural, mechanical and surface properties of foods detected by vision, hearing, touch and kinaesthetic senses".

Maillard reaction affects the texture of foods through protein crosslinking. The manipulation of the size and nature of such protein cross-linking during food processing gives an opportunity to the food industry for changing the functional properties of food. However, it is not yet known how protein crosslinking affects food texture in processed foods and how this parameter is to be controlled to maximize food quality (Gerrard, 2002b). Protein crosslinking with Maillard reaction affects not only texture but also protein digestibility.

2.2.4 Biological activity of maillard reaction products

Maillard reaction is extremely important for flavor and color formation of heated foods. Despite their indisputable beneficial effects on the sensory quality of food products, Maillard reaction products can be harmful to human health (Brands et al., 2000; Torres et al., 2001). In the past, many scientific studies have focused on the negative biological effects of Maillard reactions, and the formation of MRP, which is nutrient-reducing and toxic, has been frequently expressed. In vitro studies show certain harmful effects of them, including mutagenic, carcinogenic (Yen et al., 1993) and cytotoxic (Vagnarelli et al. 1991). It is stated that advanced glycation causes the destruction of essential amino acids, a decrease in digestibility, inactivation of enzymes, inhibition of regulatory molecule bonds, cross-linking of the glycated extracellular matrix, decreased sensitivity of proteolysis, abnormalization of nucleic acid functions, disruption of the identification of endocytosis and macromolecules and weakening of immune system (Brownlee et al., 1984). It is argued in some studies that MRPs, especially formed in the early stages of the reaction, are useful compounds contrary to popular belief. In this sense, there are findings that some MRPs are antioxidant and beneficial products (Chevalier et al., 2001; Carmelina et al., 2007).

The antioxidant activities of the Maillard reaction product mixture are explained by the superior radical scavenging properties of the melanoids, their reactions with oxygen and their reactions with products such as O_2 and OH^- (Hayase, F., Hirashima, S. et al., 1989). It has been reported in the studies that Hydroxymethylfurfural (HMF), which is an

intermediate product of Maillard reaction, also has antioxidant properties (Turkmen et al., 2006; Morales and Jimenez-Perez, 2001). HMF was found in honey (Spana et al., 2006; Tosi et al., 2002), pekmez (Aliyazicioglu et al., 2009), juices (Burdurlu and Karadeniz, 2003), wines (Guerra-Hernandez et al., 1990), beer (Harayama et al., 1994), coffee (Kanjahn et al. 1996.), baby recipes (Albala-Hurtado et al., 1997) and baked products (Rami, 2000). In a study on the relationship between storage and non-enzymatic browning of apple juice concentrates (Burdurlu and Karadeniz, 2003), browning index has revealed that the correlation coefficient between the amount of Hunter L, a, b, and HMF has changed depending on apple variety, storage temperature and brix value. In addition, it was proven in a study that DPPH radical scavenging activity has increased in connection to the increase in HMF amount as a result of heating the honey (Küçük et al., 2007).

2.2.5 Hydroxymethylfurfural analysis methods

HMF determination: spectrophotometric method by White and Winkler and reverse phase high performance liquid chromatography (HPLC) method (Zappala et al., 2005). Although the HPLC method is relatively more expensive, it is advantageous in both labor and time. In addition, the method is considered an automated and sensitive method that can exclude many interactions from other related compounds (Wootton and Ryall, 1985). Reyes-Salas et al. reported an electrochemical approach for HMF detection. In this method, borate was used as a supportive electrolyte while generating a single, sharp reduction signal against argentum or argentum chloride at gent1100 mV (Reyes-Salas et al., 2006). Another method is the ion exchange liquid chromatography - photodiode array detection technique described by Yuan and Chen , consistent with the Winkler method (Yuan and Chen 1998). Another method, Iglesia et al., is based on the working principle of the Winkler method and provides a detection range of 5-40 ppm. Micellar electro kinetic capillary chromatography (MEKC) is another fast method that uses caffeine as a standard (Iglesia et al.,1997). The technique is suitable for rapid measurement of HMF without requiring sample pre-treatment, especially in honey samples (Rizelio et al., 2012). A unique and effective rapid scanning technique is real-time (DART) direct analysis, with flight time mass spectrometry (TOF-MS), reported to provide a high resolution chromatogram (Rajchl

et al., 2013). This method can quantitatively analyze HMF concentrations more precisely than other methods and requires no (or very little) sample pre-treatment.

2.3 The Feature that Distingıieshes Electrochemistry from Other Devices

Non-enzymatic and enzymatic electrochemical sensors have found a more advantageous and fast application area with their high sensitivity, fast response, wide linear detection range and low concentration detection limit (LOQ, LOD) of modified electrodes in electrochemical sensors with its advantages that do not require a simple and long preparation compared to other methods (Chaudhary et al., 2017; Chin Wee et al., 2013).

2.4. Effects of Hydroxymethylfurfural on Human Health

HMF have both harmful and positive effects on human health.

2.4.1.Negative effects on human health

It has been confirmed that HMF and its derivatives provide genotoxic, mutagenic, carcinogenic, DNA-damaging, organotoxic and enzyme-inhibiting effects.

a) Hydroxymethylfurfural, an indirect mutagen

Florin et al. made research on the mutagenic activity of some compounds, including HMF, towards the four mutant strains of *Salmonella typhimurium* (Florin et al., 1980). Liver extracts from rats induced with methylcholanthrene were used for the metabolic activation of the examined compounds, and it was confirmed that HMF was not a mutagen. On the contrary, Lee et al. proved that HMF is an indirect mutagen because it is converted to an active metabolite, sulphuric acid ester 5-sulfo-oxymethylfurfural (SMF), and has mutagenicity to *S. typhimurium* TA104 (Lee et al., 1995). HMF is enzymatically activated to SMF by sulfotransferases (SULT) found in rat liver extracts enriched with sulpha group donor 3p-phosphoadenosine-5p-phosphosulfate (PAPS). This finding also proved that HMF, 2,5-bishydroxymethyl-furan (HMF metabolite), furfuryl alcohol (FFA) and 5-methyl-FFA mutagenized against *S. typh-imurium* TA100 expressing human ty SULT1C2, however, towards parent strains. (Glatt et al., 2011).

Another study using FVB / N (FVB) mice expressing hSULT1A1 / 1A2 revealed that HMF has a genotoxic effect. Mice given orally at a single dose of 900 or 1300 mg / kg showed significant DNA damage in kidney cells, as determined by the alkaline single cell gel electrophoresis test. Another furan derivative, 2,5 dimethyl-furan (DMF), also causes DNA damage in the kidney and colon (Høie AH et al.,2015). In an in vitro erythropoietic micronucleus analysis, rat bone marrow cells were exposed to DMF at a concentration of 0.1 mM for 1 hour. The study proved that DMF shows genotoxicity to hematopoietic cells (Fromowitz et al., 2012).

SULT1A1 activity at different levels (mouse L5178Y, no activity; Chinese hamster: V79-Hp-PST, high activity; V79, negligible activity; human: HEK293, higher activity; and Caco-2, low activity) HMF caused DNA damage when exposed to 100mM concentration for 3 hours (Durling et al., 2009). However, it has also been determined that HMF has damaging DNA effects, regardless of the SULT1A1 activity of the cell lines. In addition, 5-HMF was found to cause chromosomal abnormalities in a Chinese hamster V79 derivative cell line expressing human sulfotransferase SULT1A1 and CYP2E1. In fact, 5-HMF is a potent inducer of sister-chromatid exchange at a concentration of 19.8-3808.0 μM in cells exposed for 32 hours (Glatt et al., 2005). Nishi et al. have confirmed that 5-HMF causes chromosomal abnormalities of V79 cells at 15.8 mM (Nishi et al., 1989). In both preclinical and clinical studies, Pastoriza et al. stated that orally administered HMF is converted into reactive SMF after absorption from the gastrointestinal system (Pastoriza de la Cueva et al., 2017). SMF is present in the kidneys, leukocytes and liver cells of mice, as well as in the leukocytes of the pre-adolescent population. In addition, SMF is not properly excreted through the urine due to renal reabsorption, thereby allowing SMF to accumulate in plasma, which allows it to react with cellular proteins and DNA.

b) Hydroxymethylfurfural as a double actor in carcinogenesis

HMF and its derivative SMF have been confirmed to be potent carcinogens in several studies at the preclinical level. The compounds cause neoplastic transformations, including colon and skin. Colon cancer involves the development of a multi-stage universe where micro-adenomas and abnormal crypto foci (ACF) are morphological markers (Archer et

al., 1992). HMF functions both as the initiator and stimulant of ACF (Bruce et al., 1993). A study was performed on multiple intestinal neoplasia mice administered subcutaneously at a single dose of HMF (500 mg / kg) or SMF (25 mg / kg) within 3-6 days after delivery. After 12 weeks, both HMF and SMF administration increased the number of small intestinal adenomas and flat dysplastic lesions (flat ACF) in the large intestine (Svendsen et al., 2009). Mice had a mutant copy of tumor suppressed gene adenomatous polyposis coli (APC). The mutation in the APC gene leads to the development of adenomas in the small and large intestines, the development of the human family in a similar way to adenomatous polyposis syndrome (FAP) (Paulsen et al., 2005; Preston et al., 2007).

Zhang et al. has found that 45% of F344 female rats administered with 250 mg / kg HMF twice a day through oral gavage develop a large intestinal ACF on the 30th day (Zhang et al., 1993). HMF increases both the number and size of ACF. In contrast, a comprehensive study (with two mouse models: genetically engineered mice with wild-type FVB / N and several copies of SULT1A1 and SULT1A2 on chromosome 9), Florian and others gave opposite findings (ie neither HMF nor metabolite SMF did not induce ACF and colon tumors). HMF also induces skin papilloma (Florian et al., 2012). Upon topical application of sulfoxylmethyl and chloromethyl derivatives of HMF, papillomas were found in the skin of mice.

Another derivative of HMF has been found to induce hepatocarcinoma at a very early age in 5-chlorometiffurfural, B6C3F1 male rats (Surh et al., 1994). Schoental et al. have proven that rats administered subcutaneously with HMF (200 mg / kg) develop renal lipomatous tumors (Schoental et al., 1971). Contrary to these studies, Zhao et al. demonstrated that HMF can induce apoptosis and G0 / G1 arrest in DNA damaged cells via reactive oxygen species (ROS) mediated signal transduction by using the A375 cell line (Zhao et al., 2014). Thus, it was concluded that HMF is a potent anti-carcinogen.

c) HMF as an organotoxic agent

HMF has high concentrations of cytotoxic effects because it causes irritation on the compound mucous membranes, skin, eyes and upper respiratory tract (Morales, 2008). In an in vivo study in which SMF was administered intraperitoneally to male FVB / N mice

with SMF (250 mg /kg), it turned out to be a potent nephrotoxic agent. 5-11 days after treatment, mice either died or became worse, possibly due to liver damage in the proximal tubules or more severe kidney damage. In a histopathological study, large protein rashes and necrosis were detected in the affected area (Bauer et al., 2012). Organic anion carriers of Type 1 and 2 (OAC1 and OAC2) are highly conserved in different species, including rats and humans, and are generally expressed in the cellular basoral membrane of the proximal tubules. Carriers mediate the uptake of different organic anions, including SMF, from the bloodstream as their substrates and are mostly concentrated on tubular cells affecting the proximal tubules (Burckhardt and Burckhardt, 2003). Another study using human embryonic kidney cells (HEK293) structurally expressing OAC1 and OAC2 carriers supports this mechanism (Bakhiya et al., 2009). Glutathione is an important endogotic antioxidant in the body. An ex vivo study involving two mammalian cell lines V79 and Caco-2 showed that HMF lowered cellular glutathione levels in a concentration dependent manner at 50 mM and 120 mM, respectively (Janzowski et al., 2000).

d) Hydroxymethylfurfural as an enzyme inhibitor

The human genome encodes 16 DNA-dependent DNA polymerases. Three of them are involved in nuclear DNA replication, the rest are in the repair system (Bebenek and Kunkel, 2004; Friedberg et al., 2000). Human polymerase is a multifunctional enzyme with DNA, DNA polymerase, terminal transferase, 5p-deoxyribose phosphate (dRP) lyse and polynucleotide synthetase activities. The primary structure of the polymerase includes a nuclear localization signal, a BRCA1 carboxy terminal domain, a proline-rich region, a pol-like region and a pol X region (Ramadan et al., 2004). DNA polp shares a number of homologies that catalyze the addition of deoxyribonucleotide to the 3 'end of the dsDNA or ssDNA with terminal deoxynucleotidyl transferase (TdT) (Pandey and Modak, 1987). HMF competitively inhibits DNA pol γ and TdT according to the deoxynucleotide substrate and the DNA template primer with values of 26.1 and 5.5 μM with 50% minimum inhibitory concentration (IC50) values (Mizushina et al., 2006).

2.4.2. Positive effects of hydroxymethylfurfural on human health

a) Hydroxymethylfurfural as an antioxidant

ROS is produced as toxic by-products of the body's aerobic metabolism. Its derivatives oxidize cellular macro molecules, such as proteins, membrane lipids, and DNA, and cause cellular damage. The results range from stress to metabolic disorders, neurodegenerative diseases and even neoplastic transformations (Sies, 1985). Zhao et al proved HMF dose-dependent (0.8-6.4 mM) free radical scavenging capacity (Zhao et al., 2013). HMF also has significant protective effects against ROS-induced damage on erythrocytes. The protective effect and oxidative stress induced by the production of 2,2′-azobis (2-amidino-propane) dihydrochloride, ROS and malondialdehyde (MDA: indirect determinant lipid peroxidation), the activity of antioxidant glutathione peroxidase in HMF pretreated. GPx), superoxide dismutase (SOD) and catalase (CAT) enzymes were determined. It has been demonstrated that ROS and MDA content decreases in HMF-treated cells, and the activities of these enzymes increase compared to those of negative control cells (Zhao et al., 2013). The ROS cleaning activity of HMF is given by its structure, which has functional reactive groups such as aldehyde oxygen, double bonds and another oxygen in the furan ring. These properties can easily attract electrons and kill ROS (Ulbricht et al., 1984).

HMF also has a protective effect at the morphological and biochemical levels on hepatocytes damaged by oxidative stress induced by hydrogen peroxide (H_2O_2); under these cells wrinkle, condensation of chromatin and separation of nuclei - apoptotic and necrotic cells. However, in a H_2O_2 -stressed human liver cell line (L02), cells treated with HMF (0.79 µM) can better preserve their morphology than untreated cells. HMF also reduces caspase 3 and 9 (apoptosis practitioner) levels in these cells (Wang et al., 2010). Ding et al. found that there was a decrease in nitric oxide and caspase levels 3, as well as the underlying protective biochemical mechanism, which may be due to inhibition of apoptosis by accelerating the transition of cells in S phase to G2 or M phase.

b) Hydroxymethylfurfural against hypoxic damage

Oxygen is required for the cell to survive. Oxygen deficiency (hypoxic state) has many harmful and even life-changing effects on health. Hypoxia can be caused by many factors such as altitude and ischemia, spontaneous associated conditions such as atherosclerosis and cancer (Pattinson et al., 2005). Several cellular mechanisms are triggered, and among them can eliminate hypoxic conditions thought to involve extracellular signal regulated kinase (ERK) mediated transactivation and hypoxia inducible factors (HIF) of the transcription factor (Sang et al., 2003). Mitochondial membrane potential is also reduced and that adversely affects hypoxic cells (Iijima, 2006). In in vitro studies of ECV304 (human umbilical cord vascular endotinal cell) cell line, Li et al. proved that pre-treated cells by HMF (200 μg / ml for 1 hour) before exposure to hypoxic conditions (0.3% oxygen for 24 hours) demonstrated increased mitochondrial membrane potential and decreased phosphorylated ERK levels (Li et al., 2011). The number of apoptotic and necrotic cells also decreased significantly. In their subsequent studies with the Kunming mice model, the authors proved that prior exposure to HMF (100 μg / ml, 1 hour) significantly reduced the size of the traceability of the blood-brain barrier (BBB) caused by hypobaric hypoxia. Pre-expression also reduces the degree of neuronal damage in the CA1 region of the hippocampus. Therefore, HMF can be a potent therapeutic agent against acute mountain sickness (AMS), high altitude cerebral edema (HACE) and high altitude pulmonary edema (HAPE), since it increases survival under hypobaric hypoxic conditions (Li et al., 2011).

c) Hydroxymethylfurfural as an anti-allergen

Basophils and mast cells participate in the pathogenesis and symptoms of allergic reactions such as asthma, atopic dermatitis and allergic rhinitis. RBL-2H3 cells are mast cells located in the mucosal layer. These cells express their immunoglobulin Fc epsilon receptor type I (FcγRI) on their surface. Crosslinking of IgE with specific protein antigens and binding of crosslinked IgE to FcγRI triggers intracellular signal transduction cascades. These events lead to Ca^{2+} flow and release of mediators by degranulation, MAPK phosphorylation, up-regulation of cytokine gene expression, and increased ROS production (Kim et al., 2008). Yamada et al. proved that HMF acts at different stages to inhibit degranulation at doses of 0.01-0.30 μg / ml. In addition, HMF interferes with antigen-

antibody cross-linking and antibody-receptor binding (Yamada et al., 2011). HMF also inhibits the flow of calcium (Ca^{2+}) into RBL-2H3 cells stimulated with IgE sensitized bovine serum albumin. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase plays an important role in the production of ROS in IgE-mediated RBL-2H3 cells. It is known that H2O2 and NO, two major ROS, regulate degranulation and Ca^{2+} signal in mast cells (Kim et al., 2008). There is a significant inverse relationship between histamine and Ca^{2+} release from intracellular stores and superoxide anion or DPPH removal activities. The anti-allergenic effect of HMF on cells is due to the blocking of histamine release and Ca^{2+} signalling through the compound's free radical scavenging activity (Li et al., 2009; Suzuki et al., 2005).

In another study using BALB / c mice immunized with ovalbumin (OVA), HMF reduced total IgE and OVA-specific IgE levels. The study also proved that immunized mice treated with HMF showed lower levels of IFNy (Interferon gamma) and IL-4 (Interleukin 4) than untreated mice. Consequently, HMF can be a powerful anti-allergic compound (Alizadeh et al., 2017).

d) Use of hydroxymethylfurfural for other pathological conditions

Uric acid is the final product of purine catabolism. The last two stages of the purine catabolic pathway are catalyzed by a critical enzyme, xanthine oxidase (XO). Uric acid is mainly excreted in the urine. High levels of uric acid in the blood lead to the development of hyperuricemia, which is the main cause of gout (Pacher et al., 2006). Additionally, many other pathological conditions, such as metabolic syndrome, heart failure, pulmonary disorder, and type 2 diabetes mellitus, are associated with hyperuremia (Hayden and Tyagi 2004). Increased XO activity decreases anti-inflammatory transcription factor peroxisome proliferator-activated receptor-P (PPARγ) and accelerates the inflammatory effect (Gibbings et al. 2011). XO is the producer of an endogenous superoxide, which is also a potent nuclear factor kappa B (NF aB) activator (Lorne et al., 2008). NFκB acts as a transcription factor and regulates the expression of nitric oxide synthase 2 (NOS2) and interleukin 8 (IL-8). HMF regulates NFκB (Kitts et al., 2012), exerts an anti-inflammatory effect and inhibits the activity of XO (Lin et al., 2012).

e) Hydroxymethylfurfural as an anti-sickling agent

Hemoglobinopathies are life threatening as sickle cell disease. The underlying mechanism is polymerization of abnormal hemoglobin (sickle hemoglobin, HbS) under hypoxic conditions. Pathophysiology occurs with deformation of red blood cells, loss of resistance and blockage of small blood capillaries, which leads to morbidity (Goldberg et al., 1992). Although there are many anti-sickling agents, these agents do not have side effects. Many agents also reduce bioavailability at minimum doses and react to non-target proteins. In addition, HMF can function as an effective anti-reagent. Abdulmalik et al. proved that HMF orally administered at low doses are absorbed into the bloodstream from the gastrointestinal system in transgenic (Tg) sickle mice (Abdulmalik et al., 2005). HMF can then penetrate the erythrocytes and form a stable Schiff-based adduction symmetrically with the N terminal αV11 nitrogen of HbS. Following the formation of such an adduction, HMF shifts the oxygen balance curve allosterically to the left and prevents the erythrocytes from becoming sick. In fact, the authors also proved that HMF pre-treated Tg sickle mice survived longer than untreated mice under hypoxic conditions, and that HMF showed its potential as anti-reagent agent.

2.5 Tolerable Daily Intake of Hydroxymethylfurfural

Many studies have also proven that different cells induced by 5-HMF respond differently to cytotoxicity. The susceptibility of cells to HMF depends on the presence and expression levels of receptors (Ohmi et al., 1998), metabolism (Severin et al., 2010), structure (Safo et al., 2004) and enzyme activity of HMF (Shinohara et al., 1990). At the preclinical level, no toxic effects were observed between 80 and 100 mg / kg body weight at daily doses (Abraham et al., 2011). Zaitzev et al. proved TDI for HMF to be 132 mg / day by using a 40-fold safety margin (Zaitzev et al., 1975). The European Food Safety Authority (EFSA) 2005 attracted a concern of 0.54 mg / day for the intake of furan derivatives used as flavoring agents in Europe. However, in their study involving 268 Spanish school children, Pastoriza et al. found that the students had a statistically significant SMF level in their plasma, a strong toxic metabolite of HMF, although their daily HMF intake was 10-70 mg (Pastoriza et al., 2017). Moreover, most of the experiments on the effects of HMF on health were performed at the level of in vitro, ex vivo and experimental animals.

Therefore, it is not possible to determine a TDI based on the data obtained to date. Additionally, more research is worth considering and appreciated, especially at the clinical level, to pave the way for proposing a TDI for HMF.

2.6 Electrochemistry

Electrochemistry is defined as the branch of chemistry that examines the interrelation of electrical and chemical effects (Bart and Faulkner, 2001). Electrochemistry is concerned with electron transfer at the solution / electrode interface. In 1800, Alessandro Volta invented the first battery, known as the voltaic battery, consisting of copper and zinc discs separated by papers soaked in acidic solutions. As of 1835, Micheal Faraday defined the terms such as anode, cathode, electrode, electrolyte and ion, without a specific definition of electrochemistry. Many of the basic principles and correlations were defined before J.J Thomson discovered the electron in 1893 (Zoski, 2007).

The branch of science, which examines the interaction between matter and electrical energy, and the chemical and physical changes such as the conversion of chemical energy into physical energy, is defined as electrochemistry (Skoog et al., 1996). Electrochemical reactions are oxidation-reduction type reactions, electron transfer is in question, and are carried out in a cell called electrochemical cell.

When the solution to be analyzed is part of an electrochemical cell, the examination of a group of quantitative analytical methods based on the electrochemical properties of the solution falls within the scope of "electroanalytical chemistry." Electroanalytical techniques can reach very low detection limits, and they give information about systems in which electrochemical methods can be applied, characterizing too many systems.

Electroanalytical methods have some advantages over other analysis methods. First of all, electrochemical measurements are often specific for an element, molecule, or product-specific oxidation step formed at the end of the reaction. A second important advantage of electroanalytical methods is that the devices used are relatively inexpensive (Skoog et al., 1996).

In order for an electrochemical reaction to occur, a solution containing the substance under study, the electrode system where the substance is chemically transformed, and a conversion system that connects these electrodes are required. Buffer solution is used to provide electrical conductivity as a solution. Current intensity is measured by scanning in a certain potential range at direct current (DC), differential pulse (DPV), cyclic voltammetry (CV) and etc. with various electrolytic methods. It is the diffusion current measured here since the current is formed due to diffusion. Diffusion consists on the diffusion layer near the electrode surface, and the intensity of the current is directly proportional to the diffusion rate.

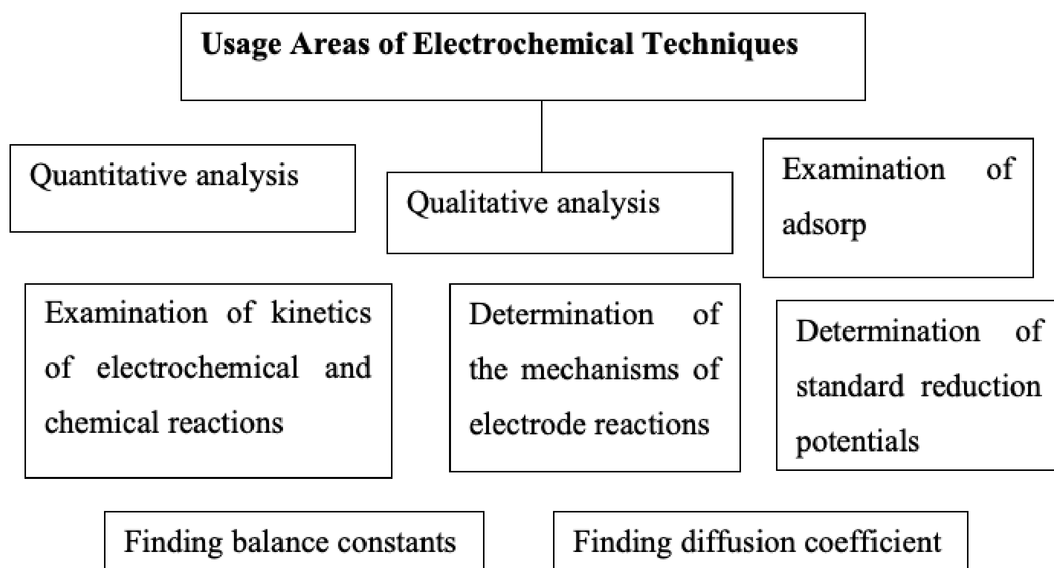


Figure 2. 5: Usage areas of Electrochemical Techniques

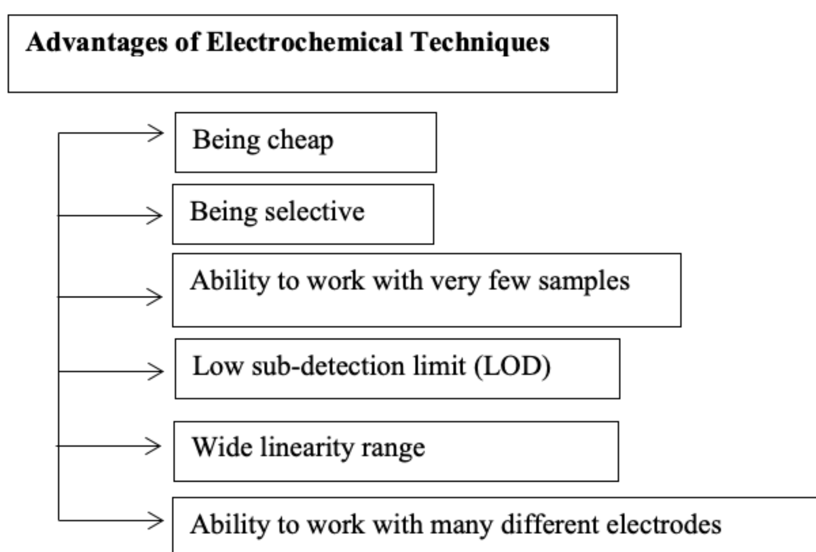


Figure 2. 6: Advantages of Electrochemical Techniques

2.7 Chemical Reactions

2.7.1 Oxidation reduction potential

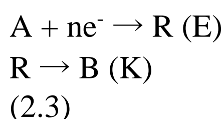
Oxidation reduction (redox) potential is an important physicochemical parameter that determines the oxidation or reduction tendencies of chemical or biochemical systems. A compound in the system gives electrons in the oxidation process. Reduction is defined as the reverse of this process. When a compound in a system is oxidized, another compound is reduced. The relationship between reduction and oxidation can be written as follows 2.2.

oxidized form + electron(s) $\leftrightarrow$ reduced form (2.2)

The oxidizing agent can be defined as the compound that receives electron(s) from another compound in a redox reaction, and the reducing agent is the compound that transfers electron(s) to another compound. While pH determines the acid-base characteristics of a solution, E_h determines its reduction and oxidation characteristics.

Products formed by electron transfer may not be stable. The unstable electroactive product turns into an intermediate product and then turns into the final product with the help of chemical reaction.

While few products are generally made up of organic materials with chemical steps, a product is generally made up of organic materials.
The reduction oxidation reaction is given below 2.3.



$A + ne^- \rightarrow R$ (E)
$R \rightarrow B$ (K)
(2.3)

The conversion of the intermediate product "R" to the final product "B" may occur in 3 ways:

- The intermediate product "R" can be converted to the substance "B" by homogeneous reaction in solution after distancing from its adsorbed electrode surface state,

- The intermediate product "R" can be converted to the substance "B" by the heterogeneous reaction of the substance "R" on its electrode surface,
- It can turn into “B” by reacting with another reagent in the medium.

In this type of reaction, the chemical step determines the reaction rate.

2.7.2 Adsorption controlled mechanisms

Adsorption is defined as the adsorption of molecules and ions in the solution to the surface of the electrode in different ways. As a result of adsorption, different types of bonds are formed between the ion and molecules adsorbed to the electrode surface and the electrode surface. Unexpected electrochemical behaviours are generally explained by the adsorption event. Adsorption of electroactive types causes a change in reaction kinetics and a decrease in reaction rate. This change is due to the reduction of the active surface that provides electron transfer and the occurrence of electron transfer away from the electrode surface.

Adsorption of electroactive substances leads to changes in the thermodynamics of electron transfer in the environment. Those types adsorbed to the electrode surface can be found as no-charge organic molecules, metal cations, and inorganic types.

The adsorption of anions increases as the positivity of the electrode increases, and the adsorption of uncharged organic molecules increases as the hydrophobic property increases (the nitrogen containing pi electrons and unbounded electron pairs in organic substances increases the adsorption of the molecule).

Uncharged organic molecules usually adsorb to uncharged electrodes. As the electrode surface is loaded with different charges, polar water molecules interact with the electrode and the uncharged organic molecules in the environment are replaced by water molecules (Zorluoğlu 2012).

2.8 Electrochemical Layers

When performing electrochemical measurements, heterogeneous layers form between the electrode surface and the sample solution to be analyzed. This is due to the fact that the electrode can supply electrons to a type adsorbed to it in the solution layer, or it can take electrons from that layer. In general, the composition of heterogeneous layers in mixed systems is shown in Figure 2.7.

Turbulent flow layer: Observed in the solution group away from the electrode.

Laminar flow zone: When it approaches the surface, there is a transition to a laminar flow. In laminar flow, the liquid layers slide over each other in a direction parallel to the electrode surface.

Nernst diffusion layer: The velocity of the laminar flow approaches zero due to friction between the liquid and the electrode at δ cm away from the electrode surface, resulting in a thin, stagnant solution layer around the electrode.

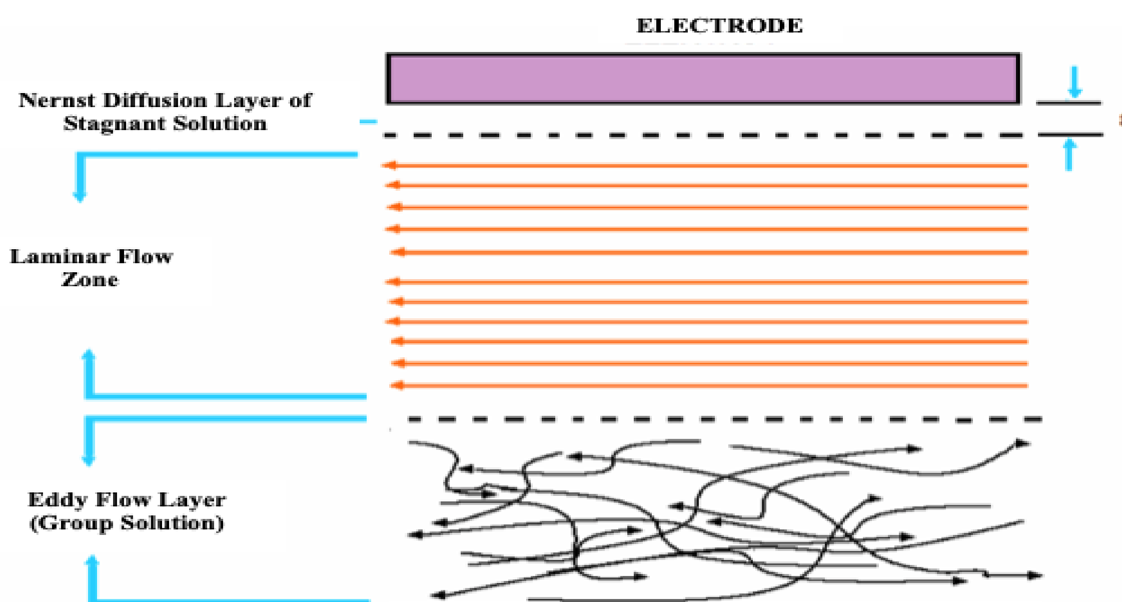


Figure 2. 7: Schematic representation of the layers on the electrode surface.

2.8.1 Electrical examination of electrochemical layers

A momentary current wave will occur which will quickly drop to zero immediately after a positive potential is applied to the electrode if there is no active types that can react on the surface of the electrode. This current is a charging current that creates a negative charge surplus (or deficiency) on the surface of both electrodes. However, as a result of ionic mobility, an opposite charge occurs immediately in the solution layers adjacent to the electrodes. As a result of the positive potential applied, positive charge excess occurred on the surface of the electrode. The charged solution layer consists of two parts:

1) a dense inner layer decreases in direct proportion to the potential distance that occurs as distanced from the electrode surface on this layer,
2) a diffuse layer decreases exponentially as distanced from the electrode surface here. This group of charges on the electrode surface and adjacent solution is called an *electric double layer*.

2.8.2 The channels of mass transfer in an electrochemical event

During the operation of an electrochemical cell, the channel of transferring the material to the electrode surface takes place in three ways (Yıldız & Genç, 1993). The channels of mass transfer are:

Electrical migration (migration): This is a transmission channel created by the effect of the electrical field.

Convection (convection): This is the mass transfer channel formed as a result of convection or vibration.

Diffusion: This is a mass transfer channel caused by the concentration differences between the fluid film on the electrode surface and the solution. One or more of them may contribute to mass transfer depending on the experimental conditions.

2.9 Electroanalytical Methods

A wide variety of electroanalytical methods are suggested. The most common of these are shown below. These methods are divided into two as the methods that take place at the interface and the methods that take place in the whole analysis environment.

The methods performed on the interfaces have a more general use. Interface methods are based on events occurring at the interface between the electrode surfaces and the thin solution layer immediately adjacent to these surfaces. It is based on events occurring in the entire solution unlike all analysis environment methods and every way is used to avoid interface effects.

Interface methods are divided into two main classes as static and dynamic according to the functioning of electrochemical cells in the presence or absence of current.

2.9.1 Voltammetry and principles

Electrochemical method based on measuring current as a function of the voltage applied to the electrode is called voltammetry (Skoog et al., 1996). The graph of the applied voltage against the measured current values is called voltamogram. In voltammetry, the limits of the voltage range that can be applied to the electrode to study the electrochemical behavior of any substance depend on the working electrode used and the types of solvent and electrolyte used.

Historically, voltammetry was developed based on the polarography technique, a special type of voltammetry, developed by the Czechoslovakian chemist Jaroslav Heyrovsky in the early 1920s (Skoog et al., 1996). The biggest difference of polarography, which is still an important field of voltammetry, from other voltammetric techniques is the use of a drip mercury electrode as the working electrode.

Voltammetry is also widely used by inorganic, physico and biochemists for non-analytical purposes, such as the investigation of oxidation and reduction processes occurring in

various environments, investigation of adsorption processes on the surface, and elucidation of electron transfer mechanisms occurring on chemically modified electrode surfaces.

2.9.2 Actuating signals used in voltammetry

Signals with changeable potential are applied to the electrochemical cell. These actuating signals form characteristic current responses (Skoog et al., 1996). The four most used excitation signals in voltammetry are the differential pulse, square wave and triangular wave.

2.9.3 Voltammetric devices

The system used in voltammetric analysis was prepared by connecting electrochemical cell, substance to be analyzed and the system consisting of three electrodes immersed in a solution called support electrolyte to the potentiostat.
The triple electrode system consists of a working electrode, a reference electrode and an auxiliary electrode.

Working electrode
It is the electrode on whose surface the substance to be analyzed is oxidized or reduced. In biosensor design, it may vary depending on the purpose of the study.

Reference electrode
The reference electrode is an electrode, the potential of which remains constant throughout the experiment. The Ag/AgCl reference electrode or saturated calomel electrode (SCE) can be used.

Auxiliary electrode
It is a counter-electrode, which is in the form of a pt-wire or a pool of mercury and allows electricity to be transferred from the solution to the working electrode. This is an electrode which forms a pair with the working electrode, but does not play a role in determining the magnitude of the measured potential.

2.9.4 Reference electrodes used in voltammetry

These are the electrodes that are insensitive to the composition of the solution studied and whose potential is not affected by the external environment during electrochemical studies (Yıldız & Genç, 1993).

In Hydrochemistry, the Standard Hydrogen Electrode (SHE) was first used as a reference electrode. However, it is not very common to use as it is a difficult electrode to prepare.
The reference electrode types used are as follows;

a) Calomel reference electrode

It consists of a mixture of calomel (Hg_2Cl_2) and metallic mercury and KCl solution. The potential of this electrode depends on the activity of chloride ions. It is very easy to prepare.

The most common one and including saturated KCl solution in it is the Saturated Calomel Electrode. Its potential was found to be + 0,244 V at 25^0C compared to the Standard Hydrogen electrode (SHE). Its temperature coefficient is higher compared to other calomel electrodes.

b) Silver-silver chloride reference electrode

The silver-silver chloride reference electrode, one of the most commonly used reference electrodes, is obtained by coating a silver wire electrolytically with AgCl and immersing it in a solution containing chloride ion. When saturated KCl solution is used, its potential is +0,222 V compared to the standard hydrogen electrode.

c) Mercury-mercury sulphate reference electrode

This electrode is similar to the saturated calomel electrode. Its potential is determined by the activity of sulphate ions.

A reference electrode should be easily prepared, variation coefficient of potential with temperature should be small, it should act reversibly in a certain current range, in other words, its voltage must remain constant even when small currents flow through it. It

should be a non-polarized electrode, its potential should not change over time, and it should read a correct and repeatable potential value quickly.

2.9.5 Working electrodes used in voltammetry

The conductive material used in the production of the working electrode may be an inert metal like platinum or gold; carbon, pyrolytic graphite or glassy carbon; a semiconductor such as tin oxide or indium oxide or a metal coated with a mercury film. These electrodes can be of various shapes and sizes and are optimally developed for biosensor design.

It is very important to determine the potential range in which such electrodes are used. In particular, this potential range varies depending on not only the electrode material in aqueous solutions, but also the composition of the solution into which these electrodes are immersed. The positive potential limits are usually determined by the large currents that occur at the end of the oxidation of water, in a way to give molecular oxygen. Negative potential limits also arise from hydrogen formed as a result of water reduction.

a) Carbon electrodes

Carbon electrodes are frequently used in electrochemical analysis, especially since they are very cheap and allow working in a wide potential range. However, carbon has a high surface activity and is therefore easily contaminated by organic compounds. Hydrogen, hydroxyl and carboxyl groups and even quinones can form bonds on the carbon surface. Due to the presence of these functional groups, many different substances can be adsorbed to the carbon surface.

It is necessary to properly prepare the surfaces of all types of carbon electrodes, which are very important in the field of electrochemistry. Types of carbon electrodes:

Carbon paste electrode (CPE)

The arrangement of carbon molecules in graphite powder into planar and aromatic rings is shown in Figure 2.8. A fast electron exchange can occur between these layers, which are connected by weak π bonds. Figure 2.9 is an overview of a carbon paste electrode.

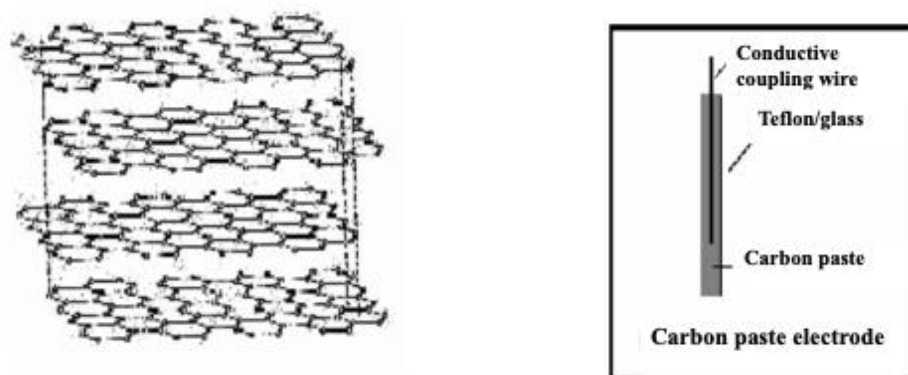


Figure 2. 8: Arrangement of carbon paste molecules

CPE is preferred due to the fact that it is inexpensive, that its surface renewal is easy, and that it creates low residual currents. As binders, Nujol (mineral oil), paraffin oil, silicone oil and bromonaftalene are used. Paste composition has a great effect on electrode activity. As the binding organic liquid ratio increases, the electron transfer rate decreases. As a major disadvantage, the carbon paste can be dispersed in the solution when the CPE is immersed in a solution containing organic matter (Cai et al., 1996).

Screen printed carbon (Graphite) Electrodes (SPCE)

In recent years, single-use screen printed carbon electrodes have been widely used. These electrodes, which give very successful results especially in terms of their applicability to DNA microchip technology, the future of DNA biosensor technology, are shown as the electrodes of future (Carpini et al., 2004). Besides their advantages such as having good repeatability and using very low amounts at 20-40 µL, these disposable electrodes have disadvantages such as long fabrication time and high cost compared to other electrodes.

Pencil graphite electrode (PGE)

While cleaning the surface of other solid electrodes between measurements, the PGE surfaces do not need to be cleaned, thus provides saving considerable time in PGE-related studies since it does not include long-term cleaning and polishing processes.

PGEs have many superior features, such as high precision, less ground current and good repeatability, good mechanical stiffness, disposability, ease of modification, wide potential range, commercial availability, low technology, low cost, easy accessibility and well-defined porous surface area. Commercially available pencil tips are available in a variety

of hardness and structures, depending on the content of graphite and clay. Therefore, pencil tips containing different amounts of graphite and clay have sp^2 hybridized carbons to facilitate surface modification due to graphite and allow easy modification of monometallic, bimetallic, hybrid nanostructures and polymeric structures to these surfaces (Gördük et al., 2018; Jesus et al., 2018 ; Nacef et al., 2019; David et al., 2017; Oriňaková et al., 2019; Pundir et al., 2019; Rosales et al., 2018; Ertürk and Keskin, 2018; Teoman et al., 2019; Karakaya and Dilgin, 2017; Ayaz and Dilgin, 2017; Emir and Dilgin, 2018; Aziz and Kawde, 2013a; Dilgin G.D and Karakaya, 2016; Dilgin et al., 2013; Dilgin et al., 2012a ; Dilgin et al., 2012b; Dilgin et al., 2012c).

b) Metal electrodes

Platinum and gold are the most preferred types of electrodes. These electrodes have high electron transfer kinetics and a wide range of positive potential ranges (Carpini et al., 2004).

2.9.6 Voltammetric currents

When voltage is applied to the electrode system, two types of currents are formed, capacitive current and Faradaic current.

Capacitive current (i_c)

Positively charged ions in the solution are drawn towards the electrode by immersing an electrode in an electrolyte solution and charging with a negative charge. Thus, a voltage difference occurs at the interface. An electrical double layer is formed in this area by the accumulation of loads with reverse signs on both sides of the interface. This double layer formed acts like a capacitor. A current is created to load this capacitor even if there is no substance to be oxidized or reduced. This current is not reaction dependent; it originates from the system, which is called capacitive current. The lower it is, the more precision measurement it makes. Capacitive current is one of the factors that cause funds current to form.

Faradaic current (i_f)

It is the current arising from the reaction (from the substance to be analyzed).

Since $i = i_f + i_c$, the precision increases if the i_c decreases $i= i_f+i_c$, the precision increases if the i_c decreases.

Usually 10^{-3} M and above; $i_C << i_f$ and can be studied. Partially good results are obtained at 10^{-4}. It cannot be studied at 10^{-5} and above since $i_c >> i_f$.

2.9.7 Faradaic processes in an electrochemical event

During the transmission of current from the surface between the solution and the electrode, oxidation reactions occur in one of the electrodes, while a reduction reaction occurs in the other. In these reactions 2.4;

$$O+ne^{-} \leftrightarrow R \quad (2.4)$$

It is shown by the reaction that O and R express oxidized and reduced form of redox pair respectively.

During the redox reaction occurring at the electrode interface, the current is transmitted by direct transfer of electrons. These types of processes are called *faradaic processes*, which state that the amount of chemical substance in an electrode is directly proportional to the current passing, and currents formed in this way are called *faradaic currents*.

Concentrations of substances and products to be analyzed only change as a function of distance from the electrode surface and within the Nerst layer.

2.9.8 Voltammetric techniques

a) Cyclic voltammetry

With this technique, current is measured as a function of voltage. Cyclic voltamogram is obtained by graphing the change in the current in a certain range against the constantly changing potential values. Cyclic voltamogram is studied at stationary system with triple electrode system. Diffusion determines the velocity here. Oxidation and reduction of the analyte can be observed on the voltamogram. First, it increases up to a potential maximum, then reverts back to the initial value linearly.

As in direct current, it is studied in the region where the capacitive current is smallest. Precision is limited to 10^{-5}M. Cyclic voltammetry is not a suitable method for quantification, however, it gives information about at what potential and how the substance to be analyzed behaves.

Besides the chosen potential range, the selected scanning speed and the number of scans are also influenced in the form and structure of cyclic voltamograms.

The voltage difference between reduction and oxidation in a cyclic voltamogram is expressed by ΔEp. Formula as shown in 2.5.

$$\Delta E_p = 57\ mV\ n \qquad (2.5)$$

The closer ΔE_p to this value, then the reaction is called reversible; the farther ΔE_p to this value, then the reaction is called irreversible.

b) Differential pulse voltammetry (DPV)

With this technique, peak maxima can be obtained in different positions, even for substances with different half-wave potentials of 0.04 - 0.05 V. Differential pulse polarography is a very sensitive method and its detection limit is between 10^{-7}-10^{-8} M.

A pulse mercury drop of 10 mV or 50 mV is applied. Before and after the applied pulse, the difference in the current obtained per pulse is recorded as a function of the linearly increasing potential. The observed differential curve is in the form of a peak and its height is directly proportional to the concentration.

The high value of the Faradaic current and the low value of the non-Faradaic current can be explained by the increased sensitivity. For example, if there is an electroactive type in the surface layer surrounding the electrode when the potential is suddenly increased by 50 mV, a current increase is observed which will reduce the concentration of substance to be analyzed to the level desired by the new potential. However, once the equilibrium concentration required for this potential is reached, the current drops to a level that will meet the diffusion, which is called *diffusion-controlled current*. In pulse polarography,

current measurement is made before this current increase is completely over. The total flow is greater than the diffusion flow. When the drop changes, the solution becomes homogeneous in terms of the substance to be re-analyzed.

When the voltage pulse is first applied, there is a fluctuation in the non-faradic current due to the load increase on the drop. This current decreases over time and the drop, where the surface area changes little, approaches zero towards the end of its life. Therefore, the residual non-fluorescent current is greatly reduced by measuring the current at this time and the signal/noise ratio increases. As a result, sensitivity increases as well.

CHAPTER 3

RELATED RESEARCH

There are various researches about electrochemical determination of hmf some of researches (Table 3.1) are summarized below:

In addition to being present in honey, HMF is released from dried fruits (> 1 g / kg), caramel, instant coffee (up to 6.2 g / kg), apple juice, citrus juices, beer, brandy, milk, cereal for breakfast, baked foods and tomato products and from sugar and carbohydrates after cooking at home, and HMF is found everywhere in the diet.

In our daily life, we consume many different food products including bakery products, milk, juice, cereals, coffee, chocolate, soft drinks, vinegar, wine, nuts and grilled meat. Most of these products are subjected to thermal treatments such as boiling, cooking, roasting, pasteurization and other processes before consumption. These processes are carried out not only to make the products more edible but also to protect the products (by reducing microbial load and / or eliminating enzymatic activities) and to develop the desired sensory (color, flavour and taste), texturizing properties. However, some unwanted harmful compounds may come out as a result of these processes, and can create negative effects by reducing the nutritional value, fresh appearance and taste. During the thermal treatment and protection, Maillard reaction or non-enzymatic reddening may also occur, where HMF is a product whose degree of formation depends on processing and protection conditions (Kowalski et al. 2013).

3.1 Sugar

Sugar is a disaccharide consisting of fructose and glucose. It is produced only from sugar beet and sugar cane. Although its extraction and purification processes are very simple, HMF occurs as a result of heat. Polovkova and Simko analyzed brown (n = 25) and white sugars (n = 13) from local markets in the Bratislava region, the capital of the Slovak

Republic (Polovkova and Simko, 2017). Upon the preparation of sugar samples, HMF levels were determined by high performance liquid chromatography combined with a diode array detector (HPLC-DAD) at 284 nm. Surprisingly, white sugar did not contain HMF, but brown sugar contained HMF (0.17-6.45 mg / kg). The presence of HMF in brown sugar can result from the addition of deception to the preparation stored at 50 ° C to maintain its liquidity. Similarly, Risner et al. found similar HMF concentration ranges (11.9-16.4 and 12.3-23.3 mg / kg) respectively for light and dark brown sugars by using HPLC (Risner et al., 2006).

3.2 Cereals

According to Norwegian and German researchers, cereals and cereal products, including bread, are some of the most important sources of human exposure to HMF (Abraham et al. 2011; Husoy et al., 2008). The degree of HMF formation in cereal products depends on many factors including temperature, dough fermentation process, water activity and fruits, cereals and other aroma or additives (such as cocoa, malt, sucrose, glucose, salt).

Mańkowska et al. made a research on HMF concentrations of 41 food products. It has been stated that cranberry wheat bread contains the highest amount of HMF (210 mg / kg) followed by breakfast cereals, that is, honey wheat loops (whole wheat bread) (85.09 mg / kg) (Mańkowska et al., 2017). The lowest amount of HMF has been reported to occur in gluten-free sponge cakes and whole grain oatmeal. Flavoured breakfast cereals contained HMF at 25.55 mg / kg, which is higher than the average HMF concentration (18.40 mg / kg) in bakery products. In another study, mixed cereals (240 mg / kg), corn flakes (7-114 mg / kg) and wheat-based cereals (6-132 mg / kg) were found to have high HMF concentrations in average grains (Rufian and Cueva, 2008). It has been underlined that the dough has been particularly affected by the fermentation process and flour type. HMF concentrations were examined in different types of flour, including wheat, whole wheat, bread flour, and rye flour. The HMF concentration in bread made with rye flour was determined to be the highest (average 26.88 mg / kg) due to its high amino acid content. Cereal products with high HMF concentrations are added to dried fruit types (raisins, cranberries, palms, strawberries, red currants and apples). On the other hand, red fruits

(47.62 mg / kg), such as apples, strawberries and red currants added to oatmeal, give the highest concentrations of HMF (Mańkowska et al., 2017). Similarly, bread with dried fruits has a higher HMF concentration than white bread without dried fruits (Ramirez et al., 2000; Serpen et al., 2012). This proves that dried fruits affect HMF concentration.

3.3 Coffee

Coffee is one of the most common beverages reported to contain HMF. The concentration of HMF in coffee depends on brewing processes or the types of coffee used (mocha, espresso, filtered coffee or sink brewed coffee) and the amount of sugar added to it. Mortas et al. researched the HMF concentrations (prepared traditionally or with momentary variety) in Turkish traditional fees using HPLC combined with a diode array detector (Mortas et al., 2017). The authors argued that ready-made and traditional Turkish coffee samples contain HMF in the range of 336.03-362.05 and 213.02-238.99 mg / kg, respectively, before brewing. However, after brewing, the HMF concentration increased by 32.29-55.83% (instant coffee) and 74.12-224.75% (traditional coffee), respectively.

Arribas-Lorenzo and Morales used reverse phase HPLC with UV detection to detect HMF concentrations in three ground coffee consumed by the Spanish, and found significant differences in the observed concentrations (Arribas and Morales, 2010). HMF levels are 110 mg / kg (natural coffee: coffee beans are produced by traditional roasting), 625 mg / kg (torrefecto coffee: obtained by adding sucrose before roasting) and 1734 mg / kg (blended coffee: natural and torrefacto ground coffee in various proportions). However, soluble coffee contained the highest level (2480 mg / kg). The authors concluded that the daily HMF intake of a high coffee consumer in Spain was about 122.42 µg / kg, which showed that HMF is generated by heat.

3.4 Dairy Products

HMF occurs as a result of side reactions during heat sterilization and browning processes. Albala-Hurtado et al. examined the formation of HMF during powder storage (up to 9 months) in powdered milk and liquid milk at different storage temperatures (20, 30, 37 ° C). They found that HMF formation followed a zero-grade kinetic profile regardless of storage temperature and milk type (Albala-Hurtado et al., 1998). A sample of powdered baby milk (31.5 µmol / L) stored at 37 ° C for 12 months contained greater amounts of HMF and furfural compounds than those found in liquid milk (2.5 µmol / L).

In another study of ultra-high temperature processed (UHT) milk, no significant change was observed in HMF levels of samples stored at 4 and 8 ° C. However, storing UHT milk at room temperature caused two times more HMF formation (Cais-Sokolińska et al., 2004). When it comes to traditional Indian dairy products, there was a strong positive correlation between the HMF concentration and the background, colors and textures of the products (Aktar et al., 1999).

3.5 Fruit and Vegetables

Due to their rich sugar and amino acid content, fruit and vegetables contain high HMF levels. In a study, a relationship has been established between the storage time and the formation of temperature-dependent HMF of the strawberry products stored at 20 and 35 ° C for 12 months (commercially and under laboratory conditions) (Rada et al., 2004). Furthermore, a positive correlation was observed between storage time and temperature and HMF formation in two apple juice varieties (Golden amasia and delicious (Burdurlu and Karadeniz, 2003). Ordóñez-Santos et al. stated in a study investigating changes in HMF levels in bottled tomato puree stored at 20 ° C for 180 days that there is a negative correlation between HMF formation and the content of organic acids such as ascorbic, citric and malic acids (Ordóñez-Santos et al., 2009).
Murkovic and Pichler analyzed HMF concentrations in dried apricots, peaches, pears, figs, dates, apples and pineapple products. As a result, HMF concentrations were highest (1000

mg/kg) and it was plum (1100–2200 mg/kg) (Murkovic and Pichler, 2006). The average range of HMF concentrations in other dried fruit is 1-780 mg/kg.
The oil concentration in the products can also affect the HMF formation. Fallico et al. examined roasted lean hazelnuts and sucrose and / or hexanol lean hazelnuts products in different amounts in high fat ratios to examine this hypothesis. As a result, they observed that the lean hazelnut with sucrose contained the highest concentration of HMF (372 mg / kg) and the lean hazelnut with the sucrose contained the lowest concentration of HMF (33.5 mg / kg) (Fallico et al., 2003).

The authors concluded that HMF levels increased with the duration of the heat treatment applied to the products since the HMF concentration increased from 66.5 to 144.0 mg / kg in lean samples after prolonged roasting (between 30 and 60 minutes, respectively).

Table 3. 1: The place of some studies on HMF in the Literature

Sample	The amount of HMF	References
Honey	10.4–58.8 mg/kg	Zappala et al., 2005
Honey	0.6–31.3 mg/kg	Kuş, 2003
Baby food	0.4–65.5 mg / kg	Ramírez et al. 2003
Pasteurized milk	2.5 µmol/l	Morales et al., 2000
Milk powder	0.50 µg/g	Chen and Yan, 2009
Hard and soft brown cheese	0.08–0.21 mg/kg	Husoy et al., 2008
Bread	2.2–87.7 mg/kg	Ramírez et al., 2000-2001
Breakfast cereals	6.9–240.5 mg/kg	Rufian et al., 2006
Wheat biscuits	0.16 mg/kg	Husoy et al., 2008
kinds of biscuits	0.5–74.5 mg/kg	Ameur et al., 2007
Jams	0.03–0.08 mg/kg	Husoy et al., 2008
Biscuits containing chocolate and hazelnuts	0.07 mg/kg	Husoy et al., 2008
Raisins	5 mg/kg	Husoy et al., 2008
Instant coffee	91.3–3060 mg/kg	Husoy et al., 2008
Coffee	100–1900 mg/kg	Murkovic and Pichler, 2006
Ready-to-drink cappuccino with added sugar	1.72–143 mg/kg	Husoy et al., 2008
Ground coffee	262–547 mg/kg	Husoy et al., 2008
Beer	0.2–13.3 mg/kg	Husoy et al., 2008
Prune	237 mg/kg	Husoy et al., 2008
Canned peach	5.8 mg/kg	Husoy et al., 2008
Some dried fruits and caramel products	More than 1 g/kg	Rada et al., 2004
Fruit juice concentrates	0.5– 7.0 mg/kg	Kuş, 2003
Halva	49.0–173.3 mg/kg	Kuş, 2003
Pepper paste	0.7–11.0 mg/kg	Kuş, 2003
Tomato paste	6.7–62.0 mg/kg	Kuş, 2003
Powder sahlep	131.3 mg/kg	Doğan et al., 2008

3.6 Electrochemical Determination of Hydroxymethylfurfural (HMF) and Honey Samples

In the study of Reyes et al., A direct electrochemical method was developed for the determination of 3 different unprocessed honey samples. HMF presented a single well-defined reduction signal at -1100 mV versus Ag / AgCl. It has been verified that borate is the most suitable supporting electrolyte for determining the HMF content in honey as it allows better identification and selectivity of honey. Standard addition method was applied to honey samples. The equipment consisted of a Pedestal Tacussel Potentiostat model PRG5. A three-electrode system was used: a mercury drop electrode (MDE) was used as the working electrode; an Ag / AgCl redox system was used as the reference electrode; and a Pt wire was used as the auxiliary electrode.

Example 1 shows the polarogram (Figure 3.1), a lower current density for HMF (2120 nA) and a higher current density (2150 nA) for the second signal. However, the current density for Sample 2 was higher for HMF (2190nA) than for the second signal (2110nA). For example 3, the second signal was not recorded and the HMF intensity value was much higher (21200nA) than for the other samples (Figure 3.2). This showed that as the HMF signal increased, the second signal decreased or disappeared completely. Both samples with low HMF content (Examples 1 and 2) had a second reduction signal that decreased in intensity as the HMF content increased and eventually disappeared. This signal may be due to enzymes (diastase or invertase) in honey and content levels can be defined as the opposite of HMF (Reyes et al., 2006).

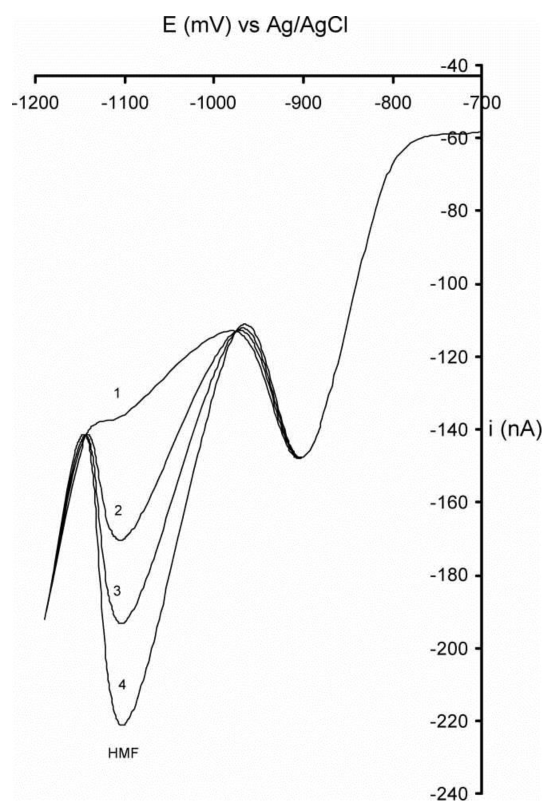


Figure 3. 1: Sample 1 polarograms using DPP and standard addition method.

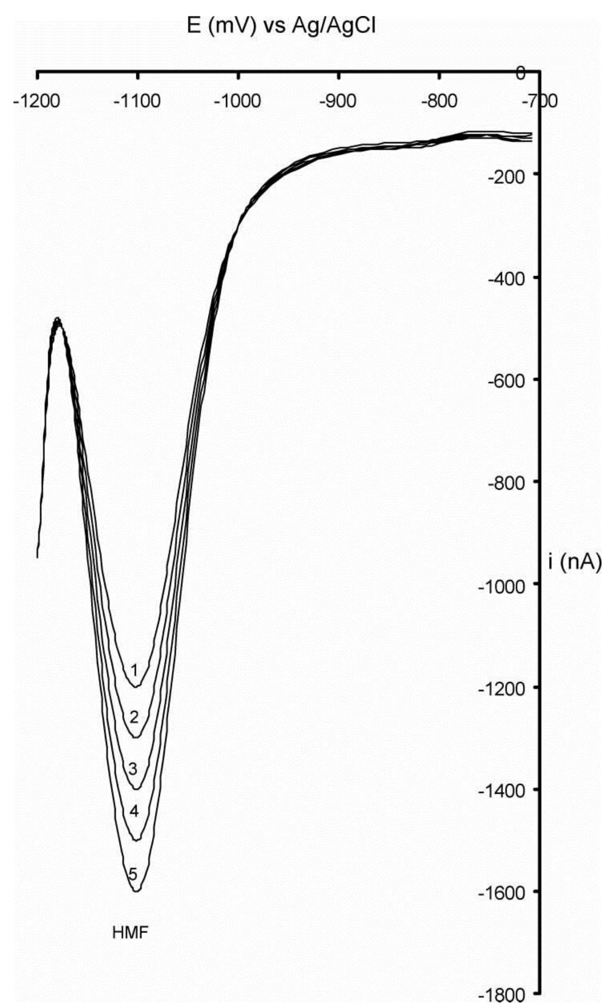


Figure 3. 2: Sample 3 polarograms using DPP and standard addition method.

CHAPTER 4

MATERIALS AND METHODS

4.1 Materials

4.1.1 Honey and pekmez samples

In this research, 2 of the honey samples with high HMF content, which were resulted in the State Laboratory and recently published in the newspaper, were selected from the market, the other 2 honey samples, a total of 4 different honey samples were randomly selected from the market.

One homemade carob pekmez was prepared from ground carob-water mixture in traditional method at home by boiling for 90 minutes, and then the puree was filtered from cheese cloth and then boiled again after adding some sugar to get desirable consistency. This home-made carob pekmez was used for the HMF analysis.

4.1.2 Chemicals

a) HMF standard preparation

The HMF standard was supplied by SIGMA and was used for the preparation of stock solution and calibration solutions. 1000 mg / mL the stock solution was prepared by disolving with 0.5 M acetate buffer.

b) Preparation of buffer solutions

Acetate buffer solution (ABS) 0.5 M

Acetate buffer solution is prepared by measuring 14.45 ml of concentrated glacial acetic acid and complete to 250 ml with distilled water. Weighed 10 g of 1 M NaOH (Sodium Hydroxide), completed it with 250 ml of distilled water and mixed it and then took it to the balloon joje. We completed the balloon with 250 ml distilled water. After weighing 0.84 g

0.02M NaCl (sodium chloride) and mixing it with acetic acid beaker, NaOH is added to the beaker until it reaches pH 4.8.

Phosphate buffer solution (PBS) 0.1 M

1.3608 g of Monobasic potassium phosphate (KH_2PO_4) (136.09g) was dissolved by adding pure water to 100 ml balloon joje. On the other hand, 4.3545 g Dibasic potassium phosphate (K_2HPO_4) (174.18 g) was dissolved by adding pure water to the 250 ml balloon joje. It is prepared by mixing 6 ml KH_2PO4 and 94 ml K_2HPO_4 into the balloon joje with the stocks we have. The pH of the solution is 8.0.

4.1.3 Equipment

a) Autolab potentiostat

All electrochemical differential pulse voltammetric measurements were made using the potentiostat AUTOLAB PGSTAT 204 (Utrecht, The Netherlands) with three electrode systems. Pencil graphite electrode was used as working electrode, Ag / AgCl (silver / silver chloride) as reference electrode and platinum wire as auxiliary electrode. Pencil graphite electrode Rotring Tikky brand pencil, 0.5 HB is used as a tip. Silver wire as Ag / AgCl was obtained by immersion in a solution containing chloride ion by electrolitically coating with AgCl. With the NOVA 2.12 software, the Potentiostat-galvanostat is connected to an ACER desktop computer for electrochemical measurements and data processing (Figure 4.1). All the experiments were carried out at a constant temperature of 25 ◦C.

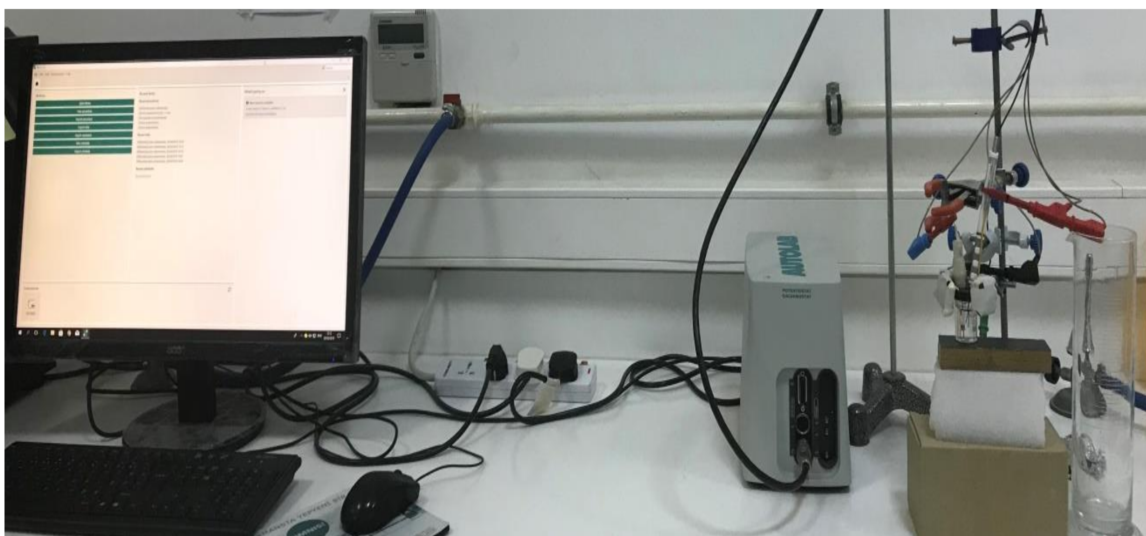


Figure 4. 1: Electrodes connecting with Nova2.12

4.2 Methods

4.2.1 Chrono amperometry

Graphite pencil leads were cut in 3 cm length and 1.5 cm of pencil lead placed into the pencil which was used as a holder for each graphite lead. Chrono Amperometry was applied to bare PGE by applying +1.4V for 30 seconds in 0.5M acetate buffer solution (ABS) for activation and cleaning its surface.

4.2.2 Electrochemical activation of pencil graphite

Graphite pencil tips 0.5 HB 3 cm long were placed in the Rotring Tikky model pencil used as a holder for graphic lead (Figure 4.2). The pencil graphite electrode pencil tip was attached to the metal top of the holders to the metal wire device. One end of the pencil tip was connected to a copper wire for electrical contact, and a 1.5 cm pencil graphite tip was inserted into the solution to be analyzed while holding the holder upright to prevent a short circuit during analysis.

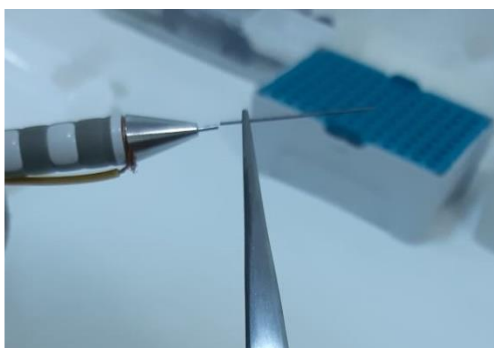


Figure 4. 2: Replacing leads into pencil to form PGE

4.2.3 Cyclic voltammetric (CV) and differential pulse voltammetric (DPV) measurements

The electrochemical behavior of HMF was investigated by using cyclic voltammetry in negative and positive region in acetate buffer. Cyclicvoltammetry (CV) measurements were performed with upper vertex potential of 1.5V, lower vertex potential of -1.5V, 0.005 V step potential, 10 mV/s scan rate.

Based on CV results, HMF calibration standards, various honey and pekmez samples were studied using differential pulse voltammetry techniques in acetate buffer system. Differential pulse voltammograms was performed with a potential range from 0 to +1.2 V, with 0.005 V step potential, 10 mV/s scan rate.

4.2.4 HMF analysis by immersion method

The HMF standard obtained from the prepared stock solution was analyzed by dipping analytes prepared according to the desired concentration. HMF sensitivity was determined by measuring peak current from calibration curves.

4.2.5 HMF analysis by immersion and holding method

Pencil tips were kept in Eppendorf tubes with diluted hmf concentrations of 200µl for 20 minutes (Figure 4.3), then dried for 20 minutes to proceed under differential pulse voltammetry.

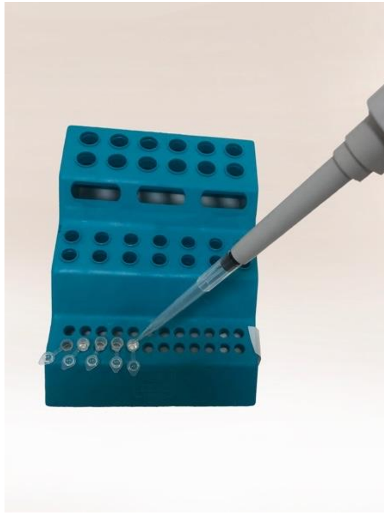


Figure 4. 3: Pencil tip kept in Eppendrof tubes

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Selection Buffer Solution

In order to determine the electrochemical signal of HMF, firstly, the selection of the appropriate buffer is aimed. For this, 0.5 M acetate buffer and 0.5 M phosphate buffer were used. As a result of the experiments, it was observed that an identifiable peak was given in the acetate buffer as a buffer solution (Figure 5.1, Figure 5.2) (Table 5.1).

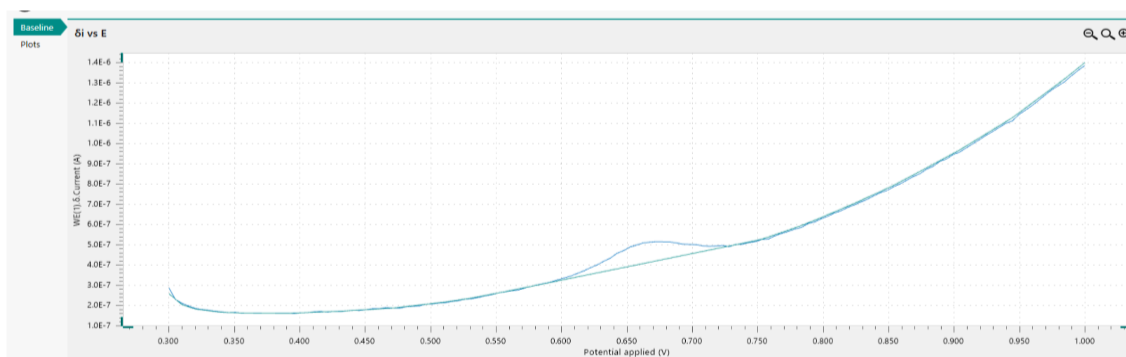


Figure 5. 1: Electrochemical signal of HMF in the presence of 0.5 M acetate buffer

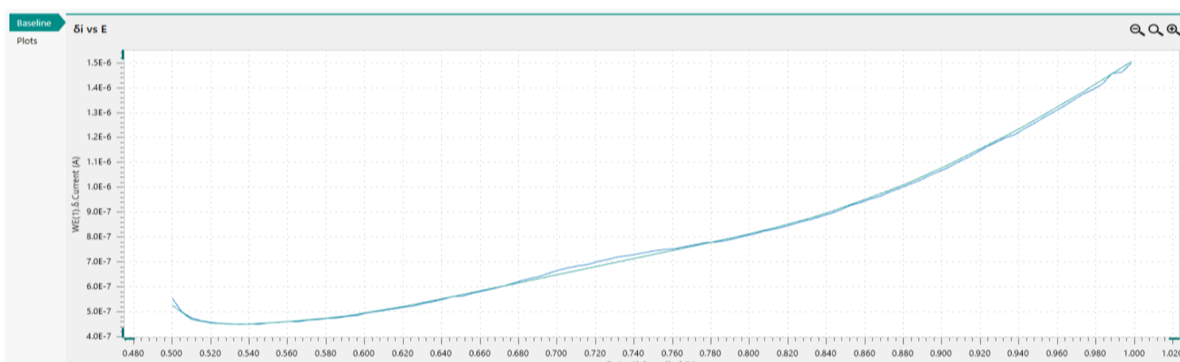


Figure 5. 2: Electrochemical signal of HMF in the presence of 0.5 M phosphate buffer

Table 5. 1: Applied buffer solutions and electrochemical results

		Peak Position	Peak Height (x10^{-8})	Peak Area (x10^{-8})
0.5 M Acetat Buffer	1. TEST	0,663	10,93	0,808
	2. TEST	0,658	3,638	0,271
	3. TEST	0,658	9,916	0,630
0.5 M Phosphate Buffer	1. TEST	0,720	4,853	0,365
	2. TEST	0,686	13,28	1,156
	3. TEST	0,731	2,457	0,170

5.2 Direct Cyclic Voltammetric Measurements of HMF

The electrochemical behavior of HMF was investigated by using cyclic voltammetry covering negative and positive region from – 1.5 V to 1.5 V in acetate buffer. With reagent blank (ACB), there was a wide reduction peak at – 0.8 V due to dissolved oxygen (Figure 5.3) and this peak was also observed in the analysis of HMF standard of 20 ppm (Figure 5.4). No any related peak to HMF could not be observed in negative region as mentioned in a study of Reyes et al. (2006).

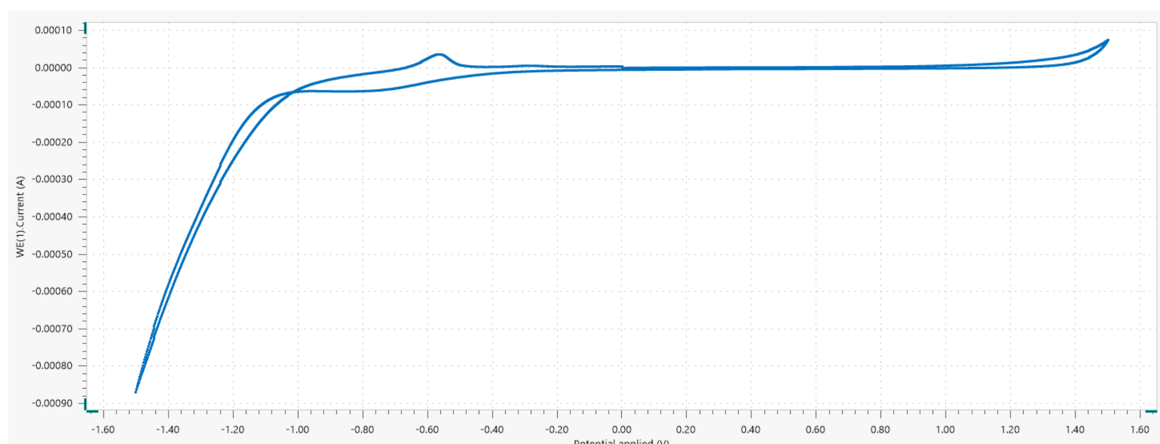


Figure 5. 3: Cyclic voltammogram of reagent blank (ACB)

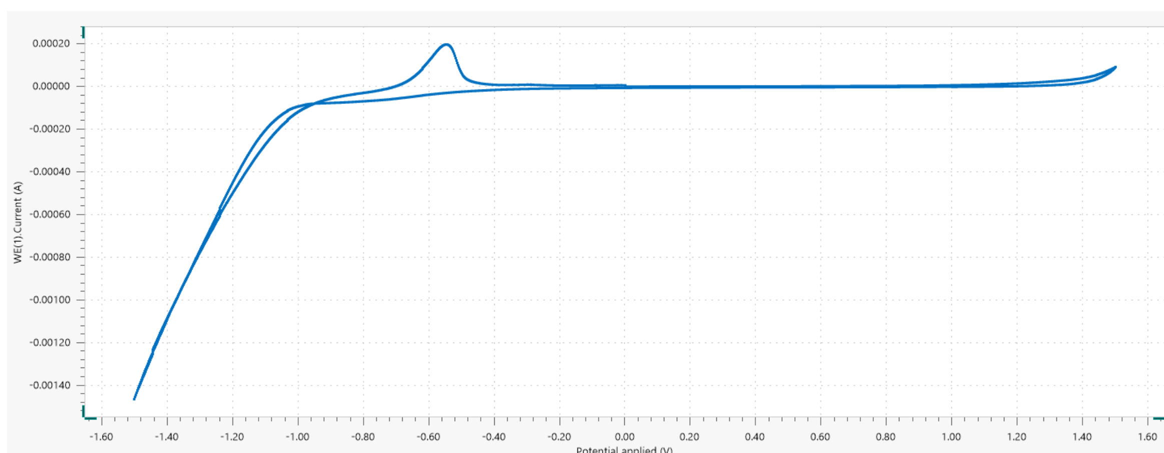


Figure 5. 4: Cyclic voltammogram of HMF standard of 20 ppm

5.3 Calibration Experiments of HMF Using Immersion Method

The standard graphic was drawn by immersion method with dilutions in the range of 10, 50, 100, 200, 400, 800, 1000 ppm from 1000 mg / mL HMF stock solution (Table5.2, Figure 5.3).

The reason we chose the immersion method is that we got accurate results in a shorter time.

Table 5. 2: Electrochemical results of concentrations of 10, 50, 100, 200, 400, 800, 1000pmm

Concentrations		Peak Position	Peak Height ($x10^{-8}$)	Peak Area ($x10^{-8}$)
10 ppm	1. TEST	0,703	2,357	0,129
	2. TEST	0,708	2,991	0,123
	3. TEST	0,698	3,680	0,219
50 ppm	1. TEST	0,703	2,708	0,162
	2. TEST	0,698	3,220	0,163
	3. TEST	0,710	1,191	
100 ppm	1. TEST	0,698	2,121	0,065
	2. TEST	0,683	2,864	0,176
	3. TEST	0,713	2,583	0,159
200 ppm	1. TEST	0,718	3,605	0,231
	2. TEST	0,698	4,193	0,236
	3. TEST	0,698	3,190	0,135
	4. TEST	0,713	4,250	0,267
400 ppm	1. TEST	0,713	2,182	0,105
	2. TEST	0,708	1,633	0,073
	3. TEST	0,720	1,630	0,038
	4. TEST	0,700	1,900	-
800 ppm	1. TEST	0,698	2,757	0,166
	2. TEST	0,703	-	-
1000 ppm	1. TEST	0,708	2,055	0,097
	2. TEST	0,688	3,520	0,243

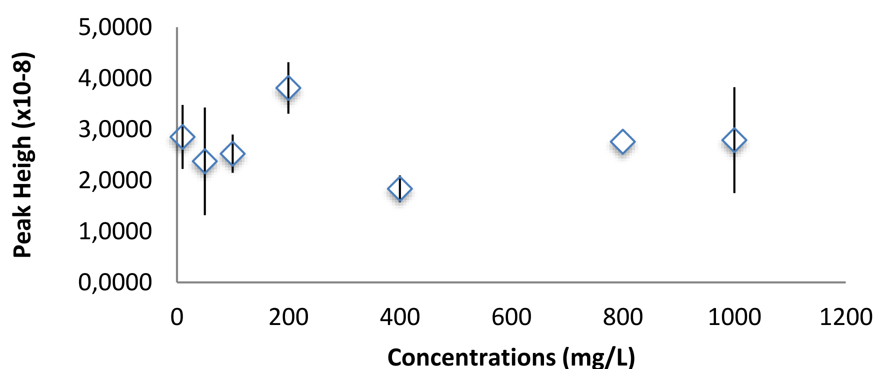


Figure 5. 5: Calibration curve for concentration of Immersion Method 10, 50, 100, 200, 400, 800, 1000pmm

Standard graphics were drawn by diluting 10, 20, 40, 80, 100 ppm from 1000 mg / mL HMF stock solution (Table 5.3).

Table 5. 3: Electrochemical results of concentrations of 10, 20, 40, 80, 100 pmm

Concentrations		Peak Position	Peak Height ($x10^{-8}$)	Peak Area ($x10^{-8}$)
10 ppm	1. TEST	0,653	1,522	0,029
	2. TEST	-	-	-
	3. TEST	-	-	-
20 ppm	1. TEST	0,688	6,936	0,493
	2. TEST	0,687	1,770	0,025
	3. TEST	0,718	3,492	0,282
	4. TEST	0,660	1,290	0,021
40 ppm	1. TEST	0,703	3,356	0,197
	2. TEST	0,693	1,184	0,050
	3. TEST	0,673	2,877	0,187
	4. TEST	0,698	2,761	0,139
80 ppm	1. TEST	0,673	1,686	0,111
	2. TEST	0,658	1,610	0,107
	3. TEST	0,683	2,960	0,024
	4. TEST	0,637	2,742	0,098
100 ppm	1. TEST	0,698	1,940	0,123
	2. TEST	0,693	1,760	0,089

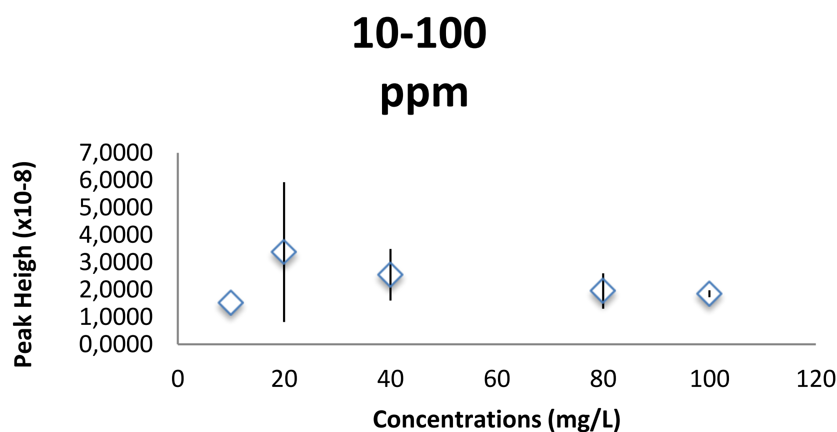


Figure 5. 6: Calibration curve for concentration of Immersion Method 10, 20, 40, 80, 100 pmm

5.4 Calibration Experiments of HMF Using Immersion and Holding Method

The standard graphic was drawn by immersion and holding method with dilutions in the range of 200, 400, 800, 1000 ppm from 1000 mg / mL HMF stock solution (Table 5.4, Figure 5.5).

Table 5. 4: Electrochemical results of concentrations of 200, 400, 800, 1000 pmm

Concentrations		Peak Position	Peak Height ($x10^{-8}$)	Peak Area ($x10^{-8}$)
200 ppm	1. TEST	0,617	1,083	0,019
	2. TEST	0,607	1,139	0,052
	3. TEST	0,612	0,645	0,015
	4. TEST	0,612	1,151	0,047
	5. TEST	0,607	1,065	0,030
400 ppm	1. TEST	0,617	1,017	0,025
	2. TEST	0,622	1,111	0,013
	3. TEST	0,612	1,212	0,020
	4. TEST	0,617	1,119	0,018
800 ppm	1. TEST	0,607	1,339	0,063
	2. TEST	0,602	1,005	0,042
	3. TEST	0,612	1,271	0,052
	4. TEST	0,602	1,592	0,053
1000 ppm	1. TEST	0,607	1,567	0,081
	2. TEST	0,612	1,549	0,074
	3. TEST	0,612	1,120	0,049
	4. TEST	0,607	1,328	0,053

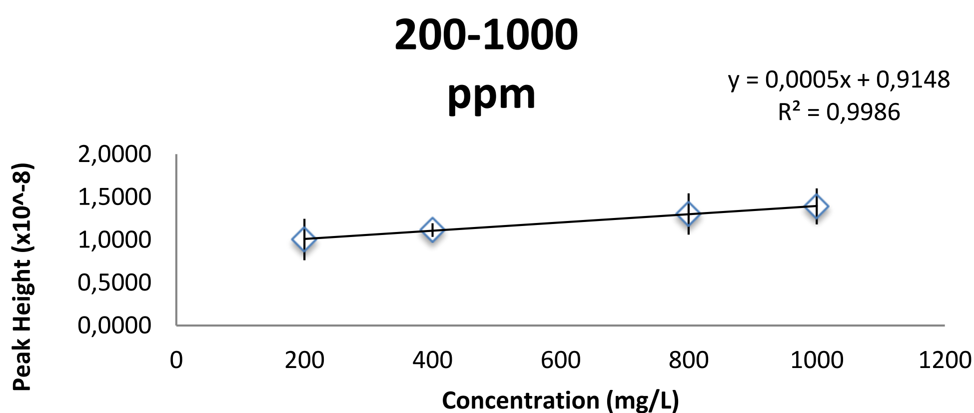


Figure 5. 7: Calibration curve for concentration of Immersion and Holding Method 200, 400, 800,1000 pmm

The standard graphic was drawn by immersion and holding method with dilutions in the range of 10, 20, 40, 80, 100 ppm from 1000 mg / mL HMF stock solution (Table 5.5).

Table 5. 5: Electrochemical results of concentrations of 10, 20, 40, 80, 100 ppm

Concentrations		Peak Position	Peak Height (x10^{-8})	Peak Area (x10^{-8})
10 ppm	1. TEST	0,623	1,410	0,061
	2. TEST	0,587	1,289	0,062
	3. TEST	0,612	1,228	0,049
20 ppm	1. TEST	0,607	2,199	0,107
	2. TEST	0,602	1,529	0,082
	3. TEST	0,623	1,293	0,084
40 ppm	1. TEST	0.612	1,790	0,096
	2. TEST	0.617	1,651	0,084
	3. TEST	0,617	1,136	0,065
80 ppm	1. TEST	0,612	1,686	0,111
	2. TEST	0,617	1,610	0,107
	3. TEST	0,612	1,890	0,121
100 ppm	1. TEST	0.592	1,206	0,075
	2. TEST	0.612	1,262	0,070
	3. TEST	0.162	1,366	0,068

5.5 Hydroxymethylfurfural Determination in Honey Samples Using Immersion Method

4 different honey hmf determinations collected from the market were measured by diluting 1g / 9 mL, 1g / 19 mL and 1g / 29 mL with acetate buffer.

5.5.1 Sample Honey-A

A peak was observed in the results obtained from sample Honey-A. The results are summarized in Table 5.6.

Table 5. 6: The results are summarized for Sample Honey-A

SAMPLE	Concentrations	Peak Position	Peak Height (x10^{-7})	Peak Area (x10^{-7})
	1g/9 mL	0,819	1,383	0,154
	1g/9 mL	0,819	1,745	0,184
Honey-A	1g/9 mL	0,814	2,232	0,252
	1g/9 mL	0,804	1,747	0,174
	1g/9 mL	0,809	1,975	0,200
	Average	0,813	1,816	0,193
	1g/19 mL	0,799	1,514	0,138
	1g/19 mL	0,819	1,367	0,119
Honey-A	1g/19 mL	0,799	1,423	0,114
	1g/19 mL	0,819	0,945	0,107
	1g/19 mL	0,819	1,192	0,104
	Average	0,811	1,288	0,116
	1g/29 mL	0,804	1,085	89,63
	1g/29 mL	0,819	0,683	0,069
Honey-A	1g/29 mL	0,809	0,632	0,051
	1g/29 mL	0,814	0,392	0,043
	1g/29 mL	-	-	-
	Average	0,811	0,698	22,448

5.5.2 Sample Honey-B

Two peak was observed in the results obtained from sample Honey-B. The results are summarized in Table 5.7.

Table 5. 7: The results are summarized for Sample Honey-B

SAMPLE	Concentrations	Peak Position	Peak Height (x10^{-7})	Peak Area (x10^{-7})
Honey-B	1g/9 mL	0,839	1,681	0,206
	1g/9 mL	0,839	1,149	0,132
	1g/9 mL	0,824	1,401	0,160
	1g/9 mL	0,849	1,226	0,156
	1g/9 mL	0,849	1,360	0,164
	Average	0,840	1,363	0,164
Honey-B	1g/19 mL	0,819	1,283	0,138
	1g/19 mL	0,819	1,010	0,110
	1g/19 mL	0,814	1,203	0,134
	1g/19 mL	0,844	1,384	0,156
	1g/19 mL	0,799	1,518	0,155
	Average	0,819	1,280	0,139
Honey-B	1g/29 mL	0,819	0,422	0,048
	1g/29 mL	0,788	0,463	0,038
	1g/29 mL	-	-	-
	1g/29 mL	0,778	0,477	0,375
	1g/29 mL	0,778	0,517	0,039
	Average	0,791	0,470	0,125

SAMPLE	Concentrations	Peak Position	Peak Height (x10^{-7})	Peak Area (x10^{-7})
Honey-B 2.PEAK	1g/9 mL	0,622	0,508	0,032
	1g/9 mL	0,617	0,777	0,044
	1g/9 mL	0,622	0,583	0,036
	1g/9 mL	0,617	0,570	0,039
	1g/9 mL	0,617	0,616	0,040
	Average	0,619	0,611	0,038
Honey-B 2.PEAK	1g/19 mL	0,612	0,497	0,031
	1g/19 mL	0,617	0,495	0,032
	1g/19 mL	0,622	0,448	0,029
	1g/19 mL	0,612	0,386	0,022
	1g/19 mL	0,617	0,561	0,033
	Average	0,616	0,477	0,029
Honey-B 2.PEAK	1g/29 mL	0,628	0,251	0,016
	1g/29 mL	0,618	0,340	0,015
	1g/29 mL	-	-	-
	1g/29 mL	0,607	0,340	0,016
	1g/29 mL	0,607	0,445	0,027
	Average	0,615	0,344	0,018

5.5.3 Sample Honey-C

Two peak was observed in the results obtained from sample Honey-C. The results are summarized in Table 5.8.

Table 5. 8: The results are summarized for Sample Honey-C

SAMPLE	Concentrations	Peak Position	Peak Height (x10^{-7})	Peak Area (x10^{-7})
	1g/9 mL	0,804	2,863	0,323
	1g/9 mL	0,809	2,931	0,327
Honey-C	1g/9 mL	0,814	2,006	0,246
	1g/9 mL	-	-	-
	1g/9 mL	0,819	1,302	0,170
	Average	0,811	2,276	0,266
	1g/19 mL	0,804	1,161	0,107
	1g/19 mL	0,809	0,897	0,108
Honey-C	1g/19 mL	0,809	0,553	0,070
	1g/19 mL	-	-	-
	1g/19 mL	0,804	2,043	0,173
	Average	0,806	1,164	0,114
	1g/29 mL	0,809	0,491	0,045
	1g/29 mL	0,778	0,744	0,058
Honey-C	1g/29 mL	0,794	1,381	1,466
	1g/29 mL	-	-	
	1g/29 mL	-	-	
	Average	0,794	0,872	0,523

SAMPLE	Concentrations	Peak Position	Peak Height ($x10^{-7}$)	Peak Area ($x10^{-7}$)
Honey-C 2.PEAK	1g/9 mL	0,617	0,656	0,032
	1g/9 mL	0,612	0,732	0,042
	1g/9 mL	0,612	0,545	0,035
	1g/9 mL	-	-	-
	1g/9 mL	-	-	-
	Average	0,614	0,644	0,037
Honey-C 2.PEAK	1g/19 mL	0,612	0,450	0,022
	1g/19 mL	0,618	0,324	0,020
	1g/19 mL	0,622	0,199	0,012
	1g/19 mL	-	-	-
	1g/19 mL	0,607	0,352	0,023
	Average	0,615	0,331	0,020
Honey-C 2.PEAK	1g/29 mL	0,607	0,247	0,012
	1g/29 mL	0,607	0,357	0,020
	1g/29 mL	0,607	0,384	0,020
	1g/29 mL	-	-	-
	1g/29 mL	-	-	-
	Average	0,607	0,329	0,017

5.5.4 Sample Honey-D

A peak was observed in the results obtained from sample Honey-C. The results are summarized in Table 5.9.

Table 5. 9: The results are summarized for Sample Honey-D

SAMPLE	Concentrations	Peak Position	Peak Height ($x10^{-7}$)	Peak Area ($x10^{-7}$)
Honey-D	1g/9 mL	0,824	1,707	0,172
	1g/9 mL	0,819	0,966	0,114
	1g/9 mL	0,809	1,105	0,131
	1g/9 mL	-	-	-
	1g/9 mL	0,809	2,996	0,318
	Average	0,815	1,694	0,184

Honey-D	1g/19 mL	0,819	0,678	0,074
	1g/19 mL	0,809	1,323	0,131
	1g/19 mL	0,799	1,856	0,169
	1g/19 mL	0,804	1,479	0,140
	1g/19 mL	0,794	1,992	0,168
	Average	0,805	1,466	0,136
Honey-D	1g/29 mL	0,809	0,655	0,056
	1g/29 mL	0,809	0,427	0,036
	1g/29 mL	0,804	0,766	0,060
	1g/29 mL	0,778	1,199	0,105
	1g/29 mL	0,799	0,707	0,059
	Average	0,800	0,751	0,063

5.6 Hydroxymethylfurfural in Various Pekmez Using Immersion Method

1 homemade pekmez was taken and diluted with acetate buffer to concentrations of 1g / 9 mL, 1g / 19 mL and 1g / 29 mL.

5.6.1 Sample Pekmez

Three peak was observed in the results obtained from sample Pekmez. The results are summarized in Table 5.10.

Table 5. 10: The results are summarized for Sample Pekmez.

SAMPLE	Concentrations	Peak Position	Peak Height ($x10^{-7}$)	Peak Area ($x10^{-7}$)
Pekmez	1g/9 mL	0,345	17,748	1,116
	1g/9 mL	0,340	15,920	0,791
	1g/9 mL	-	-	-
	1g/9 mL	0,350	19,033	1,592
	1g/9 mL	0,335	11,949	0,048
	Average	0,343	16,163	0,887
Pekmez	1g/19 mL	0,335	7,512	0,288
	1g/19 mL	0,330	5,160	0,180
	1g/19 mL	0,335	6,851	0,264
	1g/19 mL	0,335	7,451	0,273
	1g/19 mL	0,335	7,805	0,313
	Average	0,334	6,956	0,264

Pekmez	1g/29 mL	0,330	3,880	0,123
	1g/29 mL	0,330	4,901	0,162
	1g/29 mL	0,330	3,024	0,084
	1g/29 mL	0,330	2,739	0,073
	1g/29 mL	0,330	3,372	0,095
	Average	0,330	3,583	0,107

SAMPLE	Concentrations	Peak Position	Peak Height ($x10^{-7}$)	Peak Area ($x10^{-7}$)
Pekmez 2. PEAK	1g/9 mL	0,698	4,106	0,447
	1g/9 mL	0,698	4,061	0,439
	1g/9 mL	-	-	-
	1g/9 mL	0,698	3,616	0,375
	1g/9 mL	0,663	4,632	0,555
	Average	0,689	4,104	0,454
Pekmez 2. PEAK	1g/19 mL	0,688	3,220	0,241
	1g/19 mL	0,658	2,470	0,264
	1g/19 mL	0,663	2,223	0,226
	1g/19 mL	0,663	2,713	0,287
	1g/19 mL	0,693	2,547	0,288
	Average	0,673	2,635	0,261
Pekmez 2. PEAK	1g/29 mL	0,663	1,825	0,195
	1g/29 mL	0,663	2,149	0,223
	1g/29 mL	0,663	2,808	0,309
	1g/29 mL	0,653	2,588	0,284
	1g/29 mL	0,663	2,921	0,335
	Average	0,661	2,458	0,269

SAMPLE	Concentrations	Peak Position	Peak Height ($x10^{-7}$)	Peak Area ($x10^{-7}$)
Pekmez 3. PEAK	1g/9 mL	0,965	2,586	0,129
	1g/9 mL	0,970	2,693	0,129
	1g/9 mL	-	-	-
	1g/9 mL	0,965	1,926	0,083
	1g/9 mL	0,965	3,903	0,232
	Average	0,966	2,777	0,143

Pekmez 3. PEAK	1g/19 mL	0,955	0,342	0,240
	1g/19 mL	0,935	0,293	0,192
	1g/19 mL	0,935	0,296	0,212
	1g/19 mL	0,955	0,340	0,245
	1g/19 mL	0,965	0,281	0,188
	Average	0,949	0,311	0,215
Pekmez 3. PEAK	1g/29 mL	0,935	2,533	0,181
	1g/29 mL	0,935	2,319	0,161
	1g/29 mL	0,935	2,540	0,177
	1g/29 mL	0,945	2,943	0,158
	1g/29 mL	0,935	2,370	0,158
	Average	0,937	2,541	0,167

CHAPTER 6

CONCLUSION AND RECOMMENDATION

In almost all cultures, honey has a wide range of pharmacological effects such as an antioxidant, heart attack risk reducer, anticarcinogen, immune system enhancer, cataract and inflammation receptor, taken as an alternative drug and Pekmez has antioxidant source, obesity prevention, high level of vitamins and minerals. In this study, a fast, easy, cost-effective and environmentally friendly screening method has been proposed for the detection of Hydroxymethylfurfural (HMF), which is one of the most important indicators of honey and pekmez quality, and which occurs with heat treatments, under improper storage conditions and at longer storage time.

A lot of studies have been reported to determine the adverse effects of high HMF on human health. Information on whether HMF poses a potential health risk on humans, many studies on animals have shown that it harms the cell, skin and respiratory system and has a carcinogenic effect.

Two different methods were used in this study. Immersion method and Immersion/Holding method. Since we got the almost same results with both methods, the immersion method was chosen for the determination of HMF in honey and pekmez samples, as it is easier and quicker. Experimental conditions were established to obtain a linear relationship between voltammetric measurements. Measurements were made in different concentrations of HMF standard (20, 40, 80, 100, 200, 400, 800, 1000ppm). Adding honey to the electrolytes created a change in there action medium that altered both the potential range and the HMF electrochemical signal. Honey and pekmez HMF content was determinedwith DPV by using immersion technique in an aqueous acetate medium (pH 4.8, 0.5 M). For the analysis, honey and pekmez samples were diluted by adding 9ml, 19ml and 29ml of acetate buffer into 1 g sample and 5 trials were made for each dilution. The lower the honey density (1g / 29ml), the more accurate results we got.

Peak position was 0.6 V in the analysis of HMF standards.

SampleHoneyA and D were announced by Government Laboratory as free from HMF. We also could not detect HMF peak in these samples. Honey B and C were HMF positive by Government Laboratory results. We have also detected HMF in higher amounts which is 10 fold more than our calibration dynamic range. The same results were obtained with Pekmez Sample, the same peak position of 0.6 volt was detected for HMF. Some other peaks appeared at 0.3 V and 0.9 V as well. These peaks might be due to enzymes presence in these samples acording to literature (Reyes et al., 2006).

Based on our analysis results, electrochemical method for the determination of HMF in honey or pekmez can be used as screening method, since correlation coefficent of HMF in both method was 0.98. LOD of HMF was found to be 45.54 ppm from calibration curve.

It could be recommended that HMF electrochemical measurements can be performed in negative region under inert conditions by using nitrogen or argon gas flux to improve detection limits.

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APPENDIX I: ETHICAL APPROVAL DOCUMENT

ETHICAL APPROVAL DOCUMENT

Date:12/09/2020

To the Graduate School of Applied Sciences

For the thesis project entitled as "IDENTIFICATION OF HYDROXYMETHYL FURFURAL (HMF) IN HONEY AND PEKMEZ BY USING PENCIL GRAPHITE ELECTRODE", the researchers declare that they did not collect any data from human/animal or any other subjects. Therefore, this project does not need to go through the ethics committee evaluation.

Title: Assist. Prof. Dr.

Name Surname: Vasfiye Hazal ÖZYURT

Signature:

Role in the Research Project: Supervisor

APPENDIX II: SIMILARITY REPORT

Hazal Ozyurt | Kullanıcı Bilgisi | Mesajlar | Öğretmen ▼ | Türkçe ▼ | Topluluk | Yardım | çıkış

turnitin

Ödevler Öğrenciler Not Defteri Kütüphaneler Takvim Tartışma Tercihler

GÖRÜNTÜLENİYOR: ANASAYFA > AA1. > FATMA AMCA

Bu sayfa hakkında

Bu sizin ödev kutunuzdur. Bir yazılı ödevi görüntülemek için yazılı ödevin başlığını seçin. Bir Benzerlik Raporunu görüntülemek için yazılı ödevin benzerlik sütunundaki Benzerlik Raporu ikonunu seçin. Tıklanabilir durumda olmayan bir ikon Benzerlik Raporunun henüz oluşturulmadığını gösterir.

Fatma Amca

GELEN KUTUSU | GÖRÜNTÜLENİYOR: YENİ ÖDEVLER ▼

Dosyayı Gönder

Çevrimiçi Derecelendirme Raporu | Ödev ayarlarını düzenle | E-posta bildirmeyenler

YAZAR	BAŞLIK	BENZERLİK	PUANLA	CEVAP	DOSYA	ÖDEV NUMARASI	TARİH
Fatma Amca	Chapter 1	%0	--	--		1381242210	07-Eyl-2020
Fatma Amca	Chapter 6	%0	--	--		1381244482	07-Eyl-2020
Fatma Amca	Chapter 5	%1	--	--		1381244240	07-Eyl-2020
Fatma Amca	Chapter 4	%8	--	--		1381243931	07-Eyl-2020
Fatma Amca	Chapter 3	%9	--	--		1381243611	07-Eyl-2020
Fatma Amca	Chapter 2	%10	--	--		1381242897	07-Eyl-2020

Fatma Amca

Assist. Prof. Vasfiye Hazal ÖZYURT