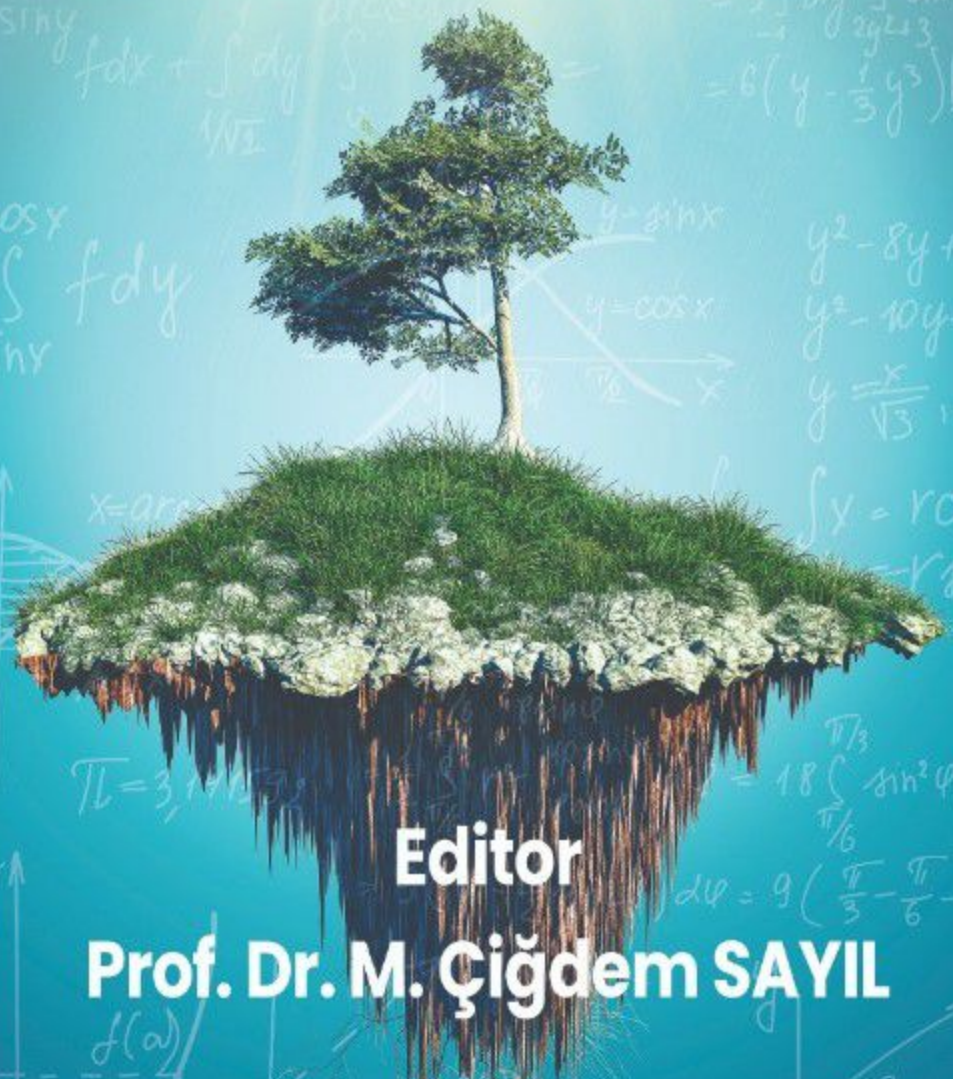
RECENT APPROACHES IN MATHEMATICS AND NATURAL SCIENCE



Editor
Prof. Dr. M. Çiğdem SAYIL

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Recent Approaches in Mathematics and Natural Science

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FOREWORD

The book “RECENT APPROACHES IN MATHEMATICS AND NATURAL SCIENCE” covers interdisciplinary studies focusing on current research for academics and researchers working in the natural sciences and mathematics, and is prepared to inform readers about current issues in the natural sciences and mathematics. In the book, researchers working in the fields of science and mathematics share their current studies with interested readers. Thus, more researchers will be aware of the academic studies published in this book and will have some new ideas for their future work.

This volume contains 12 of the chapters that were presented to editorial boards. In keeping with the formatting of the book, the papers are published in English. Selected chapters have been evaluated and approved for publication by expert referees. The authors who contributed their work to the preparation of this book have made invaluable contributions without expecting anything in return. I thank the authors for their valuable contributions. The editor would like to thank all of the authors who made this book so interesting and enjoyable. Special thanks should also be extended to the reviewers who gave their time to evaluate and to give feedback to authors of the record number of submissions with tenacity and dedication. Especially to the LVRE DE LYON Publishing House, we owe a great debt as this book would not have been possible without their consent efforts. At this juncture, I would like to thank the authors for all of their cooperation. We hope that all of those reading enjoy these chapters of the book as much as possible.

EDITOR

Prof. Dr. M. Çiğdem SAYIL

CONTENTS

CHAPTER I

A NEW HETEROCYCLIC COMPOUND CONTAINING AN IMINE BOND

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1. Introduction

Pyrimidine is the building block of thymine, cytosine and uracil used for RNA and DNA synthesis.(Termanini, 2020) Because they exhibit a wide range of biological activities, pyrimidines are widely used to produce new active molecules (Al-Issa, 2013; Karataş, Tüzün, & Kökbudak, 2022; Kumar, Khan, Tekwani, Ponnan, & Rawat, 2015; Maddila, Gorle, Seshadri, Lavanya, & Jonnalagadda, 2016; Romanowska et al., 2011; Santos et al., 2015; Tinney et al., 1981). Therefore, the assembly of a pyrimidine core has led to significant research.

Several medications that are commercially available contain pyrimidine. The most well-known are pyrimethamine (antiparasitic), sulfadiazine (antibacterial), sulfadoxine (anti-malarial), cidofovir and idoxuridine (antiviral), viomycin (anti-tuberculosis), trametinib and 5-fluorouracil (anticancer), and zidovudine (anti-HIV) agents (Figure1).

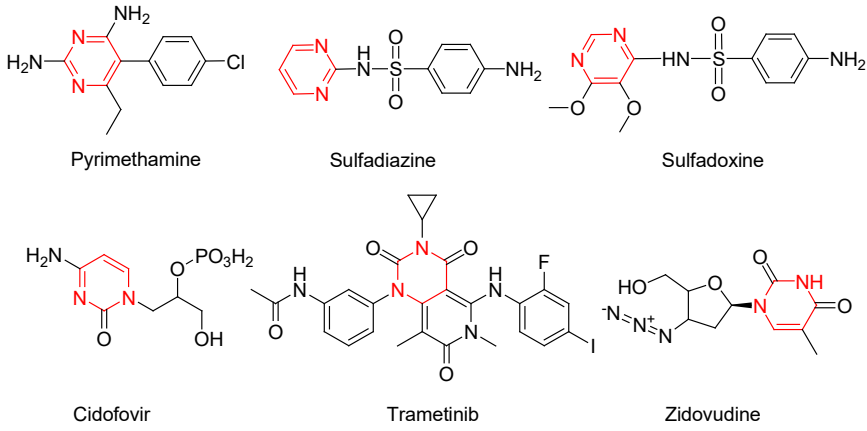


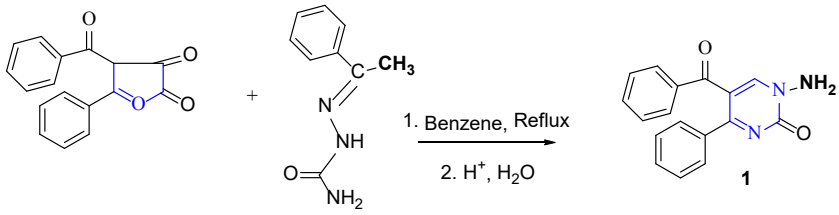
Figure 1. Some commercially available pyrimidine-incorporated drugs

N-aminopyrimidine derivatives can be used for the synthesis of heterocyclic compounds. Therefore, critical studies have been done on such compounds (G. Aslan, Akkoç, Akkurt, Özdemir, & Kökbudak, 2018; G. Aslan & Onal, 2014; H. G. Aslan, Akkoç, & Kökbudak, 2020; H. G. Aslan, Akkoç, Kökbudak, & Aydın, 2017; Çimen, Akkoç, & Kökbudak, 2018; Zülbiye Kökbudak, Aslan, & Akkoç, 2020; Zülbiye Kökbudak et al., 2020; Zülbiye Önal & Altural, 1999, 2006; Zülbiye Önal & Daylan, 2007).

Schiff base, also known as azomethine or imine, is synthesized by the condensation reaction of primary amine and aldehyde/ketone. They have versatile pharmacophore properties widely used in the design and development of biologically active compounds (da Silva et al., 2011). Schiff bases have been reported to have antimicrobial, antifungal, antibacterial, antidyslipidemic, antitumor, antituberculous, antimalarial, antidepressant, anti-inflammatory and analgesic activities (Dhar & Taploo, 1982; Przybylski, Huczynski, Pyta, Brzezinski, & Bartl, 2009). Schiff bases are also widely used in coordination chemistry. Since they contain an azomethine group, they coordinate almost all metal ions (Shivakumar, Shashidhar, Vithal Reddy, & Halli, 2008; Yamada, 1999). The popularity of Schiff base derivatives containing a biologically active pyrimidine ring is increasing (Akkoc et al., 2022; Cakmak, Basaran, & Senturk, 2022; Jasinska et al., 2022; Zülbiye Kökbudak, Akkoç, Karataş, Tüzün, & Aslan, 2022).

In the first step of the study, 1-aminopyrimidine-2-one derivative (**1**) was synthesized from methyleneaminopyrimidine derivative according to the literature procedure (**Scheme 1**) (Atioğlu, Karataş, & Kökbudak, 2021;

Zülbiye Önal & Yıldırım, 2007). Then, the (E)-N-(4-(((5-benzoyl-2-oxo-4-phenylpyrimidin-1(2*H*)yl)imino)methyl)phenyl)acetamide (**2**) was synthesized by the condensation reaction of 1-amino-5-benzoyl-4-phenylpyrimidin-2(1*H*)-one (**1**) with commercially available *p*-acetylaminobenzencarbaldehyde using *p*-toluenesulfonic acid as a catalyst at refluxing in ethanol. The general outline of the reaction studied is shown in **Scheme 2**. The structure of (**2**) was elucidated using spectroscopic techniques. The synthesized compound was optimized using the B3LYP method with the basis set 6-31G (d,p) and 6-311(d,p). Theoretical ^{1}H NMR calculations were made with both basis sets. The results were plotted.



Scheme 1. Synthesis of compound (**1**)

2. Experimental

2.1. Reagents and materials

The starting material used in the study was synthesized according to the method mentioned above. TLC monitored reaction progress. The molecule's spectra were recorded using Erciyes University (ERÜ) Technology Research and Application Center (TAUM).

2.2. Synthesis of (2)

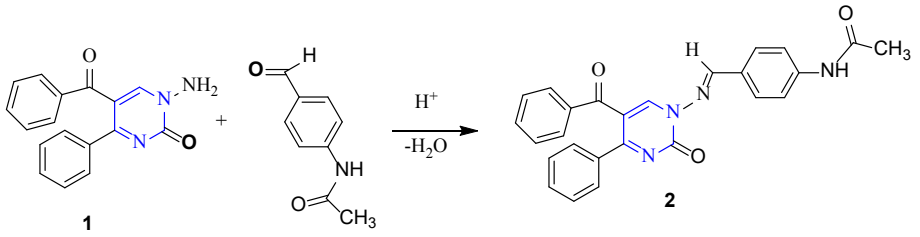
(**1**) was dissolved and a 1.2 mmol solution of *p*-acetylamino benzenecarbaldehyde was added with *p*-toluenesulfonic acid as a catalyst. It was boiled for three hours and then stirred at 25 °C for 24 hours. Ethyl alcohol was then removed, and diethyl ether was added. After stirring for 24 hours in a cold environment, it was filtered. The product (**2**) was crystallized from ethyl alcohol. The synthesis scheme of (**2**) is in Scheme 2.

Brown solid. Yield: 50%. Mp: 196-198 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 9.09 (s, 1H), 8.66 (s, 1H), 8.08 – 7.71 (m, 6H), 7.73 – 7.19 (m, 8H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 192.06, 170.99, 169.45, 167.00, 151.63, 149.17, 143.93, 137.33, 133.86, 131.08, 130.57, 130.18, 129.12,

129.06, 128.69, 126.82, 119.23, 115.93, 99.99, 24.69. IR: υ = 3158.1 (aromatic C-H), 1695.3 (NH-C=O), 1645.8-1655.1 (C=O), 1596.7, 1520.2 (C=N and C=C). Anal. Calcd. for $C_{26}H_{20}N_4O_3$ (436.462 g/mol): C, 71.55; H, 4.62; N, 12.84. Found: C, 71.36; H, 4.51; N, 12.70.

3. Results and Discussion

In the first step, compound (**1**) was prepared (**Scheme 1**), and then, (**2**) was obtained from the (**1**) and *p*-acetylamino benzencarbaldehyde in yields 50%. The structure of (**2**) was verified using spectroscopic methods. The synthesis of compound (**2**) is explained in **Scheme 2.**



Scheme 2. Synthesis of the target compound (**2**)

Compound (**2**) was synthesized from the reaction of compound (**1**) and *p*-acetylaminobenzencarbaldehyde in 50% yield. The C=O absorption bands were observed in the IR spectrum observed at 1695.3, 1655.1, and 1645.8 cm^{-1}, respectively. The peak of the N=CH proton at 9.09 ppm and -CH proton in the pyrimidine ring at 8.66 ppm were marked. Aromatic protons were observed as multiplet between δ 8.08 and 7.19 ppm. Also, methyl protons (CH_3-CO) were observed at 2.09 ppm. The ^{13}C NMR signals were observed at δ 192.06 (s, benzoly carbon's signal), 170.99-99.99 (m, aromatic carbons) and 24.69 (s, methyl carbons). The data obtained from the analysis fully confirmed the structure of the Schiff base.

3.1. Optimization And Theoretical 1H NMR Calculations

In this study, the Gaussian 09 program package was used. The experimental ^1H NMR spectrum of the compound was compared with the spectra obtained with the B3LYP 6-31G(d,p) and B3LYP 6-311++G(d,p) basis sets. The results obtained in the B3LYP/6-311++G(d,p) method provide the best fit.

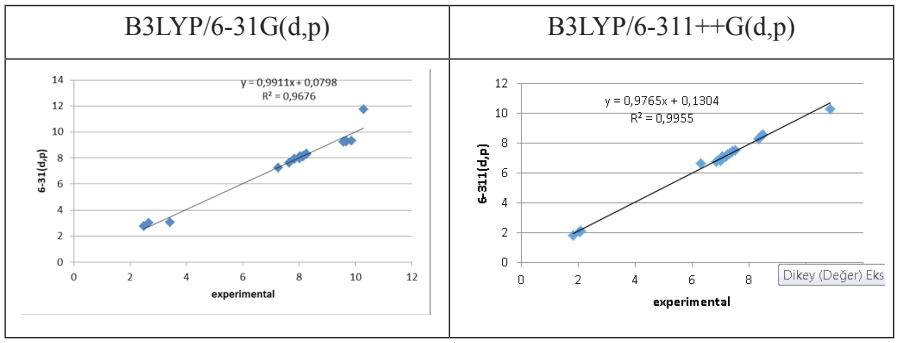


Figure 2. Comparison of ^{1}H NMR data calculated with B3LYP function 6-31(d,p) and 6-311(d,p) basis set with experimental results.

As a result of the study, R^2 values were obtained. The R^2 value was determined as 0.9676 for 6-31G(d,p) and 0.9955 for 6-311G++(d,p), respectively.

4. Conclusion

The 1-aminopyrimidin-2(1*H*)-one derivative (**2**) was synthesized starting from (**1**). The lowest energy state of the molecule was determined by quantum chemical calculations. The different biological and chemical properties of (**2**), which contain an active pyrimidine ring, will be investigated in the next steps of our studies.

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CHAPTER II

POLY(LACTIC-*CO*-GLYCOLIC ACID): FDA AND EMA-APPROVED BIODEGRADABLE ELASTOMERIC COPOLYMER

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1. Introduction

Poly(lactic-*co*-glycolic acid) (PLGA)-based polymers have been one of the most open-to-development polymers due to their biocompatible, designable mechanical properties and chemical versatility which have been approved by the US Food and Drug Administration (FDA) and European Medicine Agency (EMA). (Y. Wang, Qin, Xia, & Choi, 2021) (Operti et al., 2021) It was shown that PLGA gains improved strength ability and mechanical properties with its Young's Modulus, which varies according to the monomer composition. (Abbasnezhad, Zirak, Shirinbayan, Tcharkhtchi, & Bakir, 2021) For example in a research report, the triblock copolymer of PLGA, which was elastic and more quickly biodegradable, has been designed for macroporous scaffolds of tissue engineering applications. (Choi & Park, 2002) In a different study, it was observed that soft fibers that have lower Young's modulus produced from PLGA-PEG copolymer transform cells into a more spherical form as a result of the interaction with macrophages. (B. Zhang, Galluzzi, Zhou, & Yu, 2023)

The synthetic biopolymer PLGA, which is involved in such important current studies, is widely used in biomaterials, nanomedicine, drug delivery, tissue regeneration, and PLGA in health chemistry is on the way to becoming a particular specialty. (Hasirci, Yilgor, Endogan, Eke, & Hasirci, 2011) The

permeability of peptide nanofiber/PLGA nanocomposites designed for the encapsulation of nucleic acids, proteins, and small molecular drugs was reported by 10-times more increased therapeutic effect compared to naked PLGA nanoparticles for the treatment of lung diseases in different tissues and cells. (Chintapula et al., 2022) Customized PLGA-based nano-sized hybrid medicines developed are new treatment approaches, especially for cancer. In combination with anticancer drugs, specific peptide inhibitors and PLGA nanocomplexes were enhanced cell uptake and physiological stability. As a result of the in vivo study, it was also reported that suppressed tumor cells with the DOX/peptide/PLGA nanocomplexes with lower side effects. (N. Zhang et al., 2022)

This chapter, as described with a few examples above, it was aimed to summarize the considerable literature regarding PLGA biocompatible polymers for the last 10 years.

2. Overview of the Biocompatible Polymers

Synthetic biodegradable polymers can join the earth's innate cycles without damaging the environment and their use is rapidly expanding in every field due to their practical production. (Mtibe, Motloung, Bandyopadhyay, & Ray, 2021; Samir, Ashour, Hakim, & Bassyouni, 2022) When the production statistics of polymers for 2017 are examined, conventional polymers are 335 million tons, while biopolymers are only 2.1 million tons. However, thanks to the transformation that started with environmental policies, it is expected to reach 2.43 million tons by 2024, and 20-25% growth is expected annually. (Mtibe et al., 2021)

The most commonly studied synthetic biodegradable polymers are polylactic acid (PLA) (Y. Zheng et al., 2023), polyglycolic acid (PGA) (Wu, Wang, Ning, Jiang, & Gan, 2022), poly(lactic-*co*-glycolic acid) (PLGA) (S. Park et al., 2022), polycaprolactone (PCL) (S. Y. Zheng et al., 2023), polyurethane (PU) (Aksoy, Taskor, Gultekinoglu, Kara, & Ulubayram, 2018), polyethylene glycol (PEG) (Liu, Corciulo, Arabagian, Ulman, & Cronstein, 2019) recently.

The new generation treatment approach of multi-therapeutics, for example, nanoparticles produced from PEG-PLGA copolymer, encapsulated with anticancer drug doxorubicin and drug or prodrug activating enzymes showed the highest level of cancer cell death. (Harguindey et al., 2019)

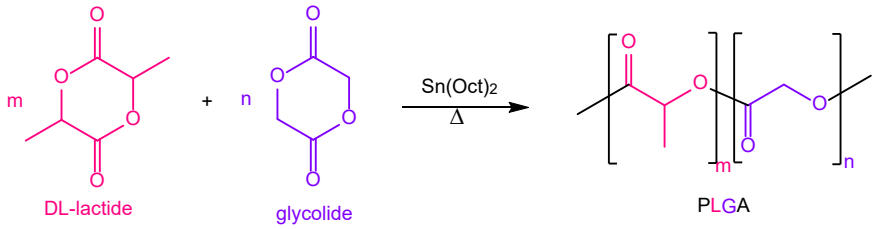
The derivatization studies of these frequently studied biodegradable polymers are also a separate research topic. For instance, it was reported that the

derivative polymer of polyurethane made with amino acid esters increases the capacity to make H bonds and gives a faster biodegradable property. In addition, as a result of cytotoxicity experiments, it has been reported that derivative PU, which could be called non-toxic level, was a new polymer suitable for tissue engineering applications. (Aksoy et al., 2018)

3. Poly(lactic-*co*-glycolic acid) (PLGA)

PLGA has been widely used in especially tissue engineering (Gentile, Chiono, Carmagnola, & Hatton, 2014) and drug release systems (Makadia & Siegel, 2011) in many different biomedical applications thanks to its biocompatibility, non-toxic and immunogenic properties associated with minimizing negative biological responses in physiological conditions. In addition, PLGA has been the most popular biocompatible and biodegradable polymer with Food Drug Administration (FDA) and European Medicine Agency (EMA) approval, particularly with its positive clinical history, non-toxic degradation products (H_2O and CO_2), and improved controlled release ability. (Operti et al., 2021)

PLGA is practically synthesized in the presence of catalysts such as tin(II) 2-ethylhexanoate by ring-opening polymerization (ROP) of DL-lactide (L) and glycolide (G). (Gentile et al., 2014) (**Scheme 1**) In order to obtain PLGA copolymer with different mechanical, thermal and physical properties, the L:G ratio is changed. The differentiation of these properties of PLGA also affects its basic properties such as solubility, hydrophilic balance, biodegradation, drug loading, and release. (Pannuzzo, Horta, La Rosa, & Decuzzi, 2020) For instance, if it is desired to prepare a PLGA with a degrade easier, the L: G ratio should be reduced. In PLGA, the fastest degradation observed was achieved when the L:G ratio is 50:50. (Makadia & Siegel, 2011) PLGA is most easily soluble in chlorinated organic solvents such as dichloromethane, chloroform and polar aprotic solvents such as acetone, ethyl acetate, tetrahydrofuran. (Körber, 2010)



Scheme 1. Synthesis diagram of PLGA

The effects of PEGylated PLGA nanoparticles produced by nanoprecipitation method on ovarian cancer were studied with the active ingredient genistein, which is a natural isoflavone. The drug release time of genistein, whose solubility in water was increased by the PEGylated PLGA nanoparticles, was increased to six days and its bioactivity in the ovarian cancer cell line was increased. (Patra, Satpathy, Naik, Kazi, & Hussain, 2022)

Below is a discussion of PLGA's considerable applications in current research.

3.1. Hydrogel

In order to treat Osteomyelitis, which is a difficult disease with treatment that involves controlling the infection and removing necrotic tissues, the application of bone cement loaded with antibiotics is performed. For treatment, the complex structure of the PLGA-PEG-PLGA triblock copolymer hydrogel with increased storage modulus with hydroxyapatite was prepared and encapsulated with vancomycin. Antibacterial experiments have shown that the hydrogel structure exhibits good bone repair properties with the effectiveness of the controlled release system in the treatment of infection. (Yuan et al., 2022)

3.2. Nano/Microparticles

It is known that epigallocatechin 3-gallate (EGCG), a polyphenol derivative isolated from green tea with anticancer and antioxidant properties, has low permeability and is chemically unstable in the physiological environment. ECGC-encapsulated PLGA nanoparticles reported findings that the cell suppression ability was enhanced both *in vitro* and *in silico* works on lung cancer cell lines. (Minnelli et al., 2023)

Biotin, folic acid, aptamer, antibodies, and peptides are specific molecules that are very often used in the active targeting of PLGAs. In order to accelerate cancer stem cells inhibition, doxorubicin (DOX) and cyclopamine (CYC) encapsulated new amphiphilic polymer hyaluronic acid- cystamine-polylactic-*co*-glycolic acid (HA-SS-PLGA) nanoparticles were prepared. In addition to being redox responsive, these nanoparticles were observed to target cancer stem cells with HA. Thus, it was reported that PLGA-based copolymeric biomaterial (HA-SS-PLGA), which was a special inhibitory effect on specific cancer stem cells, increased the anticancer activity. (Rezvantalab et al., 2018)

3.3. Tissue Engineering

The colloidal gel produced from a mixture of negatively charged inorganic hydroxyapatite nanoparticles and positively charged PLGA nanoparticles was obtained. A bone tissue biomaterial that supports regeneration was designed by transplanting stem cells into this injectable gel biomaterial. (Q. Wang, Gu, Jamal, Detamore, & Berkland, 2013)

For bone tissue engineering research, ferric hydroxide [$Fe(OH)_3$] nanoparticles-coated PLGA microspheres were designed to facilitate stem cell homing for tissue regeneration. The microsphere was coated with growth factors to improve home efficiency. Also, it was reported that the designed tissue scaffold enhanced the cell migration and osteogenesis by the polydopamine route. (Patel, Jha, & Patel, 2021)

3.4. Nano vaccines

Pseudomonas aeruginosa gram-negative bacteria is the main infectious agent in patients with cystic fibrosis. To stimulate the immunological response to these pathogens, which cause infection in many regions of the body, exotoxin A (ETA) encapsulated PLGA nanoparticles were prepared. It has been concluded that PLGA encapsulation of ETA can improve functional activity by better reducing bacterial spread. (Safari Zanjani, Shapouri, Dezfulian, Mahdavi, & Shafiee Ardestani, 2019)

3.5. Microcarriers

Collagen-patched PLGA microparticles were prepared by the microfluidic methodology to improve the proliferation and osteogenic differentiation of mesenchymal stem cells. One of the methods of PLGA micro/nanoparticle preparation was the microfluidic methodology provided well-defined functionality. (Song et al., 2022)

Microneedle structures based on PLGA and polyvinylpyrrolidone (PVP) were prepared to control the drug release kinetics. In order to facilitate the transdermal transition, needle-shaped structures were proposed to be applied by spraying. It was reported that preparing controllable multiple drug systems with the drug that were loaded on each layer. Similarly, the design of vaccines in the form of microneedles capsuled with multiple active ingredients applied by spraying was mentioned. (S. C. Park, Kim, Baek, Park, & Choi, 2019)

4. Conclusions and Future Prospects

The medical market will greatly benefit from the development of PLGA-based biomaterials and drug carriers. Further development in the future may result in a revolution in medical biomaterials and drugs in terms of economic effectiveness. PLGA clearly broadened the potential applications of biodegradable materials and drug delivery systems. Furthermore, tissue engineering applications have focused on PLGA-hydroxyapatide composite materials. In order to increase the cell affinity of PLGA forms such as fiber, scaffold, hydrogel, micro/nanoparticle, it is envisaged to develop formulations in various combinations with different substances that increase biocompatibility.

PLGA-based microcarriers, nano vaccines, hydrogel, etc. it is inevitable that biomaterials will become more stable, more effective, and cheaper to produce in coming decade.

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CHAPTER III

RECENT ANALYTICAL STUDIES FOR DETERMINATION OF ANTHOCYANINS

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1. Introduction

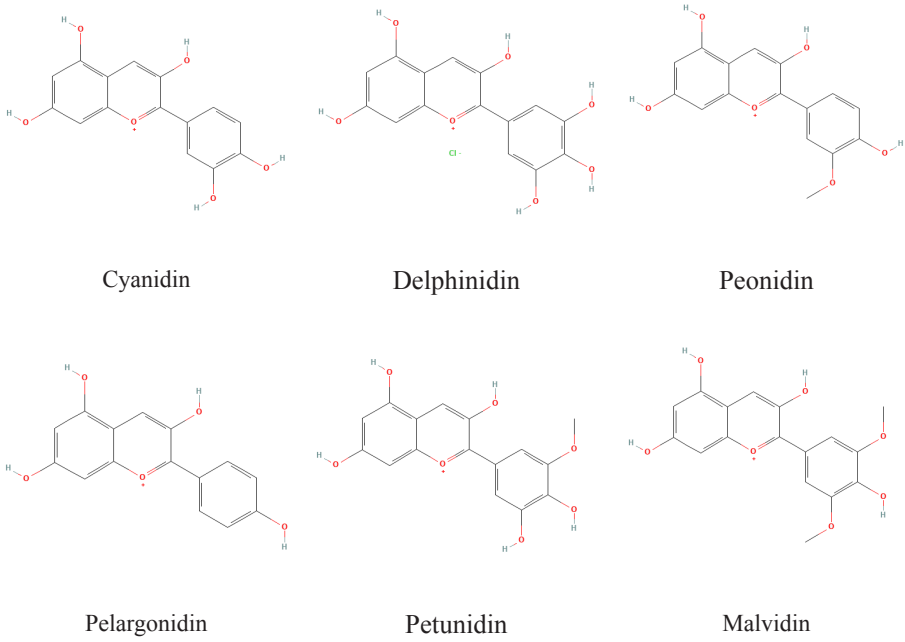
Phenolic compounds are phytochemicals with numerous bioactive properties found in most plant tissues, including fruits and vegetables. These molecules are crucial in the formation of properties such as flavor and color of fruits and vegetables, and although they do not have nutritional properties, dietary intake provides health protective effects. There are two groups of phenolic compounds: phenolic acids and flavonoids. Flavonoids are classified as flavones, flavonols, iso-flavonols, anthocyanins, anthocyanidins, proanthocyanidins and catechins (Robards & Antolovich, 1997; Khoddami et al., 2013; Laura et al., 2019).

Anthocyanins, one of the most important flavonoid groups, are water soluble natural colorants and pigments that are responsible for colors (pink, red, blue and purple) of vegetables, fruits, juices and wines. Anthocyanins,

whose basic structure consists of anthocyanidins, are not found in free form in nature, they are found as glycosides with sugars. The amount of hydroxylated groups, the number of sugars attached to the structure, the number of aromatic and aliphatic carboxylates bound to the sugar in the molecule, and the positions of these bonds are the primary variations between anthocyanins. Anthocyanins with very different structures are formed by the binding of different sugars to different anthocyanidins found in nature, and they cause a wide variety and rich color of plants, flowers, fruits and vegetables (Kong et al., 2003; Kurilich et al., 2005; Castañeda-Ovando et al., 2009). It has been reported that anthocyanins have beneficial health effects such as antioxidant (Kalt et al., 2020; Perez et al., 2022), anti-inflammatory (Klimis-Zacas et al., 2016; Rossi et al., 2003), neuroprotective activities (Strathearn et al., 2014) in the literature.

2. Anthocyanins

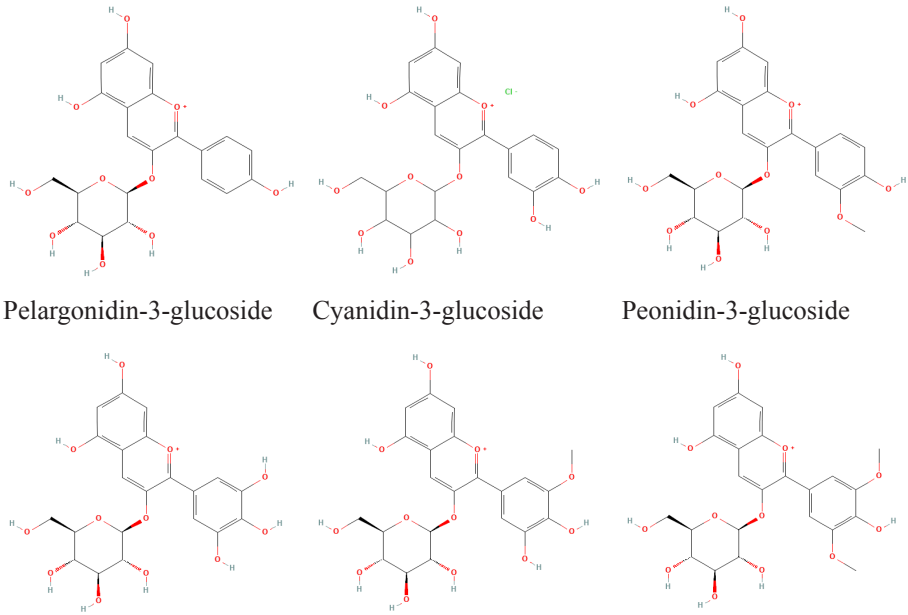
Anthocyanins are water-soluble natural pigments that are widely present in fruits and vegetables and are mainly coloured blue, purple, and red (Wang & Stoner, 2008). Anthocyanins are categorised chemically as 2-phenylbenzopyryllium glycosides of polyhydroxy or polymethoxy derivatives. These compounds are based on 6 different aglycones (anthocyanidins) shown in **Figure 1**. They are distinguished by the number and position of the hydroxyl and methoxyl groups in the molecule. Although there are more than 20 different varieties of anthocyanins in nature, the majority of plant species only have a few of them. The chemical structures of most abundant anthocyanins in fruits and vegetables are given in **Figure 2.** The most prevalent anthocyanin found in several plants is cyanidin-3-glucoside (Wu et al., 2006; Yue & Xu, 2008; Khoo et al., 2017).



Cyanidin Delphinidin Peonidin

Pelargonidin Petunidin Malvidin

Figure 1. Chemical structures of anthocyanidins (PubChem, 2022)



Pelargonidin-3-glucoside Cyanidin-3-glucoside Peonidin-3-glucoside

Delphinidin-3-glucoside Petunidin-3-glucoside Malvidin-3-glucoside

Figure 2. Chemical structures of most abundant anthocyanins (PubChem, 2022)

Anthocyanins may be in variety of forms such as protonated, deprotonated, hydrated, and isomeric, and these forms may play an important role in the antioxidant action of anthocyanins. These forms are highly pH dependent. At low pH (pH 1-3), the red flavylium cation predominates, while in aqueous media, at pH 4-5, hydration reactions produce a colorless carbinol pseudo-base. The flavylium cation can alternatively be converted to quinonoidal bases via proton transfer reactions and further converted to blue-purple quinonoid anions at pH 6-7. Since anthocyanins lose their color at high pH (>3), their use in acidic products is limited (Dangles et al., 2000; Kähkönen & Heinonen, 2003; Wahyuningsih et al., 2017).

Researchers have been trying to develop simple and effective analytical methods to purify, identify and detect anthocyanins as anthocyanins are extremely unstable substances that are influenced by factors like pH, temperature, light, oxygen, and solvents. Since HPLC is a fast, reliable, sensitive, robust and easy analytical technique to apply for target compounds determinations, it is frequently preferred in anthocyanin determination. The high chromophoric properties of anthocyanins lead to the frequent use of UV detector systems in HPLC applications. (Can et al., 2012; Castañeda-Ovando et al., 2009; Rein, 2005; Alappat & Alappat, 2020). For extraction and purification of anthocyanins in various samples, some pretreatment methods have been used such as SPE (da Silva et al., 2020; Liu et al., 2015)

Several analytical studies have been shown to determine anthocyanins in **Table 1**.

Table 1. Analytical techniques for determination of anthocyanins / anthocyanidins in the literature.

Analytical technique	Numerous of Anthocyanin	Samples	Ref.
HPLC - PDA	6	28 different food samples (vegetable fruit processed commercial products)	(Can et al., 2012)
HPLC UV-vis spectrophotometer Paper chromatography LC-MS	8	Black, red and wild rice	(Kim et al., 2008)
UPLC-Q-TOF MS Combined with QAMS	10	Grapes	(Li et al., 2022)
HPLC-DAD HPLC-ESI-MS/MS	2	Raspberry	(Xue et al., 2020)
HPLC-DAD HPLC-MS	10	Bilberry extracts	(Zhang et al., 2004)
HPLC-DAD-MS	6	Blueberry	(da Silva et al., 2020)
Table 1 (continued)			
HPLC-ESI-MS	4	Strawberry fruit	(Karaaslan & Yaman, 2017)
UPLC-MS/MS	9	American elderberry Fruit Juice	(Johnson et al., 2015)
UHPLC-PDA	14	Grapes	(Shim et al., 2014)
UHPLC-QToF UHPLC-PDA-MS	4	Elderberry	(Avula et al., 2022)
HPLC-UV	2	Purple sweet potato	(Zhang et al., 2016)
HPLC-DAD	1	Mulberry	(Guo et al., 2019)

3. Conclusion

In recent years, interest in products with antioxidant properties has increased due to healthy eating trends. Anthocyanins are one of the most important sources of antioxidants found in fruits, vegetables and various plants. Due to their health benefits, issues such as the extraction, purification

and determination of these substances are of critical importance in the field of food and health. Given the beneficial effects of these molecules on health, their use in the food and beverage industries will be of significant value. Especially considering various side effects, natural products should be preferred more than synthetic products, and more sensitive, easy and environmentally friendly analytical methods should be developed to determine the contents of natural products containing anthocyanins.

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CHAPTER IV

THE ROLE OF PHENOLIC PHYTOCHEMICALS IN FOOD PRESERVATION

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1. Introduction

In recent years, with the Covid-19 pandemic affecting the whole world, individuals' perceptions of healthy lifestyles have also changed. Societies have become more conscious about nutrition and healthy living. The fact that the "healthy diet" recommended by the WHO consists mainly of plants has increased the interest shown in plant foods significantly (Caccialanza, et al., 2020)

Phytochemicals are naturally occurring compounds in plant foods. It is mostly found in foods such as fruits, vegetables, legumes, spices, whole grains. They do not have nutritional properties on their own. Phytochemicals are bioactive compounds that have an important role in the formation of the smell, color and taste of plants, appear as secondary metabolites and have beneficial effects in terms of health when consumed as a nutrient. As the interest in the use of plant phytochemicals as an alternative to synthetic substances used in the treatment of many chronic diseases has increased, a new market has been developed. Phytochemicals in plants show biological activities such as antioxidant, antimicrobial, anticancer, antidiabetic, antifungal, anti-inflammatory, and antihypertensive (Tsao, Khanizadeh, & Dale, 2006).

Foodstuffs contain a wide variety of phytochemicals between 500 and 25000, and about 500 of them are "phenolic compounds" and constitute the most popular group of compounds with important health effects (Acosta-Estrada, Gutierrez-Uribe, & Serna-Saldivar, 2014).

Phenolic compounds, which are phytochemicals found in plants, are naturally found in various plant products such as vegetables, fruits and grains and are phytochemicals that determine the characteristics of foods such as taste, smell and color. At the same time, it protects the plant against various pests by playing a role in the defense mechanism of plants (Tufarelli, Casalino, Alessandro, & Laudadio , 2017).

The most basic functions of phenolic phytochemicals are to act as natural antioxidants, and they show different biological functions because they have different chemical structures. In particular, they play an important role in maintaining the balance between antioxidants and oxidants. Thus, it is effectively used to prevent oxidation reactions of foods, increase shelf life and increase nutritional quality with bioactive enrichments. In addition, it creates a protective shield against microbial degradation that occurs during the post-harvest storage and transportation processes of foods (Shetty, 2004).

Seafood, meats, fruits and vegetables often have very short shelf lives, as they are easily subjected to oxidation reactions. For this reason, the food industry supports the development of appropriate preservation technologies to extend the shelf life of this and similar foods, and the number of investments in this field is increasing day by day (Krishnan , et al., 2014).

Many have made use of synthetic preservatives as they are easy to obtain and low cost. However, although these synthetic chemical preservatives are allowed to be used in some countries today, it has been shown in studies by scientists that they have negative effects on consumer health. Therefore, the demand of consumers is for the development of natural preservatives that have beneficial effects on human health (Inetianbor, Yakubu, & Ezeonu, 2015).

In addition, synthetic preservatives can be associated with carcinogenic effects and cause undesirable consequences for consumer health (Nowak, Czyzowska, Efenberger, & Krala, 2016). Therefore, the perception of consumers regarding the consumption of foods containing high chemical compounds, as a result of causing other diseases, has encouraged the

search for natural preservatives, including plant derivatives (Serra, et al., 2008).

Currently, researchers are intensely searching for preservatives derived from natural bioactive plants. These natural preservatives should provide the benefit of replacing synthetic preservatives traditionally used by the food industries, as well as ensuring food safety and extending shelf life (Thielmann, Kohnen, & Hauser, 2017).

Advances in research on phenolic phytochemicals, and especially focusing on the biochemical and chemical basis of the functions of these compounds, help us to understand the basic principles of food phenolic compounds in food preservation and their role in human health. Easy identification, widespread distribution, chemical stability and chemical variability make phenolic phytochemicals useful for multiple functions (Naczk & Shahidi, 2004). In addition to preserving food, phenolic phytochemicals potentially increase the bioactive nutraceutical properties of food; this relates to especially long-term and slowly progressing noncommunicable chronic diseases such as cancer, cardiovascular disease and diabetes (Shetty, 2004)

2. Oxidative Stress and Synthetic Antioxidants in Food Industry

Oxidative stress has been defined in different ways by many researchers with studies on oxidative stress. Finally, Jones et al. (2006) defined "the shift of the balance between pro-oxidants and antioxidants in favor of pro-oxidants, an imbalance that results in interruption in signal transduction and molecular damage". As a result of metabolic reactions, including homeostatic conditions, in our body, free radicals, which are highly reactive molecules whose atoms offer one or more unpaired electrons, are produced (Kehrer & Klotz, 2015).

Free radicals were first considered as chemicals that harm the body because they are produced naturally as a result of metabolism reactions in the cell and show high oxidative potential (Di Meo & Venditti, 2020). Afterwards, as researches on this subject increased, it was seen that free radicals use important reactions such as cellular homeostasis, pathogen recognition, receptor activation, gene expression and signal transduction as triggers.

Oxidation reactions in foods reduce the sensory properties of foods. It also causes deterioration in the taste, smell and color of the food, and the resulting toxic compounds cause safety problems for consumers (Galanakis, 2021).

Food companies often add antioxidants directly to the food matrix to control oxidation reactions when foods are poor in antioxidants or contain prooxidants (Barden & Decker, 2016). In food science, the term 'antioxidant' is often used to specify compounds that prevent other oxidative reactions such as lipid peroxidation, extending the shelf life of food products (Yang, et al., 2018).

Synthetic antioxidants often used in the food industry are phenolic compounds, the most significant of which are: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertbutyl hydroquinone (TBHQ) and propyl gallate (PG) (Xu, et al., 2021) Although the use of these synthetic antioxidants is common, their misuse or overuse creates safety concerns (Liu & Mabury, 2020). Studies on this subject have concluded that high doses of synthetic antioxidants can give rise to DNA damage (Eskandani, Hamishehkar, & Ezzati Nazhad Dolatabadi, 2014) or toxicity in tissues (Ham, Lim, You, & Song, 2020).

Based on all these studies, the food industry is looking for natural substitutes to reduce the use of synthetic antioxidants based on the fact that consumers are exposed to synthetic antioxidant compounds and their harm in their daily diets (Lourenço, Moldao-Martins, & Alves, 2019).

3. Chemical Structures of Phenolic Phytochemicals

Phenolic compounds, including their functional derivatives, are structures in which one or more hydroxyl groups are attached to the aromatic ring (Robards & Antolovich, 1997). The simplest phenolic compound is benzene containing one hydroxyl group and is called phenol (Balasundram, Sundram, & Samman, 2006). Phenolic compounds containing more than one hydroxyl group are known as polyphenols (Bravo, 1998).

Over 8000 phenolic compounds have been isolated from different natural products, including phenolic acids, flavonoids, coumarins, tannins and stilbenes. Each group is divided into subgroups according to its chemical structure (Figure 1) (Manach, Scalbert, Morand, Remesy, & Jimenez , 2004).

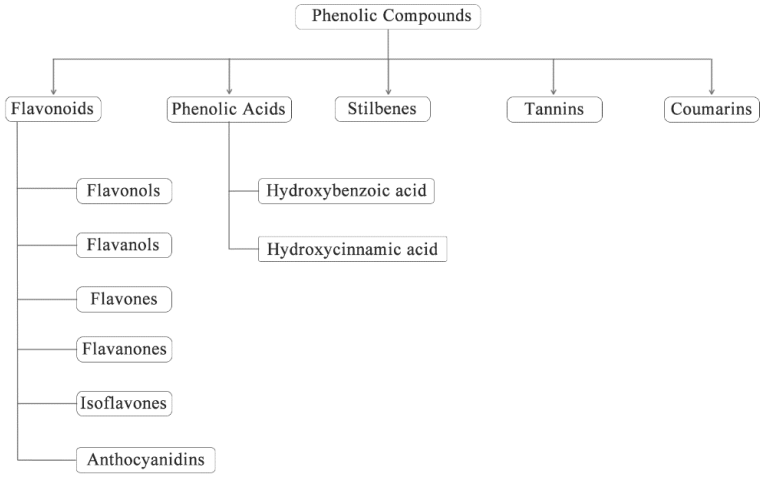

Figure 1. Classification of phenolic compounds
(Manach, Scalbert, Morand, Remesy, & Jimenez , 2004)

Phenolic compounds are one of the groups commonly found in plants (Naczk & Shahidi, 2004). To date, numerous phenolic compound structures have been identified in plants, and newly identified phenolics are constantly being added to them (Robards & Antolovich, 1997). They are found in many fruits, vegetables, cereal products, herbal products and beverages that are consumed continuously in daily life. Phenolic compounds as food ingredient; It is important in many respects, such as its effects on taste and odor formation, its participation in color formation and change (Alasalvar, Grigor, Zhang, Quantick, & Shadidi, 2001). Phenolic compounds, which constitute an important group of phytochemicals, are known to have high antioxidant activity and free radical scavenging capacity, thanks to their mechanism of inhibiting the enzymes responsible for the production of reactive oxygen species (ROS) and the mechanism of reducing highly oxidized ROS (Robards, Prenzler, Tucker , Swatsitang, & Glover, 1999).

3.1. Flavonoidler

Flavonoids are formed by the combination of two phenyl rings with the propane chain and are in the structure of diphenylpropane (C_6-C_3-C_6) containing 15 carbon atoms (Figure 2). The flavone derivatives of flavonoids, which are the most common polyphenols found in foods, are called flavonoids, and flavan derivatives are called flavonoids. Aromatic rings in flavan and flavone structures are denoted by A and B, and heterocycle by C (Figure 3,4) The carbon atoms

in the A and C rings are numbered starting from the oxygen atom. Atoms in the B ring are numbered with exponential numbers (Harborne & Williams, 2000).

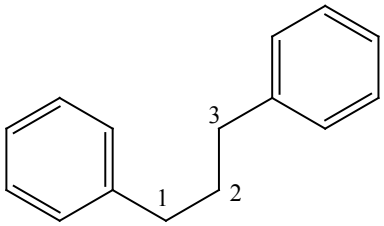

Figure 2. Diphenylpropane structure

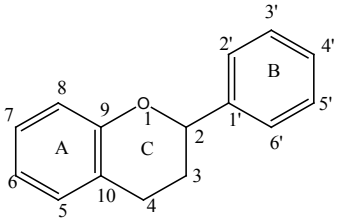

Figure 3. Flavan structure

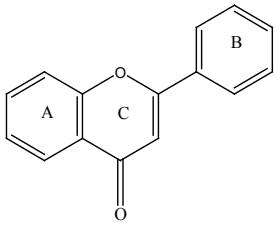

Figure 4. Flavone structure

Flavonoids are structurally divided into 6 groups: flavonols, flavanols, flavones, flavanones, isoflavones and anthocyanidins.

3.1.1. Flavonols: This group of compounds are commonly found in foods in the form of glycosides. The main ones are; kaemferol, quercetin, myricetin and isoramnetin (Figure 5) (Price, Breen, Valladao, & Watson, 1995).

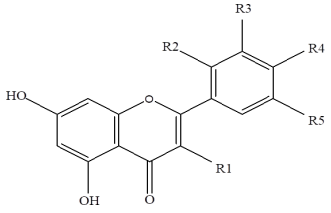

Figure 5. Flavonols

3.1.2. Flavanols: Catechins in this group are colorless compounds. They are the most commonly found flavonoids in foods. Because catechins contain an OH group on the C_3 atom, they are called flavan-3-ol (Figure 6) (Arts, Van de Putte, & Hollman, 2000).

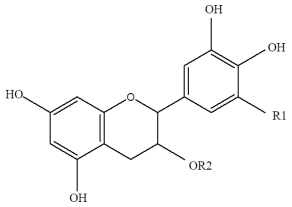

Figure 6. Flavanols

3.1.3. Flavones: They are less common in fruits and vegetables than flavanols. The most prominent flavones found in food are luteolin and apigenin (Figure 7) (Sartelet, Serghat, & Lobstein, 1996).

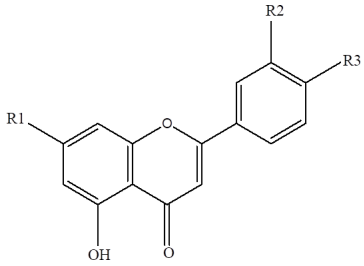

Figure 7. Flavones

3.1.4. Flavanones: This group of compounds is also found in nature in the form of glycosides. They are very common in citrus fruits. Naringin, hesperidin, naringenin are the most common flavanones (Figure 8) (Tomas-Barberan & Clifford, 2000).

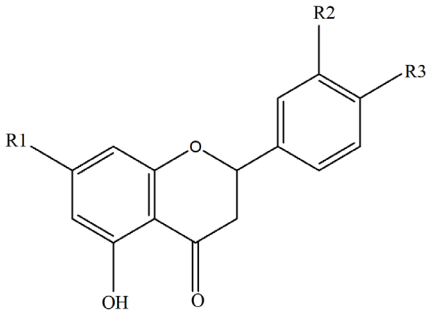

Figure 8. Flavanones

3.1.5. Isoflavones: Found in various legumes, especially soybeans, and some fruits and vegetables. Genistein and daidzein are the most common isoflavones (Figure 9) (Coward, Smith, Kirk, & Barnes, 1998).

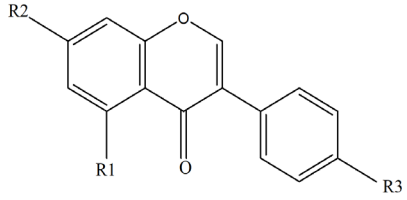

Figure 9. Isoflavones

3.1.6. Anthocyanidins: They are mostly found in berry fruits such as blackberries and raspberries and are water-soluble pigments responsible for the color of red, blue and purple fruits and vegetables (Figure 10) (Clifford, 2000).

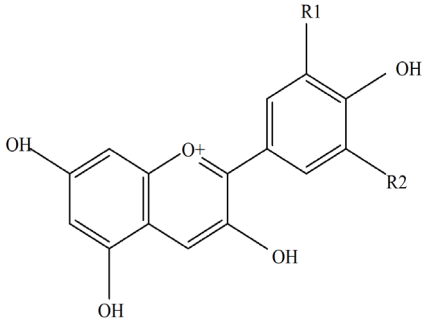

Figure 10. Anthocyanidins

3.2. Phenolic Acids

Phenolic acids are hydroxylated derivatives of cinnamic acid and benzoic acids. They are grouped as hydroxybenzoic and hydroxycinnamic acids (Spacil, Novakova, & Solich, 2008). Hydroxybenzoic acids have C_6-C_1 phenylmethane structure and vary according to the location and number of hydroxy and methoxy groups in their structures. Benzoic acid derivatives, which are colorless compounds, are usually found in trace amounts in plant foods. The most common are *p*-hydroxybenzoic acid, syringic acid, vanillic acid, gallic acid and protocatechuic acid (Figure 11) (Tomas-Barberan & Clifford, 2000).

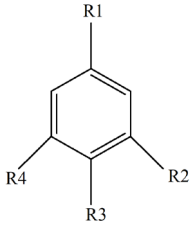

Figure 11. Benzoic acid derivative

Hydroxycinnamic acids, which have C_6-C_3 phenylpropane structure, differ according to the position and structure of the OH group. They are commonly found in plant foods. The most common ones are chlorogenic acid, *o*-coumaric acid, *p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid and cinnamic acid (Figure 12) (Sosulski , Krygier, & Hogge, 1982).

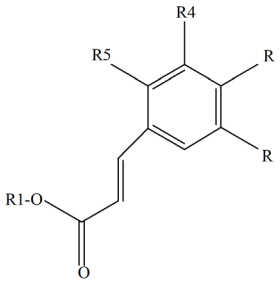

Figure 12. Cinnamic acid derivative

3.3. Stilbenes

Plants synthesize stilbenes to increase their resistance in case of disease (Goyal, Lambert, Cluzet, & Ramawat, 2012). Stilbenes are mostly synthesized by peanuts, pine trees and grape vines (Bavaresco, 2003). Their skeleton is based on 1,2-diphenylethylene structure. Resveratrol and piseid are the two main stilbenes produced from the grape vine. trans-resveratrol is the most studied compound for its effect on human health (Figure 13, 14) (Delmas, Lancon, Colin, Jannin, & Latruffe , 2006).

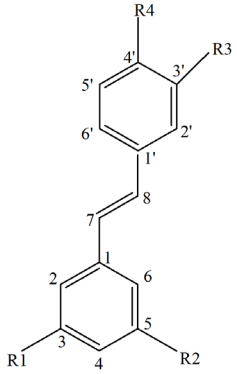

Figure 13. trans-Stilbenes

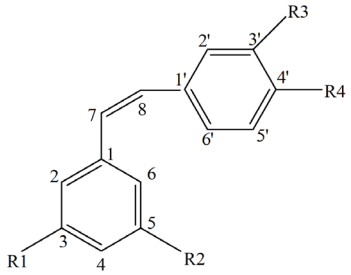

Figure 14. cis-Stilbenes

3.4. Tannins

They are known as phenolic polymers. They are high molecular weight compounds. When food tannins are mentioned, polymers of catechin and epicatechin are generally understood (Figure 15) (Ignat, Volf, & Popa, 2011).

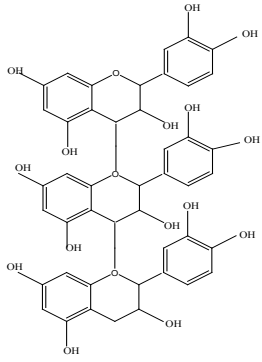

Figure 15. Tannins

3.5. Coumarins

Coumarins, also known as benzopyrones, are generally found in parts of plants such as seeds, roots, stems, flowers and fruits. It forms a broad class of phenolic compounds derived from cinnamic acid found in bacteria, plants and fungi (Lake, 1999). It has been proven that coumarins have biological activities in a wide range of areas such as antiviral, anti-inflammatory, antimicrobial, antioxidant, anti-tumor, and antidepressant (Figure 16) (Klenkar & Molnar, 2015).

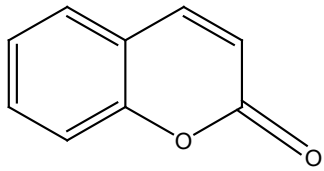

Figure 16. Chemical structure of coumarin

4. The Role of Phenolic Phytochemicals in Food Preservation

Meat products are very sensitive to oxidation reactions because their structures are rich in polyunsaturated fatty acids, and the composition of the meat has a significant effect on oxidative stability (Cunha, Monteiro, Lorenzo, & Munekata, 2018). Natural and synthetic antioxidants are used in the meat industry (Manessis, et al., 2020).

Thyme and rosemary extracts have been the subject of countless scientific studies until now. In recent years, ethanolic extracts from cork oak leaves (Quercus suber L.) with rich phenolic composition (epicatechin, gallic acid, catechin, rutin, quercetin or myricetin) have been studied to prevent oxidation in a cooked chicken model and have high showed antioxidant activity and tested conditions inhibited lipid oxidation equivalent to BHT (Durazzo , et al., 2019).

Tea polyphenols show a strong antioxidant activity with catechins, anthocyanidins, flavones and phenolic acids in their structure. Since catechins constitute 85% of the polyphenol content of green tea, the antioxidant activity is chiefly due to catechins. Phenolic acids and catechin oligomers, such as caffeic acid and gallic acid from apples, are potent inhibitors of cholesterol oxidation in meat products such as sausage, bacon, hamburger, ground beef and ham (Maqsood, Abushelaibi, Manheem, Al Rashedi, & Kadim, 2015).

Seafood is a real challenge to preserve as it has a shorter shelf life compared to red meat and chicken. Fish products have high nutritional value because they contain macro and micro elements and are an indispensable food for a healthy diet. The lipid content causes them to be easily oxidized. Phenolic phytochemicals have potential practices in the seafood industry as bioactive compounds. Citrus peel has been shown to be rich in flavonoids and phenolic acids and have higher antioxidant activity compared to synthetic antioxidants. The effects of banana peel (Musa sp. L.) and cabbage leaves (Brassica oleracea var. capitata L.) extracts on the oxidation stability of fish meats were investigated. It has been shown that phenolic antioxidants can block the food spoilage process by interacting with free radicals (Ali, et al., 2019).

Since edible vegetable oils are highly sensitive to oxidation reactions, they cause great difficulties during storage, cooking and processing. They reduce nutritional value and shelf life of foods by causing bad tastes and sour odors in foods. Many plant extracts have been successfully added to edible oils to increase oxidative stability. The essential oil of Coriandrum sativum L. was found to be rich in phenolic compounds, and it was reported that the oxidative stability of this oil increased with the addition of sunflower oil and it had similar activity with the synthetic antioxidant TBHQ (Wang, et al., 2018).

Fish oils are rich in omega-3 and are prone to oxidation reactions. A preventive effect was observed when myricetin was added (Guitard , Paul, Nardello-Rataj, & Aubry, 2016). Dihydroquercetin has been successfully used to inhibit oxidation reactions in salmon oil and extend the shelf life of food (Dragoev, Balev, Ivanov, & Vassilev, 2014).

Industrial processing of vegetables and fruits increases the risk of oxidation in these food matrices. Studies have shown that phenolic phytochemicals can be used to prolong the shelf life and maintain quality of some fruits, such as litchi (Litchi chinensis), which suffer from polyphenol oxidase activity (Jiang, Duan, Joyce, Zhang, & Liu, 2004).

5. Conclusion

Current processes used in the food industry have synthetic preservatives that can be used as antioxidants to extend the shelf life of foods. However, studies have shown that some of the synthetic preservatives can cause health problems among consumers. The idea of using natural preservatives is increasingly accepted by consumers seeking natural and healthy products. In recent years, the increasing consumer perception of the need for more natural

and safer additives and food processing techniques has led to increased efforts to use plant metabolites such as phenolic phytochemicals which are a group of phytochemicals with potential health-promoting effects.

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CHAPTER V

TECHNOLOGICAL ENZYME RESEARCH

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1. Introduction

Many articles have been made on enzyme. In this section, some articles related to enzyme have been reviewed. These studies are:

In an article, the authors explain in the summary and conclusions that; Natural selection-driven evolution is a common approach in protein engineering for enzymatic design. It allows the right iterative evolution of existing proteins to those with the desired characteristics with random mutagenesis application in the laboratory. However, library construction is the most fundamental part of guided evolution. The implementation of various building methods affects both the number and variety of variants created and the selection techniques used. Early procedures, including error-prone PCR, mutator ornaments, chemical mutagen and gene mixing, have succeeded in the entire gene mutagen, however, more selection/scanning efforts are required by the major larger libraries. On the other hand, recent approaches such as the use of degenerate primers and field saturation mutagen have reduced the scanning/selection effort by allowing random mutagen of amino acids in certain positions in the polypeptide chain. In particular, the active site remains of the bicatalytic converters have been selected as targets and their catalytic efficiency has been increased. CYP119, a member of the cytochrome P450 protein family

from Sulfolobus Acidocaldarius, is a thermostabyl enzyme that can cathalize peroxidation, monooxygenation and oxidation reactions. Here, a mutant library of CYP119 varials was created through the application of a combination active region saturation test (CAST) in 213–214 amino acid positions, and an effective fluorescent-based method was developed to scan the library for increased peroxidase activity when using hydrogen peroxide as an oxidizer. After scanning the mutant library, a variant of Thr213Arg–Thr214Ile subs showed peroxidase activity increasing by 1.32 times in the Amplex Red catalyst compared to wild type CYP119. Advances in recombinant DNA technology have allowed a high amount of protein exposure Enable the use of bicatalytical converters instead of chemical catalytic converters in industrial applications.. Benefits of substrate specifiality, enantioseccension, chemical selectivity and both temperature and pH stability have made them valuable tools. In addition, it is possible to obtain or further improve the desired characteristics with protein engineering methods. In this work, a mutant library of a thermophilic The enzyme has developed an efficient scanning method to determine the variance that exhibits higher peroxidase activity when hydrogen peroxide is used as an oxidant created by CYP119. Three different substrates were used to assess the peroxidase activity of CYP119, including ABTS, guaiacol and Amplex Red. All substrators were catapulted by CYP119 and Amplex Red was found appropriate for scanning the mutant library because of superior fluorescent efficiency over the control group. The improved method allows fast scanning of dissolved enzymes on 96-hole plates. In the screening of the 66 CYP119 variance, CYP119 Thr213Arg–Thr214Ile pairs of mutants showed 1.32 times higher peroxidase activity than the wild type CYP119. Consequently, this study first performed a combination active zone saturation test of CYP119 and developed an improved scanning method to scan enriched enzymes in supernatant rather than purify. Furthermore, the first oxidation of guaiacol was informed in this case by CYP119 (Haklı, E. ,2018).

In another article, the authors explain in the summary and conclusions that; The ligninolithic and pektinolithic enzymes, respectively, disrupt the lignin and pectin components and plant biomass used in the main feeds, food and various industries are to produce energy, wastewater treatment, textile, oil removal, plant fibers and paper wetting industries. Pektinolithic enzymes dominate %10 and %25 of the total enzyme market. worldwide food enzyme market. This is a major bottleneck in domestic industries, the supply of affordable enzymes to local markets. The heterologist enzyme production is a key. the technology that

uses the powerful tools of molecular biology and allows production is custom products for special applications, e.g. requested enzyme mixtures. Pichia pastoris is a methylotropic yeast and a promising host for industrial products. biotechnology, because it can be used for high levels of protein production. In addition, suitability for high cell density planting, the capacity to secrete proteins produced, the processing sequence downstream processes are cheaper, the presence of the genome array, the convenience of genetics manipulations and the ability of post-transmissions changes, P. pastoris is an interesting host for a heterologist protein production. In this thesis, five pectinolithic and ligninolithic enzymes; polygamacturonase, pectin methyl esters, pectin lyserase, lachase and peroxidase heterologists of ligine P. made in pastoris. Recombinant enzymes are produced on a laboratory scale under AOX1 proengines that can be stimulated by methanol, and their activities are measured by products that are released from coarse extract. As a result, five different P. pastoris ornaments that can produce target enzymes are designed using recombinant DNA technology. This thesis offers a successful implementation of recombinant DNA technology. the production of five pectinolithic and ligninolithic enzymes. In applications despite successful upstream, the final enzyme flakes are low, and therefore improvement of the downstream process is necessary for subsequent applications. In accordance with this, additional upstream manipulations must be taken into account. Increase tremors and productivity (Akçadağ, G., 2019).

In another article, the writers explains in the summary and conclusions that; The hydraulics of the biomass obtained as industrial or agricultural production waste have seen significant interest in recent years. 2 different biomass studies have been studied in this study. 1 of your work. In the sugar beet generation hydraulics are optimized using the Response surface Methodology. The sugar beet sash is a product of sugar production and its fluid allows to produce precious sugar that can be used for fermentation. The effect of the enzyme concentration, the duration of the hydraulic trace and the quantity of the trace are examined as the fluid parameters of the SBP with the RSM. The experiment design was made using a 5-level central composite design and the amount of vesubstrat, two different enzyme-Pectinex Ultra SP-L and Cellic Ctec3 concentrations and the effect of the hydraulic duration were the parameters examined. When 20% SBP is used, the output has been found to be higher than 90 g/L as a result of the increase of the Pectinex Ultra SP-L to over 250 µl. As the SBP percentage increases, it has been determined that efficiency has reached maximum, even when using Cellic Ctec3 with low volume of around 150 µl. The analysis results

show that the use of the Pectinex Ultra SP-L in combination with Cellic Ctec3 has a sinergic effect. The estimates obtained as a result of the RSM model have shown that the optimal point for maximum reduced sugar efficiency is beyond the experimental range used in this thesis. In addition to the RSM, the classification and Regression Tree method is used to investigate the effects of substrate amount, enzyme concentrations, and hydrolysis time. The results of the regression Tree analysis confirmed the estimates on the RSM. In addition, the analysis results can form a basis for future optimization studies. 2 of your work. in, the corncob is used as substrate for your hydraulics. The corncob is a content product of the high leagues and requires different pre-treatment methods to be used with the hydraulic system. In this study, instead of using the pre-processing method, surfactants are used to improve the accessibility of enzymes to the cellulose network through the ligine. Tween 80 and 20 are used as surface active material. The hydraulics were performed in the 0.05 M sodium citrate buffer with Novozyme 188 andpH of 4.8, Celluclast 1.5L, used as enzyme at a constant concentration of 150 μL and the hydraulics were monitored for 24 hours. The results have shown that the effect of the surfactant supplement is not effective on your hydraulics unless the league is removed from the environment by a pre-treatment method (Leyluhan Yurtseven, B. ,2019).

Researchers investigating the use of ligno-cellulosic biomass are focusing on eliminating costly pre-processing methods. The composition of the Lignin depends on different sources of biomass. The corncob has a higher league than most biomass sources, while the sugar beet sash has a lower amount of leagues. The surfactants have recently been used in the preconprocessing phase of lignoselulosic biomass, and some studies have shown that surfactants give promising results that increase productivity. In this article, two biomass were hydrated as enzymatic. In the first part, the sugar beet generation was optimized using your hydraulics, reaction surface Methodology (RSM) and Classification and Regression Tree (CART). No pre-treatment has been performed because the amount of leuking is low on the beet bird. The findings from the RSM showed that the change in substrate % is relatively important than the change in the amount of Pectinex Ultra SP-L, because the old one has more effect on efficiency. Due to the cellulose content is higher at the SBP than the grain, the lower substrate% and the Cellic Ctec3 volume have resulted in slightly higher efficiency. As expected, productivity increased with both higher substrate quantity and longer hydraulic time. The results showed that the combination of cellulose, hemiselulase and pectinazine creates a synergic response and

increases reductive sugar efficiency. Negative interaction between reaction time and Cellic Ctec3 content was also introduced. Inhibision was observed as expected in higher volumes of Cellic Ctec3 because selobious or glucose formation slowed the rate of hydrysis-final product inhibition. Regression tree results confirmed the findings from the RSM; maximum reductive sugar efficiency was achieved when the substrate was greater than 18%. Reduced sugar efficiency was higher when the substrate was between 14% and 18%, the reaction time was less than 15 hours, the Cellic was greater than CTec3 300 kl, and the Pectinex Ultra SP-L was greater than 300 kl. Classification tree analysis results must be greater than 14% to achieve high reductive sugar efficiency of substrate content 46, reaction time must be at least 15 hours and enzyme loads must be 250-300 liters. The amount of substrate and the reaction time have also dominated the classification results. Enzymatic hydrysis experiments were performed in both the avicel and avicel-corncob combinations to see the effects of surprise on the biomass containing the leagues. If the pre-treatment method is not used, it has been concluded that Tween 80 and 20 have no significant effect on your hydraulics. The Lignin presence is still a nunchink and has not been hung only by the lynchings. Due to the sugar beet pavement has a very low league level, the cost-effective pre-decency phase can be easily eliminated. The yield was quite high below 24 hours of reaction time. Therefore, the actual cost is the cost of enzymes. Reducing the amount of enzyme during the fluid of the SBP should also be another goal. Working on enzyme blends with Pektinaz, cellulose and hemiselulosis can also give remarkable results. For biomass rich in the perspective of the League, the addition of the phial does not necessarily make any meaningful results while eliminating the pre-treatment phase. It is recommended for further works to observe the effect of the surfactant during the pre-treatment of the Lignoselulocyclic biomass (Leyluhan Yurtseven, B. ,2019).

In another study, the writers explain in the summary and conclusions that; Enzymes are used as additives to improve the quality parameters of the muffins. However, high temperature conditions produce compounds containing carbon. pioneers of toxic maillard reaction products. In this work, three food-grade enzymes are used as agents to reduce the formation of carbon-containing compounds, while preserving cake quality factors. Transglutaminase, lipase and amylase enzymes were used for this goal. Each of the three enzymes reduced their carbonated quantities as follows: The largest decrease in lipaze by 31.83% compared to the control cake. The inclusion of transglutaminase and lipase has changed the carbon profile of the muffins. Both transglutaminase and lipase have

caused significant changes in protein secondary structures, while alpha wrap has not caused such large changes, along with large increases in rotations and anti-parallel beta structures. The three enzymes used reduce lipid/protein ratio. The lipid saturation level has not changed, transglutaminase and lipase, but the level of saturation has decreased, amylase showing the formation of dicarbonate is due to Maillard reaction which is not linked to lipid peroxidation. However, the GC-MS analysis results showed no change. In the head gap analysis of neither Maillard reaction products nor lipid oxidation products. The amorphous structure of starch in cake samples increases depending on the enzyme concentration used (Er, A. E. ,2021).

Transglutaminase, lipase and amylase enzymes were used to improve the cake. properties while reducing carbon build-up. Each of the three enzymes used form a low amount of carbon. In addition, enzymes have changed carbonate. profiles, protein secondary structures, and lipid/protein ratios. The GC-MS results indicated that there was no change in the formation of any of the Maillard reactions. Lipid oxidation products in products or top cavity analysis (Er, A. E. ,2021).

In another article, the authors explain in the summary and conclusions that; Tirozinase, cathalize L-thyrosin's transformation into L-DOPA and finally melanin type-3 is an enzyme with copper content. Melanin, biotechnology and biological functions the has a wide range of and is widely used in pharmaceutical, cosmetic and other industry areas. L-DOPA is a preferred drug for treating Parkinson's disease. In this study, a type of Bacillus containing tiroinase enzymes is isolated from nature and is defined by morphological, biochemical and molecular analysis. The production of natural insulation enzymes is based on classical and statistical methods optimized and enzyme characterized. even more, the tirozinaz-encoding gene In the two express systems using pET22b as the express vector cloned. The gene exposures in the two systems are compared. The Gen fragmenti is greeted by the Sanger sequence method. The sequence has been subjected to insilo analysis using different bioinformatics programs and websites. L-tirozin is converted into L-DOPA and melanine by HPLC and TLC methods. The melanin produced has been examined. The isolated ornament showed 99% homology with Bacillus megaterium. The production of enzymes of the natural M36 Bacillus type is 0.05 IU/mL. After optimizing cultural conditions, it reached 0.38 IU/mL. The recombinant reached 31 IU/mL after optimizing the exposure of M36 tiroinase enzymes. The optimal temperature for natural and recombinant M36 thyroinase enzymes was 40°C and 45-50°C

respectively; the optimum pH was 7.0 and 7.5 respectively. In the SDS-PAGE analysis, the natural and recombinant M36 thyroinase enzymes were determined to be 34kDa and 35 kDa respectively. According to the analysis of the M36 melanin, FT-IR and EPR produced in this study, Sigma has shown structural similarity to the standard synthetic melanin. At the same time, M36 melanin produced had significant anti-bacterial, anti-UV and anti-oxidant effects. The MTT experiment has shown that M36 melandis have an anti-cancer effect (Valıpour E. ,2015).

This is bacillus sp in the investigation. it is isolated from the moisture that has the least enzymes of tyrosine. Sus has shown homology up to 99% with Bacillus megaterium. Thyroinase enzyme, M36 Bacillus sp. successfully produced and characterized by. The natural M36 thyroinase enzymes and l-tirozin have been converted into both L-DOPA and melanine, and this conversion has been approved using TLC and HPLC. The corresponding gene trailer was cloned to E. coli BL21 pLysS and Rosetta-Gamut 2 pLysS using the pET22b vector and its exposure was successful. Comparison of the two systems showed that the enzyme in Rosetta-Gamut 2 pLysS was the street head of the statement in E. coli BL21 pLysS. Bioinformatics analysis of the M36 tiroinase genes and protein sequences showed that the enzyme is similar to the tiroinase enzymes obtained from the Bacillus megaterium reported by Shuster and others. The production of the enzyme by both natural and recombinant cells was optimized using the triala design by Design-Expert 9 program. Native M36 Bacillus sp. enzyme production by. It has reached 0.38 IU/mL with the optimization of the culture condition, set at 0.05IU/mL, and has reached 31 IU/mL with the optimization of the recombinant M36 tiroinase enzyme exposure. The M36 melanin produced in this study showed structural similarity to the standard synthetic melanin, based on resolution, FT-IR and EPR analyzes. It also showed significant anti-bacterial, anti-UV and anti-oxidant effects. The MTT test showed that M36 melanis had an antibody effect. Purified DNA processed by M36 melanin was not affected. This system is therefore suitable for the production of melanin and has many industrial functions and biotechnology of melanin (Valıpour E. ,2015).

In another article, the authors explain in the summary and conclusions that; Matrix Metallloproteins are a group of protease enzymes that contain zinc in active areas and serve as a component of extrasellular matrix fragmentation and reformation. MMPs play an important role in tumor formation, tissue invasive, angiogenesis and tumor metastases in particular. Matrix Metalloproteinase-2 is an enzyme that is a member of the Jelatinase class of the pancreas and MMP

family, skin, propropane, bladder, breast, it has been determined that there has been significant increases in activity in colon, lung and ovarian cancers. Many MMP inhibitors have been developed to control this activity increase, but there have been selective problems with the developed inhibitors. Moreover 36 amino acids of chlorotoxin is a peptide of scorpion venom. The inhibitor in its structure has high stability due to the node pattern of the sistein and selectively inhibe by targeting MMP-2. It is known that the determination of the peptitis in the plasma of the ring chlorotoxin is more stable than the linear chlorotoxin. This is thought to reduce proteolithic division. Aim of this study is to synthesize the MMP-2 enzyme, which has a significant role in cancer studies, with rings and flat chlorotoxin peptide derivatives that can inhibe in high selectivity. As a result, the derivative of straight chlorotoxin, which is only more than 7 amino acids, has been synthesized. However, the MMP-2 enzyme activity study showed that this synthesized derivative had no inhibisyon effect on the enzyme. Another zinc metallopeptidase, known as angiotensin converter, is an important component for checking enzyme, blood pressure, fluid and electrolyte balance. Its best-known function is to convert angiotensin I into angiotensin II, a powerful vasopressor in living tissue. For this reason, ACE inhibitors can be used in many areas of heart and vascular disease, from high blood pressure to vascular congestion. On the other hand, cojic acid is used as an inhibitor of the thyroid enzyme, which causes melanin hyperpigmentation disease. This inhibitor effect is caused by the ability of cojic acid to clamp the copper ion in the active area of the enzyme. It also features a clamp against ions such as cojic acid zinc, nickel, iron and gold. In addition, because the ACE active center has zinc ions, it can inhibe this enzyme by creating a cojic acid zinc clamp. The purpose of this study is to synthesize cojic acid-peptid conjugates that may have a high degree of ACE inhibitor effect. As a result, the conjugate of two cojic acid-phenylfields has been synthesized. However, the effects of synthesized conjugates on the enzyme were not studied because no activity was observed in the enzyme as a result of the activity studies with the already acquired ACE (Çiftçi, B. ,2014).

As a consequence, the two enzymes of the zinc-metalopeptidazes family, MMP-2, and peptide inhibitors for ACE, were tried to synthesize. In the first part of the study, the chlorotoxin derivative for the inhibition of MMP-2 enzyme activity was synthesized by the Fmoc-based SPPS method. Chlorotoxin is a disulsibly rich peptide and is known to have an inhibitor effect on MMP-2 activity. It is also known that chlorotoxin is more stable than the linear form of the frequency form containing 7 aminoacids. From here on, the frequency and

linear forms of chlorotoxin derivatives with these seven amino acid residues were tried to be synthesized with the SPPS. As a consequence, the linear form of the chlorotoxin derivative was successfully obtained, while the synthesis of the frequency form failed. The inhibitor effect of this linear chlorotoxin derivative on MMP-2 was measured by activity testing, but no inhibitor effect was observed. In the part 2 of the study, cojic acid-amino acid conjugates were synthesized. Cojic acid is known as a good metal selector and inhibe the activity of thyroinazine by sellinging Cu2+ ions in the active area of the enzyme. Moreover, ACE is known as a zinc metalloproteinase. Kojik acid was thought to be able to block ACE activity by sellinging Zn2+ ions in the active area of the enzyme. Cojic acid-amino acid conjugates are tried to synthesize to increase inhibission selectivity and stability. As a result, two derivatives of cojic acid-phenylalanin conjugate were synthesized. However, due to the activity problems of commercial AEs, the interaction of these conjugates with the enzyme was not examined (Çiftçi, B. ,2014).

In another study, the writers explain in the summary and conclusions that; In this study, various microbial and enzymatic methods have been developed for the synthesis of enzymatic asymatic asyclocytes for the preparation of some pharmaceutical-important intermediate productsBy carrying out biotransformation through aspergillus flavus, the enantioselective (76%) biooxidation of mézo-hydrobenzoïne was obtained with a high value. In the same bio-oxidation process, the rasematic form of hydrobenzoin was used, which caused the form of a mezo that verifies the recommended oxidation-reduction sequence mechanism of hydrobenzoin. Wieland-Miescher keton is an important start for bioactive compounds, such as the steroids and terpenoids below. Many synthetic approaches include the enantibody reduction of this compound. In this study, the reduction of the Wieland-Miescher keton with the Aspergillus niger was achieved with a high yield (80%), de (79%) and ee (94%) value, and these results were far superior to those previously reported. studies. Karbolization enzymes benzoiyl format decarboksilaz and benzaldehyde used for synthesis of bicatalytic asilolin. These enzymes are immobilized to surface modified superparamagnetic silica coated nanoparticles using the metal ion afinity technique. With this system, the recombinant histidin labeled HONEY and BFD is purified by the single container purification-immobilization procedure and immobilized to magnetic particles. The SDS page analysis showed that our surface-modified magnetic particles are suitable for specific bonding of histidin-labeled proteins. Conventional HONEY and BFD catalyst benzoin condensate

reactions and some representative asytoine reactions were performed with this system with high enzyme (99- 92%) and efficiency. The results founded with the magnetic particular enzyme system were also found to be comparable to the free enzyme catalyst reactions (Sopacı, Ş. B. ,2009).

In this study, the aim of developing oxidorized biomaterials is to develop biotransforms using biotransforming and carboligation reactions with a immobilized enzyme agent for synthesis of α-hydroxxydrocyte, the essential building block for the preparation of bioactive compounds. As part of all cell bioconversions A. Flavus and A. biooxidation and bioreduction reactions were performed through niger. Biooxidation of mezo-hydrobenzoin has provided moderately high ee (78%) values to obtain enantioxidant benzoin through biotransforming (Sopacı, Ş. B. ,2009).

In another article, the authors explain in the summary and conclusions that; Living in hot places called thermophilis is for biotechnology they were a very important matter. As a natural result of adapting to hot spaces thermoods are heat resistant to make them the target of biotechnology they produce enzymes. In our study previously, lipaz and esterase from the Balcova (Izmir) Thermal Zone Bacillus sp, isolated and characterized by their activities. we used their bacteria. To measure the activity of esters and lipase, bacteria should use tween 20 detergent, respectively, and He was incubated in five environments containing rhodamin-B. Only 3 of about 110 groups of bacteria showed high lipase and esterase activity. From different environments The different three esters collected and two lipaz consensus were cloned directly from the genomic DNA using degenerate primers using the PCR replication method. The amino acid sequences from these three different esterazes showed that there are only a few amino acids differences between them. But the characterization of the lipaz genes that were lined up was so complicated that the characterization study was only done for esters. E. coli has strong esters and lipaz genes to provide high software It was cloned to pET28a vectors containing a promotor of T7. Purification of recombinant lipase and esophone proteins was done on one step using the his-Select HF nickel afinite gel method. Enzyme Ascounts with different emergency chain lengths (C2-C16) have proven esterity activity, where various p-nitrophenyl (p-NP) esters are used as substrate. All the esters showed very specific activity against all the tested p-NP esters. The purified esters, the optimal pH and temperature required for the robustness and operation of Est2, Est1 and Est3, the effects of various metal ions, inhibitors and detergents were determined and compared (Tekedar, H. C. ,2009).

The main purpose of this study is to characterize, purify and examine it. he chose two non-cell enzymes due to potential use as a biotechnological biotechnology bicatalyst in a variety of biotechnological applications. About 110 thermomesh scones were scanned for the presence of two non-cell enzyme activities. These two enzymes are limarkets and esters substrate for an enzyme scan of Tween 20 and Rhodamin-B with olive oil used as an enzyme. The second step is to amplify the genes that encode these two enzymes with PCR technique and clone them to Escherichia coli for expression. Using the PET 28 a (+) exposure vector, three esters were completely over-exposure, while three lipase, partially over-exposure. The enzymes that were overexposure were later purified by afinite chromatography techniques. The PET 28 a (+) express system adds the corresponding gene-tag region with affinity to the beads of some metals, such as nickel. The total cell proteins were passed through the nickel column and the column content was divided into fractions with the LPLC. The highest enzyme activity was seen in p-nitrophenyl acetate, which has a relatively long, twelve carbon chains, according to substrate specificity results. It has been investigated that pH values of optimum pH esterase enzymes are relatively alkaline. It resists bacterial esterase temperature and pH change. Therefore, esters can be used and applied in many areas with advanced characterization methods and studies (Tekedar, H. C. ,2009).

In another article, the authors explain in the summary and conclusions that; Biorunas are increasingly important, with the benefits they have over oil-based products and an environmentally friendly production based on renewable resources. When we look at the classes of Biyorun, the most intensive areas of research and arrival are pharmaceutical and biofuel production. Biodiesel, which has the greatest potential as biofuel, can also be produced using microorganisms. The wax equivalent timing enzymes, which have a key role in this synthesis, have been identified in previous studies that are isolated from different organisms. In this study, two wax equivalent feeler enzymes from Psychrobacter arcticus 273-4 and Muş musculus C57BL/6 are intended to be cloned and purified. P. The arcticus 273-4-welded wax estery is cloned to the pET22bTV vector, manufactured using the cell line BL21 of the Escherichia coli bacteria and purified with approximately 1 mg of efficiency. The other wax equivalent feeler enzymes from the mus musculus C57BL/6 are cloned to the Saccharomyces cerevisiae yeast type and purified with less than 1 mg. The taxadien-5α-ol-O-acetyl-transferase, due to the last enzyme Taxus cuspidata, has an important role in the bio-synthesis of the most commonly used chemotherapy drug, Taxol.

This enzyme is cloned for pET22bTV fog-temine, which is the gene expression vector. The enzyme was then produced using BL21 (DE3) star cell lines with Escherichia coli bacteria. Approximately 23 mg of protein was obtained as a result of purification procedures. As a basic approach, all three enzymes were purged by the immobilized metal afinite chromatography method. This study has been indirectly helpful in future structural protein engineering studies, revealing the conditions for the cloning and purification of three important enzymes involved in the biosynthesis of promising products (Gürelme, E. ,2016).

In this project, three biosynthetic enzymes with promising applications in the field of biodiesel and pharmaceutical production were examined. Biosynthesis of fatty acid ethyl esters in microorganisms is more advantageous and sustainable than production with a conventional diesel method based on transesterification. The key role in this bio-synthesis is an enzyme called wax ester feeler. The cloning and purification of wax ester synthesis is the main topic of this study. Psychrobacter arcticus 273-4 based wax ester feeler, successfully cloned to pET22bTV express vector systems. The exposure tests result in paWES with 0.5mm IPTG at 20 ˚C by the E. coli BL21 ornament. Purified through IMAC with ~1 milligrams of efficiency. Also, because the PPAWES protein with a sense-mark has a problem connecting TO THE IMAC column, relocating the his-label may increase the effectiveness of the purification step. The wax ester feeler from Muş musculus C57BL/6 is the other enzyme studied. The cloning of MmWES was performed for two different organisms, the PET system in bacteria and THE GAL system in yeast. In particular, the solutions to the basic problems of the bacterial exposure of an ocaryotic enzyme have been investigated. MmWES express s. Cerevisiae was made with 2% galactic powder at 30˚C with BY4741 ornamations and purified with less than 1 mg. The exposure level was lower than expected, because of the series used it may be because the code is not optimized for cerevisiae. Exposure and purification efficiency can also be increased using different types of yeast as host organisms. To provide a model for future studies, the cloning and purification of these enzymes is required for the renewable production of biodiesel. In particular, the structural work of these enzymes can illuminate the catalytic mechanism and resolve limitations on FAEE production. In addition, the production of a large amount of novo biodiesel in microorganisms can be inspired by these studies. The third enzyme that was studied belonged to the acetyltransferratic class in Taxol's biosynthesis, a drug that was mainly used in chemotherapy. Acetyltransferers have important functions in critical steps of the Taxol biosynthesis path. The cloning and

purification of the taxaxus cuspidata-tachsadien-5α-ol-O-acetiltransferazine is one of the main objectives of this study. After previous studies, it was cloned to TcT5AT, pET22bTV vectors and 23 mg of efficiency E. Coli BL21 was successfully expressed by the star ornament at 18 ˚C at 0.5 mm IPTG. It was obtained at an estimated 80% purity according to protein purification methods performed with immobilized metal afinite chromatography and dimensional exclusion chromatography. The purification of TcT5AT continues. In protein purification applications, the purity of the protein can be increased by different chromatographic methods, such as ion exchange chromatography. In the future, this purified protein can be used in mechanical and structural analysis of TcT5AT, and can affect the development of new pioneers along with increased Taxol biosynthesis for therapeutic applications (Gürelme, E. ,2016).

2. Conclusion

A lot of research has been done in the field of biotechnology (KORKMAZ, S. A., & GÜNDOĞDU, S. G.), (KORKMAZ, S. A., & ÇAKIR, V.). However, in this study, studies in biotechnology on enzyme were examined. In an article, the authors explain in the conclusions that; The main purpose of this study was to characterize, purify and investigate. two non-cell enzyme biotechnology applications selected due to potential use as various bicatalyst. about 110 thermophilic decorations have been scanned for. the presence of 2 extracellular enzyme activities. These two enzymes are esters of substrate for an enzyme scan of Tween 20 and Rhodamin-B with libics and olive oil used as. The second step is to amplify the genes that encode these two enzymes with PCR. He was cloned to Escherichia coli to express his technique and his expression. Using the expression PET 28 a (+), the vector three esters were fully overstated, while the three lipase was partially overstated. Enzymes that are overexposure are then purified through affinity. chromatography techniques. The PET 28 a (+) exposure system adds the its-label region again. the area of interest that some metals, like nickel, have an affinity to beads. The total cell proteins were passed through the nickel column and the contents of the column were fractionated with the LPLC (Low Pressure Fluid Chromatography) the highest enzyme activity is relatively long, with twelve carbon chains, p-nitrophenyl acetate. Comparatively alkaline pH values of optimal pH esterase enzymes were investigated. It resists bacterial esterase temperature and pH change. This means that the esters can be used and can be applied in many areas with more characterization methods and studies (Tekedar, H. C. ,2009).

In another article, the authors explain in the conclusions that; In this project, three biosynthetic enzymes, which have promising applications in the field of pharmaceutical and biodiesel production, were examined. Biosynthesis of fatty acid ethyl esters in microorganisms is more advantageous and sustainable than production with a conventional diesel method based on transesterification. In this bio-synthesis, the key role is an enzyme called wax ester feeler. The study of the cloning and purification of wax ester synthesis is the main topic of this study. The Psychrobacter arcticus 273-4-based wax ester feeler (PaWES) was successfully cloned into pET22bTV express vector systems. As a result of the testifying tests, PaWES was obtained with 0.5mm IPTG at 20°C, with an E. coli BL21 (DE3). Purified by ~1 mg of efficiency via IMAC. As there is also a problem connecting the his-labeled PaWES protein to THE IMAC column, relocating the his-label can improve the efficiency of the purification step. The wax ester feeler from Muş musculus C57BL/6 (MmWES) is the other enzymes investigated. The cloning of MmWES is provided for two different organisms, the yeast GAL system and the PET system in bacteria. In particular, so-lusyon methods have been investigated for basic problems in the expression of ocaryotic and bacteria enzymes. The express of MmWES is 2% galactic at 30°C, s. Cerevisiae was made with a BY4741-bit mist and purified with less than 1 mg of efficiency. The level of expression was lower than expected, because of the series used it may be because there is no codeone optimized for cerevisiae. Expression and purification efficiency can also be increased by using different types of yeast as host organisms. To provide a model for future studies, the cloning and purification of these enzymes is required for the renewable production of biodiesel. In particular, the structural work of these enzymes can illuminate the catalytic resolve and mechanism limitations on FAEE production. In addition, the production of a large amount of novo biodiesel in microorganisms can be inspired by this work. The third enzyme examined belonged to the acetyltransferase class in Taxol's biosynthesis, a drug mainly used in chemotherapy. Acetyltransferers have important functions in critical steps of the Taxol biosynthesis path. Cloning and purification of the taxadien-5α-ol-O-acetiltransferazine (TcT5AT) from the Taxus cuspidata is one of the main objectives of this study. Following his previous work, he was cloned to TcT5AT, pET22bTV vectors and E. Coli BL21 (DE3) successfully expressed with star-like 18°C and 23 mg output at 0.5 mm IPTG. It was obtained at an estimated 80% purity according to protein purification methods with immobilized metal afiniteli chroma-tography and size exclusion chromatography. The purification of TcT5AT continues. In protein purification

applications, the purity of protein can be increased by different chromatographic methods, such as ion exchange chroma-tography. In the future, this purified protein can be used in mechanical and structural analysis of TcT5AT, and may affect the development of new pioneers along with the increased biosynthesis of Taxol for therapeutic applications (Gürelme, E. ,2016).

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CHAPTER VI

AN OVERVIEW OF THE ANTICARCINOGENIC EFFECTS OF THE ESSENTIAL OIL OF *THYMUS SERPYLLUM* L. AND *THYMUS VULGARIS* L.

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1. Introduction

Cancer is a complex disease that may be found practically anywhere around the globe. It is defined by the abnormal, uncontrolled growth and spread of cells and is one of the leading causes of mortality in humans. It can vary depending on age, genetics, diet, and environmental variables (Peto, 2001; Urruticoechea et al., 2010; Yurtaslan, 2005). All over the world, cancer is the leading serious health problem in terms of morbidity and mortality in almost every country. In the 20th century, it ranked seventh and eighth on the list of diseases that caused deaths, but currently, it comes in the second rank to cardiovascular diseases (Karabulut and Uslu 2006). Important advancements in cancer therapy have recently been made with the use of new techniques. Chemotherapy, radiation therapy, and surgery are the main cancer effective treatments. In addition to these treatments, laser treatment, hormone therapy,

and bone marrow transplantation are also used (Oylar ve Tekin, 2011; Akdemir 2011). There are several side effects to these treatments. The tendency toward natural materials like plants has accelerated as a result of the presence of these side effects (Collins et al., 2011; Monsuez, Charniot, Vignat, & Artigou, 2010; Nobili et al., 2009; Selwood, 2008). According to research, consuming phenolic chemicals found in plants is connected with a decreased occurrence of cancer in people (Ahmed ve ark., 2013).

Many different plant species are cultivated in Türkiye and are regularly used by the general public to reduce the symptoms and effects of many diseases. Many properties of these widely used plants such as antispasmodic, antidiabetic, and anticancerogenic are utilized (Abu-Irmaileh & Afifi, 2003).

There are many aromatic plant species from the Lamiaceae family, known as thyme, and used for this purpose among people. However, the species that contain thymol/carvacrol type components in the essential oil content are considered thyme. Thymus, Origanum, Satureja, Thymbra, and Coridothymus, which are known as thyme, are of great importance both in terms of distribution in the world and commercially (Baser, Özek, Kürkçüoglu, & Tümen, 1994).

Carvacrol, the primary component of thyme oil, juice, and extract, is known to have a significantly stronger antioxidant activity than several synthetic antioxidants. It possesses antibacterial, antifungal, natural food preservation, and anti-aging characteristics in humans. This molecule is a volatile monoterpene. Carvacrol is said to have strong antimutagenic and anticancer properties (Ipek et al., 2005).

2. General Properties

2.1. Thymus vulgaris L.

Thymus vulgaris L., a significant flowering plant from the Mediterranean area of Europe and a member of the Lamiaceae family, has a strong, aromatic, and spicy flavor. Its length is 15-30 cm and its width is 40 cm. The majority of European countries cultivate it (France, Svizzera, Spain, Italy, Bulgaria, Portugal). Yield and quality vary depending on the amount of oil, the genetic makeup of the ingredients, the maturity of the crop at harvest, and hardening and distillation follow-up (Reddy et al. 2014). Asthma, numerous inflammatory illnesses, and infectious diseases are all treated with this herb. Its essential oils have antibacterial, antifungal, and antioxidant effects (Amirghofran, Ahmadi & Karimi, 2012).

Essential oils such as borneol, carvacrol, cymol, linalool, and thymol; flavonoids such as apigenin and luteolin; saponins; tannin, and triterpenic acids are some of the herb's basic components. The phenolic components of *Thymus vulgaris* L. essential oil (%) were listed in Table 1.

Extracts and essential oils from its leaves and flowers can be used as aromatic additives in foods, medicines, and cosmetics. *T. vulgaris* has sedative, expectorant, diaphoretic, antispasmodic, and carminative effects. In addition to the pharmacological effects of thyme such as antifungal and antibacterial, spasmolytic, antitussive, expectorant, and secretomotor, the essential oil of *T. vulgaris* has many uses in medicine (Khosravipour ve Direkvand-Moghadam, 2016; Kovács ve ark., 2016). The distribution map of *Thymus vulgaris* L. was given in Figure 1.

Taxonomic Classification

Kingdom: Plantae

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Subfamily: Nepetoideae

Genus: Thymus L.

Species: *Thymus vulgaris* L. (Reddy et al., 2014).

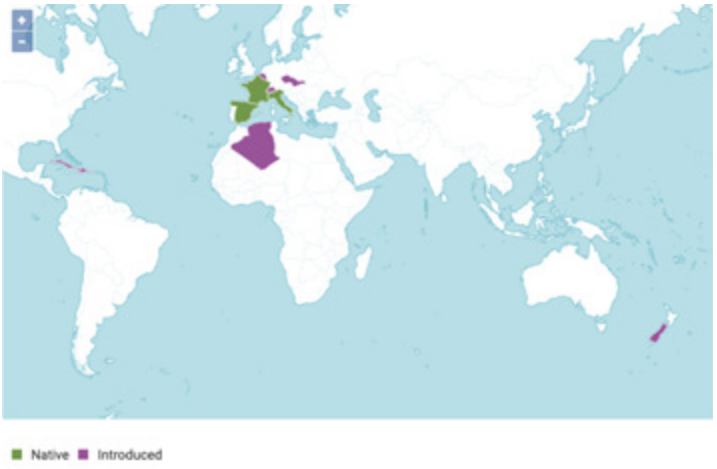

Figure 1: Distribution map of *Thymus vulgaris* L. (URL-1).

Table 1: Phenolic Components of *Thymus vulgaris* L. essential oil (%).

p-Cymene	Thymol	Carvacrol	Geraniol	P-Cymol	linalool	γ-Terpinene	*β-Caryophyllene*	Reference
15,25	52,61	-	-	-	-	-	6,77	(Contini et al., 2020)
	3	-		23,8	2,6	15,4	-	(Sertel et al.,2011)
1	1,29	-	17,91	-	42,06	2,71	-	(Amorese et al.,2018)
6,6	63,7	2	0,6		4	7,7	1,3	(Said-Al et al., 2019)
19,20	42,10	2,70	-		5,67	3,43	-	(Capatina et al.,2020)
7,85	16,54	16,78	-	-	-	8,83	20,71	(Niksic et al.,2021)
0,2	24,72	-	-	-	-	68,41	5,50	(Alexa et al.,2018)
11,29	6,12	45,53	0,07	-	9,60	3,84	-	(Dash et al.,2021)
6	55,88	8,36	1,48		13,71	1,04	2,72	(Ali;2021)
24,3	33,2	8,6	0,2	24,3	5,7	0,2	3,6	(Catauro et al.,2017)

2.2. *Thymus serpyllum* L.

The Lamiaceae family of aromatic plants includes wild thyme (*Thymus serpyllum* L.), which is recognized for its medicinal and biological qualities (Hussain et al., 2013). It is indigenous to high-altitude regions of the Mediterranean, Europe, and North Africa. It is used in a variety of conventional home treatments. This herb has stimulant, diuretic, expectorant, carminative, analgesic, antiseptic, and analgesic effects. Additionally, it can be used in mouthwash and mouthwash to treat colds and coughs (Nikolić et al., 2014). *T. serpyllum* possesses essential oil components that have been shown to be effective disinfectants and immunostimulants in fighting a variety of infections. In addition to being used in hair loss treatments, its essential oil relieves rheumatism (Aziz et al., Nikolić et al., 2014).

The use of *T. serpyllum* essential oil is increasing day by day due to its pharmacological properties. According to sources, the geographical region, the plant's stage of growth, the season of harvest, the habitat, and the meteorological conditions of the area all have an impact on the chemical components and yield of essential oil of *Thymus serpyllum* essential oil. The phenolic components of *Thymus serpyllum* L. essential oil (%) were listed in Table 2. Due to its pharmacological properties, thyme oil represents an important natural resource for the pharmaceutical industry. In the food industry, it can also form the basis of natural antioxidants, supplements, and functional foods (Jarić, Mitrović, & Pavlović, 2015). The distribution map of *Thymus serpyllum* L. was given in Figure 2.

Taxonomic Classification

Kingdom: Plantae

Order: Lamiales

Family: Lamiaceae

Genus: Thymus L.

Species: *Thymus serpyllum* L. (URL-2)

Figure 1: Distribution map of *Thymus serpyllum* L. (URL-3).

Table 2: Phenolic Components of *Thymus serpyllum* L. essential oil (%).

p-Cymene	Thymol	Carvacrol	Geraniol	P-Cymol	linalool	γ-Terpinene	*β-Caryophyllene*	Reference
8,9	38,5	4,7	-	-	2,4	7,2	0,4	(Nikolic´ et al., 2014)
26,1	35,4	62,7	-	-	-	29,5	29,3	(kulisic et al.,2005)
5,3	25,1	2,2	0,3	-	30,9	0,6	-	(Bajet al.,2020)
-	13,21	55,19	-	11,24	-	13,52	1,42	(Kirillov et al.,2016)
21,9	41,6	3,2	-	-	2,3	19,2	-	(Xie et al.,2020)
3,46	0,18	45,24			0,38	7,68	0,40	(vesolowska et al.,2014)

2.3. Anticarcinogenic Effects of the Essential Oil of Thymus vulgaris L.

2.3.1. The effects of essential oil of T. vulgaris on breast cancer cells

In a study by Najar et al., they obtained the essential oil of commercially purchased plant seeds in Italy. When the effects of essential oil on three different human breast adenocarcinomas cells (MCF7, T47D, and MDA-MB-231) were examined, the LC_{50} values were 39.9 ppm, 61.5 ppm, and >300 ppm depending on the cytotoxicity of the essential oil against MCF-7, MDA-MB-231, and T47D cells, respectively. It has been reported that the cytotoxic effect of the essential oil on MCF7, and MDA-MB-231 cells is strong, and its cytotoxicity against T47D cells is moderate (Najar et al., 2020). Berdowska et al. obtained essential oil from the commercially available *T. vulgaris* plant in Poland by hydrodistillation method. They investigated the cytotoxic effects of this essential oil on Adriamycin/MCF-7 and MCF-7 cancer cells. One of the cell lines in the study was used as obtained from ATCC and the other was MCF-7 cells grown in the presence of increasing Adriamycin (7-10 μM) concentrations for 3 months. The EC_{50} against Adriamycin/MCF-7 cells was determined to be 407 mg/mL. Essential oils have been reported to cause greater cytotoxicity against Adriamycin/MCF-7. In addition, it was determined that essential oil caused more cytotoxic effects with increasing concentration (Berdowska et al., 2013). Ali obtained the essential oil of the leaves of the *T. vulgaris* sample collected from the north of Saudi Arabia with the Clevenger type apparatus. Essential oil at a 100 μg/mL concentration has caused apoptosis in MCF-7 cells (Ali, 2021). Niksic et al. obtained the essential oil of the leaf, flower, and stems of *T. vulgaris* collected from Bosnia and Herzegovina with the Clevenger apparatus. The essential oil exhibited its potent antiproliferative effect against the MCF-7 cell line. compared to the reference drug doxorubicin. The IC_{50} value was found as 52.65 μg/mL (Niksic et al., 2021). Zu et al. investigated the anticarcinogenic effects of *T. vulgaris* essential oil commercially available in China on MCF-7 human breast cancer cell lines. In the study, which was reported to increase the anticarcinogenic effect due to the increase in concentration, the IC_{50} for essential oil of thyme was determined as 0.030% (v/v), and considering this value, it was reported that thyme essential oil caused a strong cytotoxic effect (Zu et al., 2010). Nikoli´c et al. obtained the essential oil of commercially available *T. vulgaris* sample in Greece by the Clevenger method and investigated its anticarcinogenic effects on many different cancer cell lines. These anticarcinogenic effects were also compared with different types of thyme. The GI_{50} value of this essential

oil on MCF-7 cells was determined as 180.49 μg/mL. In the same study, when the cytotoxic properties of *T. vulgaris* essential oil against colon, breast, lung, cervix, and liver cells are compared, it was reported that it exhibited the weakest effect on breast cancer cells (Nikolić et al., 2014). Hassan et al. commercially purchased Egyptian plant samples as seedlings and obtained essential oil by hydrodistillation with Clevenger. When the effect of this essential oil on MCF-7 cells is compared with the reference drug doxorubicin, it has been determined that it exhibits weak anticarcinogenic activity. The IC_{50} values of three samples under different cultivation conditions were determined as 1.43-7.19 μg/mL. The cytotoxic effect of *T. vulgaris* essential oil has been related to the monoterpenes (o-cymene) found in it (Hassan et al., 2019).

2.3.2. The effects of essential oil of T. vulgaris on lung cancer cells

Nikoli´c et al. examined the cytotoxicity of essential oil of *T. vulgaris* essential oil on NCI-H460 lung cancer cells, as well as its effects on breast cancer cells, and the GI_{50} value was determined as 160.01 μg/mL. This result shows that it is similar to its cytotoxicity on MCF-7 breast cancer cells(Nikolić et al., 2014). In the study made by Guardo et al., the essential oil of the above-ground parts of the *T. vulgaris* plant collected from Spain (Villarroya) was obtained with a Clevenger-type apparatus. The components of the essential oil were isolated and their cytotoxic effects on LC5 human lung fibroblasts cells were investigated. γ-terpinene, piperithone oxide, and p-cymene isolated from the plant exhibited close to 80% cytotoxicity only at the applied highest concentration (100 μg/mL), while β-pinene and carvacrol caused 50% cytotoxicity(Guardo et al. 2017). Niksic et al. studied the cytotoxic effects of the essential oil of the leaf, flower, and stems of *T. vulgaris* collected from Bosnia and Herzegovina on lung carcinoma H460 cells and compared them with the reference drug doxorubicin. According to the results of the study, the IC_{50} value of the essential oil on H460 cells was determined as 68.59 μg/mL and they defined the essential oil as a potent cytotoxic agent on these cells (Niksic et al., 2021). The cytotoxic effects of essential oil of *T. vulgaris* commercially available in China on human lung carcinoma (A549) cells were studied by Zu et al. According to the results of the study, it was determined that the cytotoxicity increased as the concentration increased. The IC_{50} value for thyme essential oil against A549 cells is 0.011% (Zu et al., 2010). Accordingly, *T. vulgaris* essential oil has been reported to be a potent cytotoxic agent. Hassan et al. investigated the anticarcinogenic effect of *T. vulgaris* essential oil on A-549 cells. The obtained data were compared with

the effects of the reference drug doxorubicin on the same cell line. The IC_{50} values of three samples under different cultivation conditions were determined as 1.4, 6.6, and 12.6 μg/mL. Considering that the IC_{50} for doxorubicin is 0.95 μg/mL, it can be said that the anticarcinogenic potential of only one sample is close to doxorubicin and the other two samples cause moderate cytotoxicity (Hassan et al., 2019).

2.3.3. The effects of essential oil of T. vulgaris on colon cancer cells

In addition to its effects on breast and lung cancer cells, Nikoli´c et al. also investigated the anticarcinogenic effects of essential oil of *T. vulgaris* on HTC-15 colon cancer cells. The results show that T. vulgaris essential oil causes stronger cytotoxicity on colon cancer cells compared to breast and lung cancer cells. The GI_{50} value of *T. vulgaris* essential oil for HTC-15 colon cancer cells was determined as 83.92 μg/mL (Nikolić et al., 2014). Hassan et al. investigated the cytotoxicity of *T. vulgaris* essential oil on HCT-116 and Caco2 colon and intestinal cancer cells. This effect was compared with that of the reference drug doxorubicin. The IC_{50} value of *T. vulgaris* essential oil grown under drought conditions was 0.44 μg/mL and 0.33 μg/mL for HCT-116 and Caco2 cell lines, respectively. The IC_{50} values of doxorubicin for the HCT-116 and Caco2 cell lines are 0.49 and 1.93 μg/mL, respectively. This clearly shows that this essential oil caused strong cytotoxicity on colon cancer cells. In addition, the findings of the study show that the sample examined exhibited similar cytotoxicity to the reference drug for lung cancer cells and less cytotoxicity for breast cancer cells (Hassan et al., 2019).

2.3.4. The effects of essential oil of T. vulgaris on cervix cancer cells

Nikoli´c et al. also investigated the anticarcinogenic effects of the essential oil of *T. vulgaris* on HeLa cervical cancer cells. The findings show that *T. vulgaris* essential oil exhibits the strongest cytotoxic effect (GI_{50} = 76.02 μg/mL) on HeLa cervical cancer cells when compared to colon, breast, lung, and larynx cancer cells(Hassan et al., 2019). In the study by Golkar et al., the essential oil of the *T. vulgaris* plant collected from Iran was obtained with the Clevenger-type apparatus. Researchers examined the effects of *T. vulgaris* on HeLa (cervical cancer) cells at 24, 48, and 72 hours of exposure. When HeLa cells were exposed to *T. vulgaris* essential oil for 72 hours, cell viability decreased by approximately 70%, and cell viability was determined as 55% and 80% at 48 and 24 hours of exposure, respectively (Golkar et al., 2020). In a study conducted

by Amorese et al. in 2018, the essential oil was obtained from a plant sample taken from Northeast Italy by hydrodistillation method. The essential oil of *T. vulgaris* has been reported to exhibit cytotoxicity exceeding 50% on WKD cells at concentrations lower than 0.12% (Amorese et al., 2018).

2.3.5. The effects of essential oil of T. vulgaris on human melanoma cells

In a study carried out by Alexa et al., essential oil of *T. vulgaris* was obtained from the aerial parts of *T. vulgaris* collected from Romania with the help of a Clevenger-type apparatus. At concentrations of 50 and 100 µg/mL, the percent inhibition rates of essential oil on A375 (human melanoma) and B164A5 (mouse melanoma) cells have been reported to be approximately 20% and 70%, respectively. In addition, it was reported that the inhibition rate increased 3.5 times with a 2-fold increase in concentration (Alexa et al., 2018).

2.3.6. The effects of essential oil of T. vulgaris on pancreatic cancer cells

Catauro et al. obtained essential oil from the different parts of *T. Vulgaris* plant (stems and leaves) collected from Southern Italy by hydrodistillation in n-hexane for three hours using a Clevenger-type apparatus. They investigated the anticarcinogenic effects of this essential oil on PANC-1 pancreatic cancer cells in the concentration range of 10-400 µg/mL. According to the findings, cytotoxicity increases as the concentration increases. It is also among the results of the study that the essential oil causes a strong cytotoxic effect on PANC-1 cells. Namely, even at a dose of 10 µg/mL (the lowest concentration studied), PANC-1 cell viability decreased. At the 100 µg/mL dose, the viability of PANC-1 cells was reduced by 60%. It has been reported that cell viability decreases below 10% at 400 µg/mL concentration. It has also been reported that *T. vulgaris* essential oil at all concentrations (10, 50, 100, 200, and 400 µg/mL) reduces the number of PANC-1 cells as exposure time increases (at 24, 48, and 72 hours) (Catauro et al., 2017).

2.3.7. The effects of essential oil of The effects of T. vulgaris on oral carcinoma cells

In a study by Sertel et al., the anticarcinogenic effects of essential oil obtained by hydrodistillation of flowers, leaves, and stipe of *T. vulgaris* plant grown in England (Herefordshire) on UMSCC1 8 (oral squamous cell carcinoma) cells were investigated. In 72 hours of exposure, essential oil caused a proliferation in the concentration range of 10^{-4}-10^{-1} µg /mL, while cell

viability decreased by 90% in the concentration range of 1-10 µg /mL (Sertel et al., 2011).

2.3.8. The effects of essential oil of T. vulgaris on lymphoma cells

In the study conducted by Abdelli et al., the essential oil of *T. vulgaris* leaves collected from two different regions of Algeria was obtained by the hydrodistillation method. Cytotoxic effects of essential oil on B-cell lymphoma (CLBL-1) cells were investigated and the IC_{50} value was determined as 35.3 µg /mL. It has been found that the cytotoxicity induced by the plant's essential oil differs depending on the place where it is harvested when assessed in terms of inhibition concentration (Abdelli et al., 2019).

2.3.9. The effects of essential oil of T. vulgaris on osteosarcoma cells

Catauro et al. obtained the essential oil of *T. vulgaris* stem and leaves, which they collected from Southern Italy, by hydrodistillation in n-hexane for three hours using a Clevenger-type apparatus. The cytotoxic properties of *T. vulgaris* essential oil in the concentration range of 10-400 µg/mL on human osteosarcoma U2OS were investigated. As the concentration of the essential oil increases, its cytotoxicity on U2OS cells increases. There was no decrease in U2OS cell viability at a concentration of 10 µg/mL. It has been reported that at a concentration of 100 µg/mL, U2OS cell viability was reduced by 40%. At 400 µg/mL concentration, cell viability was determined to be less than 10%. *T. vulgaris* essential oil caused a decrease in the number of U2OS cells at all concentrations studied (10, 50, 100, 200, 400 µg/mL) over time (24, 48, and 72 hours) (Catauro et al., 2017). It was reported by Abdelli et al. that the essential oil of *T. vulgaris* leaves collected from two different regions of Algeria caused cytotoxicity on osteosarcoma (D-17) cells. The IC_{50} value was found to be 90.38 µg /mL. It was determined that the cytotoxic effects of the essential oil of two plant samples taken from different regions differed. It has been reported that the IC_{50} value of the essential oil isolated from *Thymus vulgaris* from Tlemcen is lower than the IC_{50} value of the essential oil compared to the essential oil from Mostaganem (Abdelli et al., 2019).

2.3.10. The effects of essential oil of T. vulgaris on ovarian carcinoma cells

Contini et al. obtained essential oil from the aerial parts of *T. vulgaris* plant collected from Italy by hydrodistillation. They examined the cytotoxic effects

of essential oil of *T. vulgaris* on A2780 ovarian carcinoma cells and determined that cell viability decreased by 60% in 24 hours of exposure in the concentration range of 0.001 µl/mL - 1.0 µl/mL. The IC_{50} value reported in this study is 621.2 µg/mL (Contini et al., 2020). Tak et al. investigated the effects of *T. vulgaris* essential oil, which they purchased commercially in Canada, on T. ni ovarian cells. In addition, the effects of thymol, p-cymen, thymol:p-cymen mixtures, which are the major components of the oil, on this cell line were also examined in order to make a comparison. The researchers determined that the essential oil caused the strongest cytotoxic effect and the LC_{50} value was determined as 200.8 µg/mL. While the cytotoxicity of thymol and p-cymen were similar, the thymol:p-cymen mixture has a higher cytotoxic effect compared to the individual components and a lower cytotoxic effect than the essential oil (Tak, Jovel, & Isman, 2016).

2.3.11. The effects of essential oil of T. vulgaris on liver carcinoma cells

Dash et al studied the cytotoxic effects of *T. vulgaris* essential oil commercially purchased from India on human liver cancer cell line (HepG-2). In the concentration range of 6.25-100 µg/mL, the essential oil caused 35% cell death at only 100 µg/mL concentration. The essential oil has been reported to be negligibly cytotoxic at all other concentrations (Dash et al., 2021). Nikoli´c et al. evaluated the cytotoxicity of *T. vulgaris* essential oil on HepG2 liver cancer cells and determined the GI_{50} value as 95.06 µg/mL. The results of this study, which investigated the effects on different types of cancer, show that *T. vulgaris* essential oil has a stronger cytotoxic effect on liver cancer cells than on breast and lung cancer, and weaker than on cervix and colon cancer cells (Nikolić et al., 2014).

2.3.12 The effects of essential oil of T. vulgaris on prostate carcinoma cells

Dash et al. investigated the cytotoxic properties of *T. vulgaris* essential oil commercially available in India on human prostate adenocarcinoma (PC-3) cell lines. It has been determined that the essential oil causes negligible cytotoxicity in the concentration range of 6.25-50 µg/mL. It was determined that essential oil at a concentration of 100 µg/mL caused 55% of cell death on PC-3 cells (Dash et al., 2021). In a study by Zu et al., the cytotoxicity of *T. vulgaris* essential oil, which is commercially available in China, on PC-3 cells was investigated. While it was determined that cytotoxicity increased as

the concentration increased, the IC_{50} was determined as 0.010 % (Zu et al., 2010).

2.3.13. The effects of essential oil of T. vulgaris on leukemia cells

Najar et al. investigated the cytotoxic effects of *T. vulgaris* on human chronic myelogenous erythroleukemia (K562) cells by using the essential oil obtained from commercially purchased seeds in Italy and determined the LC_{50} value as 67.2 ppm. In the study, it was reported that the cytotoxic effect of essential oil on these cells was strong (Najar et al., 2020). The essential oil of the leaf, flower, and stems of *T. vulgaris* collected from Bosnia and Herzegovina by Niksic et al. was obtained using the hydrodistillation method with the Clevenger apparatus. The cytotoxicity of *T. vulgaris L.* essential oil on acute lymphoblastic leukemia MOLT-4 cells was compared with the reference drug doxorubicin and it was reported that the oil caused moderate cytotoxicity (IC_{50} 228.78 µg/mL) on these cells (Niksic et al., 2021).

2.3.14. The effects of essential oil of T. vulgaris on neuroblastoma cells

The essential oil of *T. vulgaris* seeds sold commercially in Italy was obtained by Najar et al. It was stated that the obtained essential oil caused strong cytotoxicity on SH-SY5Y human neuroblastoma cell lines, and they reported the IC_{50} value as 49.3 ppm (Najar et al., 2020).

2.4. Anticarcinogenic Effects of the Essential Oil of Thymus vulgaris L.

2.4.1. The effects of essential oil of T. serpyllum on breast cancer cells

Nikoli´c et al. investigated the effects of commercially purchased T. serpyllum essential oil from Greece on MCF-7 breast cancer. According to the results of the study, the cytotoxicity of essential oil on breast cancer is weak compared to its effects on colon, lung, liver, and cervical cancer cell lines. However, *T. serpyllum* essential oil was found to be more cytotoxic on MCF-7 cells compared to *T. vulgaris* essential oil. The GI_{50} value for *T. serpyllum* was determined as 52.69 µg/mL (Nikolić et al., 2014). The essential oil was obtained from the aerial parts of *T. serpyllum* plant collected in Pakistan by Hussain et al. using the hydrodistillation method. The antiproliferative activity of *T. serpyllum* essential oil on MCF breast cancer cells was investigated and compared with the reference drug doxorubicin. The IC_{50} was 95.8 mg/mL and moderate cytotoxicity was reported compared to the reference drug (Hussain et al., 2013). Berdowska

et al. obtained the essential oil of *T. vulgaris* and *T. serpyllum* by hydrodistillation method and compared their toxicity on Adriamycin/MCF-7 breast cancer cells. The EC_{50} value has been reported as 399 mg/mL. It was determined that the essential oil caused a more cytotoxic effect with increasing concentration. The essential oils of *T. vulgaris* and *T. serpyllum* have been reported to be similarly cytotoxic on MCF-7 cells (Berdowska et al., 2013).

2.3.2. The effects of essential oil of T. serpyllum on colon cancer cells

Nikoli'c et al. was obtained the essential oil of *T. serpyllum* plant sample purchased commercially from Greece by hydrodistillation using a Clevenger type apparatus. This essential oil has been reported to exhibit potent cytotoxicity on HTC-15 colon cancer cells. In the study, the anticarcinogenic activity of different thymus species against different cancer cells was investigated. According to the results of this study, which is one of the two studies in which the anticarcinogenic potentials of the two samples (*T. vulgaris* and *T. serpyllum*) we determined in our study were compared, *T. serpyllum* essential oil (GI_{50}= 7.02 µg/mL) has a potent cytotoxic effect on HTC-15 colon cancer cells, approximately 10 times stronger than *T. vulgaris* essential oil (GI_{50}= 76.02 µg/mL) (Nikolić et al., 2014). Azabayeva and Sylibayeva obtained the essential oil by hydrodistillation using the aerial parts of the *T. serpyllum* plant collected from East Kazakhstan. Researchers investigated the cytotoxicity of flavonoids isolated from essential oil on HCT-15 colon cancer cells. According to the data obtained, IC_{50} values for thymol, luteolin, and quercetin were determined as 40 µM, IC_{50} = 50 µM and 100 µM, respectively, and IC_{50} for apigenin-7-glucoside could not be determined in the applied concentration range. Since the effects of the essential oil on this cell line have not been studied, there is no data on this. However, the isolated anticarcinogenic activity of *T. vulgaris* essential oil has been attributed to the flavonoids in the oil content (Tazabayeva & Sylibaeva, 2018).

2.3.3. The effects of essential oil of T. serpyllum on prostate cancer cells

Hussain et al. investigated the anticarcinogenic potential of Pakistani *T. serpyllum* essential oil against LNCap prostate cells and compared it with the reference drug doxorubicin. The determined IC_{50} value was determined as 105.0 µg/mL and the essential oil was reported to be moderately cytotoxic (Hussain et al., 2013).

2.3.4. The effects of essential oil of T. serpyllum on lung, liver, and cervix cancer cells

Nikoli´c et al. studied the effects of commercially purchased *T. serpyllum* essential oil from Greece on lung (NCI-H460), liver (HepG2), and cervix (HeLa) cells. The IC_{50} values of *T. serpyllum* for these cells were determined as 37.17, 34.96, and 17.71 μg/mL, respectively. All these IC_{50} values are lower than those of *T.vulgaris*. As a result, it has been reported that *T. serpyllum* is more effective against these three cancer cells at lower concentrations and is more cytotoxic than *T. vulgaris* (Nikolić et al., 2014).

3. Conclusions

In conclusion, the effects of essential oil of *T. vulgaris* and *T. serpyllum* plant on cell lines of many cancer types such as breast, colon, cervix, leukemia, lung, liver, lymphoma melanoma, neuroblastoma, oral, osteosarcoma, ovarian pancreatic, prostate were investigated in vitro. Although the majority of researchers obtain essential oil by hydrodistillation, some researchers have purchased essential oil samples commercially. The above-ground parts of these two plants, whose essential oil is extracted by hydrodistillation, are generally used, but some studies have used leaves, stems, flowers, and seeds. Data for the cytotoxic profile are expressed as percent cell viability, IC_{50}, LC_{50}, and GI_{50}. MTT, XTT, Alamar Blue, and DNA Ladder assay were used as methods for determining cytotoxicity. Considering all these parameters, of course, it is difficult to make comparisons. Because in the evaluated studies, there are many parameters such as samples taken from different countries of the world, different parts of the plant, different experimental methods, and different concentrations. Among the factors affecting biological activity are altitude, the place where the plant is collected, soil quality, climate, the season in which the plant is harvested, the parts of the plant used, and the method used to obtain the extracts, etc(Fieldsend & Morison, 2000; Yang et al., 2018; Zargoosh et al., 2019). Despite all these differences, both *T. vulgaris* and *T. serpyllum* essential oil are thought to be promising natural remedies for many types of cancer. Because the idea that alternative medicine can be supportive alongside modern medicine is spreading more and more every day. For this reason, advanced in vitro/in vivo studies should be done for all plants to be used in herbal therapy and their toxic doses should be determined.

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CHAPTER VII

INVESTIGATION OF STUDIES ON GENETICALLY MODIFIED ORGANISM (GMO)

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1. Introduction

Many articles have been made on Genetically Modified Organism (GMO). In this section, some articles on GMO have been examined. These studies are:

In an article, the authors explain in the summary and conclusions that; As more than a hundred genetically modified organism (GMO) events have been developed and approved for commercialization globally, GMO analysis methods are essential for the application of GMO labeling regulations. Protein and nucleic acid-based detection techniques have been developed and used to identify and quantify GMOs. However, knowledge is needed for the global harmonization and standardization of GMO analysis methods. GMDD will be a platform for analysts to share their data, issues and ideas, with comprehensive information of almost all current GMO detection methods, including GMO basics, conversion information and clearly classified detection methods information, and new method submission and comment functionality. This will make the development and validation of new detection methods much easier. It would be more appropriate for GMO detection laboratories to choose appropriate detection methods. As most of the GMO sequences are still unknown to us and the sequence information contained in the GMDD database is of

great importance for the development of new methods, the GMDD consortium plans to organize a project to sequence GMO insertion boundary sequences or even entire insertion sequences. alternatively, initiate collaboration with GMO insertion boundary sequences. developing and licensed companies to get more sequence information. EThe GMDD consortium will also continue to cooperate with its European partner RIKİLT – Food Safety Institute (part of Wageningen University and Research Centre) by creating an additional database. This joint database will focus specifically on risk assessments for food and feed use of transgenic events disclosed by GMDD. In this way, the data provided by GMDD will create a unique combination of sequence data, detection methods and safety data, thus providing a useful tool for analytical method developers, regulatory authorities, risk assessors, risk managers and other interested parties from both industry and governments (Loureiro, M. L., & Hine, S. , 2002).

In another article, the authors explain in the summary and conclusions that; A large community of independent scientific researchers and academics has been challenging recent consensus claims about the safety of genetically modified organisms (GMOs). In the joint statement below, it has been shown that the alleged consensus is an artificial construct that is incorrectly maintained through various fora. As documented below, the argument that there is now a consensus on the safety of GMOs continues to be widely and often uncritically published, regardless of the conflicting evidence in the peer-reviewed literature. For decades, the safety of GMOs has been a hotly debated topic around the world. Published results are contradictory, in part due to the diversity of different research methods used, the inadequacy of available procedures, and differences in data analysis and interpretation. This lack of consensus on safety allows policymakers from more than 160 countries – in the UN's Cartagena Protocol on Biosafety and Codex Alimentarius Guidelines – to carefully evaluate each GMO on a case-by-case basis by national authorities to determine whether the particular construct meets national criteria. To 'safe' is evidenced by the grant agreement. Rigorous assessment of GMO safety has been hampered by a lack of funding independent of property interests. Public interest research has been further constrained by property rights issues and the denial of access to research materials that give proprietary interests unacceptable control over publication to researchers who do not wish to contract with developers. Developed and signed by more than 300 independent researchers and reproduced below, the joint statement does not claim that GMOs are unsafe or safe. Rather, the paper concludes that the scarcity and contradictory nature of scientific evidence

published to date preclude any definitive safety claims or lack of safety of GMOs. Claims of consensus on the safety of GMOs are not supported by an objective analysis of the peer-reviewed literature. Within the scope of this document, we can highlight only a few examples to show that the overall nuance of scientific research results in the field of GM crop safety; complicated; often contradictory or inconclusive; confused by the researchers' choices, assumptions, and sources of funding; and overall it has raised more questions than it currently answers. Whether to continue or expand the inclusion of GM crops and foods in the human food and animal feed supply and whether the identified risks are acceptable are decisions that involve socioeconomic issues and currently unresolved biosafety research agendas beyond the narrow scope of scientific debate. These decisions should therefore involve the wider community. However, they must be supported by strong scientific evidence on the long-term safety of GM products and foods for human and animal health and the environment, obtained in an honest, ethical, rigorous, independent, transparent and sufficiently diversified manner. to compensate for the bias. Decisions about the future of our food and agriculture should not be based on misleading and misrepresenting claims by an inner circle of like-minded stakeholders that there is a 'scientific consensus' on the safety of GMOs. This document was then opened for approval by scholars from around the world in their personal (not institutional) capacity reflecting their personal views and based on their personal expertise. There is no suggestion that the views expressed in this statement represent the views or positions of any institution or organization to which individuals are affiliated. The qualifying criteria to sign the statement were deliberately chosen to include scientists, physicians, social scientists, academics and experts on the legal aspects and risk assessment of GM crops and foods. Scientist and academic signatories were asked to hold a doctorate or equivalent qualification from accredited institutions. Legal professionals were required to have at least one JD or equivalent. By December 2013, more than 300 people had signed the statement, meeting strict qualification requirements. The paper received wide media coverage and numerous press coverage, and the evidence provided here continues to be widely cited. At a time when there is great pressure on the scientific community from institutional and political interests, it is critical that scientists working in the public interest take a stand against attempts to reduce and jeopardize the rigor of scrutiny in favor of the rapid commercialization of new applications. ensure profits and economic growth. The document remains open for signature on the website of the initiating scientific organization Ensser.

(Hilbeck, A., Binimelis, R., Defarge, N., Steinbrecher, R., Székács, A., Wickson, F., ... & Wynne, B. ,2015).

In another article, the authors explain in the summary and conclusions that; As more than a hundred genetically modified organism (GMO) events have been developed and approved for commercialization globally, GMO analysis methods are essential for the application of GMO labeling regulations. Protein and nucleic acid-based detection techniques have been developed and used to identify and quantify GMOs. However, knowledge is needed for the global harmonization and standardization of GMO analysis methods. GMDD will be a platform for analysts to share their data, issues and ideas, with comprehensive information of almost all current GMO detection methods, including GMO basics, conversion information and clearly classified detection methods information, and new method submission and comment functionality. This will make the development and validation of new detection methods much easier. It would be more appropriate for GMO detection laboratories to choose appropriate detection methods. As most of the GMO sequences are still unknown to us and the sequence information contained in the GMDD database is of great importance for the development of new methods, the GMDD consortium plans to organize a project to sequence GMO insertion boundary sequences or even entire insertion sequences. alternatively, initiate collaboration with GMO insertion boundary sequences. developing and licensed companies to get more sequence information. The GMDD consortium will also continue to cooperate with its European partner RIKİLT – Food Safety Institute (part of Wageningen University and Research Centre) by creating an additional database. This joint database will focus specifically on risk assessments for food and feed use of transgenic events disclosed by GMDD. In this way, the data provided by GMDD will create a unique combination of sequence data, detection methods and safety data, thus providing a useful tool for analytical method developers, regulatory authorities, risk assessors, risk managers and other interested parties from both industry and governments (Dong, W. , Yang, L., Shen, K., Kim, B., Kleter, G. A., Marvin, H. J., ... & Zhang, D. ,2008).

In another article, the authors explain in the summary and conclusions that; Ontology mapping is an important task to ensure interoperability between semantic web applications that use different ontologies. Structural similarity plays a central rolerole in ontology matching. However, current approaches rely heavily on lexical similarity and these get mixed up. Lexical similarity with structural similarity. In thispaper, we present a graph matching approach

for ontologies called GMOs. Uses two-part graphs to represent ontologies and measures structural similaritybetween charts with a new measurement. Further, GMO can externally take a set of matched pairs typically found with other approachesentered in the matching process. Our application and Experimental results are given to demonstrate the effectiveness of the graph matching approach. GMO is also a complement to other related products. work in the field of ontology matching. As we noted in Chapter 3, ontologies must be coordinated before comparison due to their heterogeneity. Ways of expressing semantics and the ability to infer from ontology languages. However, it is not easy to choose the appropriate coordination rules trade-off between extraction cost and extraction cost mapping quality. Another issue is interaction. between the conceptual layer and the similarities in the example layer. At the current stage of our implementation, we separate these two layers and use the mappings conceptually. layer as input to calculate similarities in the sample layer. In the case of comparing example-dense ontologies, machine learning (e.g. GLUE) is a promising approach to leverage sample knowledge in alignment classes or properties. As part of future research, GMO approach and in some ways related algorithms, eg coordination problem and layers problem. We are planning integrate GMO with techniques in machine learning and natural language processing to perform more powerful ontology matchers (Hu, W., Jian, N., Qu, Y., & Wang, Y. ,2005, October).

In another article, the authors explain in the summary and conclusions that; A possible consequence of planting genetically modified organisms (GMOs) in centers origin of the crop is undesired gene flow to traditional land areas. A 2001 study reported: Presence of transgenic 35S promoter in maize fields sampled from Sierra in 2000 Juarez from Oaxaca, Mexico. Analysis of a large sample taken from the same region in 2003 and 2004 failed to confirm the presence of transgenes, thus casting doubt on previous results. These two studies relied on different sampling and analytical procedures and therefore difficult to compare. Here we present new molecular data for this region confirming its existence. of transgenes in three of the 23 regions sampled in 2001. Transgene sequences not detected in the samples taken in 2002 from nine regions and in the oriented samples taken in 2004. Two of the positive regions in 2001 were again found to contain transgenic sequences. These findings indicate persistence or re-emergence of transgenes in this area until 2004. We address variability in recombinant sequence detection by analyzing consistency current molecular assays. We also present theoretical conclusions about the limitations

of estimation. Possibility of transgene detection in terrestrial samples. Inclusion of A limited numbers of female gametes and, more importantly, bulk transgene distributions can significantly reduce detection probabilities. Our analytics and sampling considerations help explain inconsistencies between different detection efforts, including those presented here and to provide considerations for the establishment of monitoring protocols to detect the presence of transgenes among the constructed black race populations. Evidence of 35S promoter found By PCR and SB at sites 7, 11 and 23 from 2001 collection. No transgene was detected in the 2002 localities. Second, it didn't include any places that were positive Examples for the 35S promoter in 2001. 7 for settlements and A new directed survey and corn leaf collection was conducted on 11 December. The existence of the organizer of the 35s, undertaken in 2004 It was detected in 11 of 60 sites. Analyzes on the INE-CONABIO 2001 collection One of the first independent PCR analyzes performed EAB and RRB laboratories, at least 10 settlements seedlings positive for the 35S promoter in both laboratories found (settlements 2, 4, 5, 7, 10, 11, 14, 17, 21 and 23). Inside a second round of analysis, a total of 21 plantlets (1867 analyzed plant clusters from three (7, 11) of 23 sites and 23) were positive for 35s in two independent DNAs Extractions and PCR experiments in EAB and RRB laboratories. Therefore, in this study, these individuals and localities as confirmed positives, 0.011 overall (21/1867) frequency of transgenes in total number analyzed plants. To further confirm the presence of the 35S promoter in positive PCR plantlets, we selected 10 positives. Random individuals from districts 7, 11, and 23, and additional independent DNA extractions were performed and PCR experiments in Eab followed by SB hybridization experiments for 35s (see Table 1 and Fig. 1). 11 for locality, one very low germination rate and high mortality and therefore we can reach enough tissue for only one person to have Approved by the SB (example 6 in Table 1). Additional PCR assay yielded positive results for all (Fig. 1a), positive SB results were obtained for only eight 10 samples. We performed PCR and SB for a second group of plants these were considered negative controls. these plants Had at least three negative PCR results for 35S First round of analysis by EAB and RRB Checked with 2001 samples or landrace samples known to be non-transgenic (provided by JASH laboratory). All these examples yielded negative results both types of molecular tests. We cloned and sequenced eight PCR bands. Positive B individuals, plus sample 5. We got three Types of 35S CAMV promoter-like sequences (Fig. 1b). We present an alignment of three samples, including three Different sequences retrieved using the 35S promoter

CaMV genome as reference (PIÑEYRO-NELSON, A., Van Heerwaarden, J., Perales, H. R., SERRATOS-HERNÁNDEZ, J. A., Rangel, A., Hufford, M. B., ... & Álvarez-Buylla, E. R. ,2009).

In another article, the authors explain in the summary and conclusions that; This article reviews the history of federal regulatory oversight of plant agricultural biotechnology in the United States and focuses on the scientific and political forces that shape the ever-evolving regulatory landscape today. Unlike many other jurisdictions, the US has decided to adapt pre-existing legislation to cover biotech products. In doing so, it established a comprehensive committee (the Office of Science and Technology Policy) to review and distribute various regulatory responsibilities among relevant agencies: the Food and Drug Administration, the Environmental Protection Agency, and the U.S. Department of Agriculture. This article reviews each agency's history and procedures in fulfilling its regulatory duties and explores the advantages and disadvantages of US regulatory strategy. The US has a detailed but coordinated regulatory system for evaluating new crops and foods. The scientific basis for assessing risks, combined with the coordinated framework that establishes regulatory responsibility, gives the United States the functional, though flawed, bureaucracy to allow the environmental and market release of biotechnology agricultural products. This does not mean that the US system is efficient or fair. Indeed, there are significant inefficiencies and at least one major flaw in the US regulatory system. Most importantly, the scientific community, both in the US and around the world, has concluded that using the biotechnology process as a trigger for regulatory review is scientifically invalid (McHughen, 2007). Rather, regulation should be based on the risks posed by the characteristics of the product, not the breeding process. In particular, the USDA ignores the findings of the scientific community, as well as its own OSTP, by using the process-based regulatory trigger, thus placing unnecessarily significant regulatory requirements on some non-risky GE facilities and failing to catch the occasional risky facilities. simply because they are not products of biotechnology. In addition, current regulatory policies pose an almost insurmountable barrier for low-risk GE crops and foods from small market and specialty crops, perhaps unwittingly, due to the high financial cost of regulatory compliance and low overall value from small plantation or small market potential. custom gmo's. In other words, the additional market value attributable to improvements to the GE plant or crop is insufficient to justify spending to meet regulatory demands. This is particularly frustrating for improvements that are considered very low risk, even

by regulatory agencies. GE facilities with significant health or environmental benefits are denied market access, not because they pose undue risk, but because the developer cannot afford to jump through unnecessary regulatory circles with little or no confidence in product safety. However, at least some biotech products have passed the US regulatory bureaucracy since 1994, are widely grown and heavily consumed, and there are still no documented cases of adverse health or environmental effects from any approved biotech product. Although the rapid adoption of biotech crops by farmers around the world (Brookes and Barfoot, 2006) suggests a potential problem, particularly with the concomitant inevitability of herbicide resistance and the evolving herbicide resistance of weeds, it raises this concern, where conventional breeding also produces crops with new herbicide resistance. must be put in context. and indeed, weeds that are resistant to these herbicides. The emergence of weeds that acquire herbicide resistance from GE plants largely supports and reinforces the early scientific predictions of OECD (1982, 1986), NAS (1983, 1987, 1989) and others that the risks associated with GE plants are the same as conventional breeding (McHughen, A., & Smyth, S. ,2008).

In another article, the authors explain in the summary and conclusions that; Biotech crops are the fastest adopted crop technology in the history of modern agriculture. The commercialization of GMO is tightly regulated in many countries, which has demonstrated the need for traceability and labeling. Detection methods are needed to comply with this legislation. To date, GM events have been developed by inserting a transgenic insert (i.e., promoter, coding sequence, terminator) into the plant genome, and real-time PCR is the preferred detection method. However, new types of genetic elements will be used to build new GMOs and new crops will be transformed. In addition, the presence of unauthorized GMOs in food and feed samples may increase in the near future. Intensive screening will become necessary so that application labs can continue to detect all GM incidents and gain insight into the possible presence of unauthorized GMOs in a food and feed sample. A pragmatic, cost-effective and time-saving approach is presented here, along with an overview of the evolution of GMO and future needs. The number of GMOs grown for commercial or research purposes continues to increase worldwide. Not only will these contribute to the complexity of detecting GMOs authorized by application laboratories in food and feed samples, but also to the emergence of UGM, which will increase steadily over the coming years. Unwanted escapes and interference with UGM can never be ignored. There will likely also be a

growing number of GMOs that have industrial or pharmaceutical properties but are not intended for food or feed use. They can also enter agricultural supply chains and cause ethical and religious concerns and even pose a significant risk to human and animal health. Therefore, there is a clear need for continued development of appropriate detection methods and strategies. Existing detection/identification approaches can continue to be used, even for stacked events, as long as the development of GMO follows the same path and genetic modifications are created using a transgenic cassette originating from a foreign organism. However, gene stacking poses a new challenge for GMO detection laboratories, as there is no way (except when analysis is done on individual seeds and plants) between GMO being found separately in a sample or combined as a stacked event. In response to the increasing diversity of GMOs on the market, new screening markers will need to be designed and developed for new species-specific sequences (e.g. eggplant and melon) and new genetic elements (e.g. encoding pathogen resistance). The challenge will be to collect large datasets (especially for UGM) to develop standardized real-time PCR methods and then combine the results into an appropriate decision system such as CoSYPS for effective GMO testing. Eventually, event-specific methods must also be developed to clearly identify the GMO in step two. The developed SYBR Green screening methods will allow detection of potential allelic variation in GM markers compared to the natural (non-GM) donor Bt ssp, for example the cry1Ab gene in Bt11, Bt176 and MON810 maize. in the course. The haplotypes of the gene in the three GM maize events are differentially optimized for expression in plants / maize. However, SNP can be detected using a technique derived from human genetic diagnosis, namely High Resolution DNA Dissolution Analysis (HRM), to screen for mutations in DNA pools. This requires only a simple real-time PCR step (using an interfolding saturated dye such as SYTO9 or LCgreen Plus+ and primers developed for screening markers) followed by DNA melting curve analysis. Samples containing a mutation form heteroduplexes in the post-PCR fragment mix. These are defined as differential melting temperature curves compared to homoduplexes. Therefore, HRM is a cost-effective, high-throughput mutation screening method with high potential for GMO analysis, including UGM analysis. HRM can be completed by validation of the mutation by sequencing. However, the screening methodology will no longer be fully suitable for the detection of new types of genetic modifications under development with new plant breeding techniques. In the case of sysgenesis, for example, the detection of inserted elements alone can

no longer be used as evidence of genetic modification. However, the order of different elements and loci of insertion into the plant genome may still offer an opportunity for detection of these modifications. Provided this information is available, these event-specific sequences can be used to develop new real-time PCR-based methods if needed. Also, for other types of genetic modifications introduced by these new plant breeding techniques (e.g. ZFN technology, ODM), minimal information about the DNA sequence of the modification and the neighboring sequence to detect them is required. Without prior knowledge, detection of these minor changes would be unlikely to be used in routine laboratories, as more sophisticated technologies are needed (for example, whole genome sequencing). For others, detection of genetic modification is currently not possible (eg, RdDM, grafting, reverse breeding, and agricultural filtering). In addition, crops resulting from most techniques are indistinguishable from conventionally grown crops or crops produced by natural genetic variation, and therefore identification is not possible. These new developments in molecular biotechnology pose challenges for regulators as to whether they fall within the scope of their regulatory authorities. In 2011 the European Commission published an overview of these techniques in collaboration with the different member states. However, it is currently unclear whether these new breeding techniques lead to organisms to be classified under the currently used definition of GMO. and so on GMO legislation. If they were to be classified as GMO, they would require control and traceability. New approaches need to be developed, possibly involving a combination of different analytical methods, as existing methodologies will fall short. If they are not classified as GMOs, the detection methods currently used will remain appropriate for now. However, the development of additional screening markers, potentially in multiplex setups (which may include technologies beyond real-time PCR platforms), should continue to be achieved in the near future to predict the increasing number of GM events. A robust and cost-effective solution to the GMO problem (Broeders, S. R., De Keersmaecker, S. C., & Roosens, N. H. ,2012).

In another article, the authors explain in the summary and conclusions that; There is a growing need for quantitative technologies suitable for molecular detection in a variety of applications, including food traceability and tracking of genetically modified (GM) crops and their products through the food processing chain. Traditional molecular diagnostics using real-time polymerase chain reaction (RT-PCR) and fluorescence-based determination of amplification requires temperature cycling and relatively complex optics. In contrast,

isothermal amplification coupled to a real-time generated bioluminescent output (BART) takes place at a constant temperature and requires only a simple light sensing and integration device. Loop-mediated isothermal amplification (LAMP) demonstrates robustness against sample-derived inhibitors. Here we demonstrate the feasibility of combined LAMP and BART reactions (LAMP-BART) for the detection of genetically modified (GM) maize target DNA at low contamination levels (0.1-5.0% GM) using certified reference material and compare it to RT-PCR. The results show that conventional DNA extraction methods developed for PCR may not be optimal for LAMP-BART measurement. In addition, we show that lamp is more tolerant to plant sample-derived inhibitors, and we show that it can be used to develop rapid extraction techniques suitable for simple field-based qualitative testing for determination of GM status. We also evaluate the effect of total DNA assay load on LAMP-BART quantification. LAMP-BART is an efficient and sensitive technique for GM detection with significant quantification potential even in samples from crops such as maize with low contamination levels and large genome size. LAMP-bart's resistance to acidic polysaccharides makes it well-suited for rapid sample preparation techniques and therefore for both high-throughput laboratory environments and portable GM detection applications. The effect of the plant sample matrix and genome loading in a reaction must be controlled to ensure quantification at low target concentrations (Kiddle, G., Hardinge, P., Buttigieg, N., Gandelman, O., Pereira, C., McElgunn, C. J., ... & Murray, J. A. ,2012).

In another article, the authors explain in the summary and conclusions that; The relatively low acceptance rate of GMO products in the European Union (EU) market appears to be linked to the strictness of authorization regulations and the inefficiency of the authorization process itself. These issues will apply to any product considered a GMO potentially marketable in the EU. As modern plant breeding methods involving oligonucleotide-directed mutagenesis (ODMs) or site-directed nucleases (sdns), including Clustered Regularly Spaced Short Palindromic Repeats (CRISPR), are becoming increasingly popular, it is critical to determine whether such products exist. whether they are exempt from EU legislation on GMOs, including new breeding techniques (NBTs), in particular precise mutagenesis methods. Legal uncertainty regarding their status may cause reluctance to invest in and further develop such methods. Here, developments in the legal classification of certain NBTs products are presented in the context of recent decisions and case law. The socioeconomic aspects of GMO adoption in both global and European

contexts are discussed. The legal and practical environment of GMO regulation in the EU and how it can be a barrier to investment and the development of new products is presented. The most recent case-law on the interpretation of the legal concept of GMOs and the scope of legislation (for example, Case C-528/16) is analyzed, concluding that strict regulations will likely also apply to products of NBTs that include strict mutagenesis methods. This will likely limit their application in the development of new plant varieties in the EU. Plant breeding companies may be enthusiastic about introducing innovative technologies into their breeding programmes, but for the purposes of the EU market it would be possible for such technologies not to be classified as leading to the production of GMOs. The current situation may affect the selection of breeding methods for plant breeders, not only because of risk assessment requirements, high costs and the long time required for authorization in the EU, but also because there is no real market for GM food in the EU. GMO food is barely available in EU stores because consumers don't want it. Whether a product is regulated by EU legislation on GMOs largely depends on the interpretation of the GMO definition in Directive 2001/18 / EC and the exclusion of this directive. The final decision in Case C-528/16 went against the Counsel General's view and favored the broad application of GMO legislation, and SDN appears to be addressing the products of genome editing techniques such as ODM or CRISPR as covered by the GMO-related regulatory regime. The decision could have far-reaching consequences both for research (experimental release of GMOs requires approval) and for the development of new products. In some cases, mutagenesis may limit the applicability of NBTs to basic research only. Specifically, the limitations include: Limit access to innovative technologies for EU breeders, may limit access to new varieties for farmers (due to lack of authorization applications), farmers may not be able to use permitted varieties as they are preferred by many EU countries, The EU's dependence on foreign protein sources (mainly imported soybeans) will be maintained and, Mandatory labeling associated with the social stigma of GMOs may mean that products of new varieties can be used primarily as feed. It seems that without further regulatory action that would include separate approval procedures for some NBT products or even a move from a process-based approach to a product-based approach, definitive mutagenesis products may be difficult to market in the EU. Such changes in legislation are unlikely in the near future (the current term of the Commission is coming to an end). They will also need widespread political consensus, which can be difficult to achieve, given the controversial

nature of the issue (Zimny, T., Sowa, S., Tyczewska, A., & Twardowski, T. ,2019).

In another article, the authors explain in the summary and conclusions that; The increasing use of genetically modified organisms (GMOs) is accompanied by the increasing complexity of matrices containing GMOs. The most common DNA-based approach for the detection and quantification of GMOs is real-time quantitative polymerase chain reaction (qPCR). However, since qPCR is sensitive to inhibitors and relies on standard curves for quantification, it has limited application in GMO quantification for complex matrices. To overcome this hurdle in DNA quantification, we present the 'Ready to Recover' soybean and droplet digital PCR (ddPCR) analyzes designed to target the soybean reference gene. Three ddPCR assays were transferred from qpcr to QX100/ QX200 ddPCR platforms and characterized. Together, the fit-for-purpose study on four real-life examples and the use of a room-based PCR system showed that dpcr has great potential to improve such metrics in GMO testing and food authenticity monitoring. The aim of this study was to develop a DNA-based method for the quantification of GMOs in complex samples. This goal was achieved by transferring qPCR assays targeting the soybean endogenous and the GM-soybean line MON40-3-2 to a ddPCR platform. Both simplex and duplex ddPCR analyzes (Decathlon Project, 2015, ENGL et al., 2015) performed within the required parameters and measured MON40-3-2 in complex samples even when quantification failed by qPCR. In addition, direct transfer of qPCR methods to a cdPCR platform was evaluated. Although the transfer from qpcr to ddpcr was relatively simple, this was not the case for transfer to cdpcr. Indeed, transfer of simplex assays was successful for both the ddPCR and cdPCR platforms. However, transfer of the bidirectional assay was not possible using the cdPCR platform. However, further optimization, i.e. switching to one that is more suitable for multi-target analysis in mastermix, will likely make transfer possible. This study demonstrated that there is great potential for using dPCR to improve GMO testing and that qPCR methods can be directly transferred to alternative platforms. However, as transfer is not always straightforward, tests will need to be validated before being implemented in any GMO testing scheme (Košir, A. B., Demšar, T., Štebih, D., Žel, J., & Milavec, M. ,2019).

In another article, the authors explain in the summary and conclusions that; This article provides an overview of the legal and procedural uncertainties associated with genome-edited organisms and possible avenues forward for European GMO policy. After a recent decision of the European Court of

Justice (CJEU judgment of 25 July 2018, C-528/16), organisms obtained by genome editing techniques are GMO and subject to the same obligations as transgenic organisms. Uncertainties arise if genome-edited organisms cannot be distinguished from organisms grown by conventional techniques such as crossing over or random mutagenesis. In this case, identical organisms may be subject to GMO law or exempt from regulation due to the use of an unidentified technique. Regulators may not be able to enforce GMO law for such situations in the long run. Because other jurisdictions do not regulate organisms such as GMOs, accidental imports may occur and undermine European GMO regulation. In the near future, the EU Commission, European and national regulatory bodies will decide how to implement the updated interpretation of the law. To reduce existing legal and procedural uncertainties, the first step forward lies in updating all guidance documents to specifically address genome editing, including a solution to provide a unique identifier. In part, the authorization procedure for GMO release can be adapted to different types of organisms, taking advantage of existing flexibility in GMO law. However, only a change in regulations governing the authorization process for the release of GMOs could significantly reduce the burden on innovators. In the second step, any way forward should aim to amend, supplement or replace the European GMO Directive (2001/18/EC). The policy options presented in this article presuppose the political preparation for reform. In the current political situation, this may not be realistic. However, Europe's competitiveness and green biotechnology research will suffer if the problems of current GMO law are ignored. Companies and research institutions using new breeding techniques face significant legal uncertainties after the latest CJEU decision. In principle, uncertainties associated with the application process for GMO release can be handled at a lower level by procedural changes, while application problems with organisms that are indistinguishable from the result of conventional breeding techniques cannot be resolved without changes in European GMO legislation. There are several legislative amendment options that share the common requirement to treat organisms indistinguishable from non-GMOs if they lack known additional risks, i.e. to exclude them from most or all obligations of GMO regulation. This is by no means a statement for less rigor, as organisms with novel traits associated with risks still need to be extensively evaluated and regulated. But such a solution requires the constructive participation of European institutions and member states. Therefore, the paths forward presented in this article are only possibilities in an optimistic scenario that predicts the will to act politically. In the current political situation, this may

not be realistic. However, a crisis situation will arise if the problems in GMO law are ignored: Regulators will struggle to enforce GMO regulation, international trade relations will be affected, European agriculture will lose the opportunity for sustainable innovation, and jobs in research and development will move elsewhere. (Wasmer, M. ,2019).

In another article, the authors explain in the summary and conclusions that; This article examines the potential impact of both mandatory and voluntary labeling schemes on the research and commercialization of process-based and product-based GM crops. The analysis concludes that mandatory labeling will impose exorbitant costs on GMO producers. This result will threaten the research and commercialization of GM foods. In contrast, voluntary positive labeling of non-GMO goods or the presence of certain GMO characteristics in goods will limit producer costs. This result will be optimal both commercially and socially. In the long run, the issue of labeling may become less important when biotechnology is used to develop new crop-based GM crops with desirable properties rather than simply reducing production costs. Labeling goes to the heart of the private sector, biotech-based research and development agriculture-food business. Mandatory labeling is clearly a threat to the continued development of biotechnology products and processes. However, in the absence of industry action, positive The label can be forced to enforce by governments, consumers, and various lobbying groups. labeling to ensure that companies are held accountable for product-specific confidence uncertainties (Phillips, P. W., & Isaac, G. ,1998).

In another article, the authors explain in the summary and conclusions that; Won anti-GMO (genetically modified organism) movement since 2012 Significant grassroots momentum in efforts to demand mandatory GMO food labels through state-level ballot and legislative efforts. Major food and agriculture companies include opposed mandatory GMO labels and successfully defeated most of these attempts. However, these wars attracted significant media attention and Discussion on GM crops and foods. In this article, one of the most important The results of this struggle are the results of food retailers and value-based food companies. voluntarily apply non-GMO labels and brands. We leverage governance and policy consumerism literature and how to investigate (against) movement efforts for imperative labels these efforts are institutionalized through private voluntary management institutions. Our assessment is based on in-depth, semi-structured interviews with key informants. consumer and environmental organizations, agriculture and biotechnology companies and

Content analysis of food industry websites as well as government regulatory agencies. A growing number of food retailers recognize the new reputation and economic value While it can offer niche markets for non-GMO foods, the anti-GMO movement is looking into these strives as a step towards mandatory GMO labels. Your volunteer tags can take action to resolve the labeling debate by easing concerns about the agri-food industry mandatory labeling and meeting the political consumer's desire for more choice and transparency, but without addressing broader social and environmental sustainability concerns driving the anti-GMO movement in the first place. The recent expansion of specific governance initiatives for GM foods is one of the most important developments in the two-decade war against GMOs. This change is not just the result of abstraction. market forces or consumer demand, rather political processes. As Boström and Klintman (2008) stated, labeling always takes place in an "organizational context" that includes social action. mobilization and coalition building for or against labeling". In this case, the intransitive federal level to ban or label GMOs caused anti-GMO movement to join sub-policy level. While the grassroots is mobilized to campaign for mandatory labels in individual states, it also Targeting key food industry actors to ban or label GM products. with the agri-food industry tens of millions of dollars poured into them, overwhelmingly against mandatory labeling High-stakes, state-level battles, successfully defeating every ballot for tagging. Yet while these attempts for mandatory tags failed at the polls, they appear to have It energized the anti-GMO movement, attracted significant media attention, fueled a national debate, and Raising public awareness about GMOs. After the Oregon ballot, George Kimbrell, attorney The Center for Food Safety stated: "Every person in Colorado and Oregon has a GMO now". Increasing public awareness of GMOs has turned into the fact that it is non-GMO Labels are one of the fastest growing consumer trends in the food industry. Key players in the food industry are willing to respond to this trend and help guide it in the development of non-GMO institutions, like labels and branding. While responding to calls for greater transparency and elections Recognize the potential of non-GMO products, food retailers and value-based food companies Non-GMO institutions will develop valuable niche markets for political consumers. non-GMO institutions also provide opportunities for food companies to gain trust, value and status. brand names potentially undermine calls for mandatory government labels for GMOs Ironically, the non-GMO governance efforts of food retailers and others for the anti-GMO movement may arise to undermine the broader and longer-term goals of their aims. GMO

labeling The movement emerged from an anti-GMO movement that was fundamentally opposed to GMO products and foods and ultimately tries to banish them entirely from the agri-food system. This stance is rooted broad-based concerns that technology is inherently linked to and empowering an agri-food system It supports large-scale, corporate-controlled industrial production with little social or social concern. environmentally sound and fair outcomes for farmers, consumers and the environment. From this In prospect, GMOs are problematic because they perpetuate a system based on a silver bullet. rather than technological fixes, approaches to address complex social and environmental issues. Emphasize holistic and systems thinking For the anti-GMO movement, non-GMO labels are seen as a proxy for concerns about: sustainability of the agri-food system. However, non-GMO labels can ultimately function as poor proxy. In its efforts to mobilize political consumer support for labeling, much of the movement sustainability concerns are cast aside in favor of a discourse that emphasizes individualistic concerns about choice, the right to know and transparency. These arguments appeal to the political minority consumers adopting labels as a way to increase transparency and reduce information asymmetries to reflect the social and ethical values of consumption practices. As a result, Niche markets for non-GM crops will expand and present a significant market opportunity for minors segment of manufacturers to meet this demand. However, if political consumers feel they can fulfill social and ethical values by making good choices in the market for themselves and their families then there is little incentive for them to act on the broader structural changes that could lead to agri-food just the system that is more social and environmental for everyone. In summary, voluntary labels can act to resolve the GMO labeling debate by satisfying political desire. consumers for greater choice and transparency while providing a profitable niche market for retailers. it also removes the agri-food industry's concerns about mandatory labeling. Still, voluntary tags are unlikely fundamentally addressing anti-GMO's social and environmental sustainability concerns The movement with GM crops is likely to continue to expand as a mainstay of the US agri-food system, and "fix" for most of what's bothering him (Bain, C., & Dandachi, T. ,2014).

In another article, the authors explain in the summary and conclusions that; Environmental contingent valuation (CV) studies in the context of developing countries have been advancing over the past two decades. However, these studies are prone to bias due to their hypothetical nature, and many of these studies lack a properly designed and implemented curriculum vitae. This

article conducted a systematic review of 36 previous forest background studies conducted in the context of developing countries and focused on two main objectives: (i) examining communities' preference and willingness to pay (WTP) for forest conservation in developing countries, and (ii) assessing content and validity of what was included. create studies using indicators selected from best practice guidelines recommended in the literature. The overall annual average WTP ranges from $0.01–75.36 per household and 7.17–94.34 man-days for monetary and labor-time payment patterns, respectively. The results reveal that WTP estimates differ slightly between individual studies in the same country, while estimates vary widely between countries. These mean that policy designs for forest protection programs must emphasize local contexts and other relevant parameters. In addition, the finding indicates that most of the CV studies included in this review fail to document and report the validity test fully and clearly as recommended in the literature, despite some efforts to improve the quality of the studies. Therefore, it is strongly recommended that future studies on the topic should be validated, documented and fully reported. The finding provides available evidence of the accuracy of previous CV forest studies that could inform policy/users to use the results in decision making This study carried out a systematic literature review of forest-related background studies in the context of developing countries. The main objective of the study is to examine the quantifications of resources where communities are WTP for forest protection and to evaluate the validity of value estimates from previous CV forest studies using validity indicators selected from best practice guidelines suggested in the literature. Validity assessments (content and construct validity) made in the review are limited to the data/information documented and reported in the included studies. Some content and construct expiration indicators are selected and used as checklists from which validation is evaluated. The finding reveals that the required information is not fully documented and reported in most of the studies reviewed. Findings from a review of the background studies included suggest that communities in developing countries value the environmental services of forests and are willing to contribute significant resources to forest management and conservation on a sustainable basis despite their relatively low levels. income. The current inconsistency in WTP estimates is partly explained by the difference in the location of the studies, the difference in the proposed background scenario or proposed forest protection context, the change in the income level of the households and other parameters. WTP value estimates vary slightly between personal studies within the same country and vary widely

between countries. This suggests that policy designs for public participation in forest conservation programs should emphasize the local context and other relevant parameters. The result shows that there is a difference between CV studies in reporting validity tests. Some studies including content and construct validity tests, although not fully and clearly reported as recommended in the literature. This means that the importance of explicitly including, documenting and reporting validity tests as recommended in the literature received insufficient attention in most of the included studies. Therefore, the absence of validity tests hinders the precision of WTP value estimates from studies. This may lead to a lack of data/information that CV end users need to assess the reliability and validity of value estimates from these studies. This may create a sense of skepticism for using the value estimates from such studies in decision making, which may preclude wider application of the method itself in the economic appraisal of environmental services. Therefore, it is arguably correct to conclude that most of the CV studies included in this review fail to fully and clearly document and report validity (content) tests as recommended, despite some current attempts to improve the quality of the studies. Based on the findings of this review, policy recommendations and future research direction are presented and generalized as the following points: (a) Only a few of the existing studies on household dtp for forest resource conservation in the context of developing countries consider dtp in the contribution of labor time. Therefore, it is crucial for future research to consider non-monetary forms of payment, such as labor time participation, in addition to monetary payment, as poor and large labor households tend to contribute more labor in human days. (b) The result of this study indicates that previous forest background studies were limited in documenting and reporting validity testing. Therefore, future studies are strongly recommended to document and fully report validity testing and other essential information in CV studies as suggested in the literature. (c) Furthermore, the finding indicates that the evidence for a significant portion of validity testing, such as criterion and convergent validity tests, has received insufficient attention and is limited in the context of emerging economies. This necessitates the need for further research in the future to provide an evidence base on the topic (Clapp, J. ,2008).

In another article, the authors explain in the summary and conclusions that; This study focuses on Italian undergraduate students to identify and characterize different hypothetical customer segments with their knowledge of GM food. We used data from a survey of 243 undergraduate students from three Italian universities to profile consumer groups based on their views on GM crops. The

main findings show that the type of education plays a large role in students' perception of GMOs. In particular, students in technical and natural science programs (60.90% of the sample) differ from those enrolled in Social Sciences programs (22.63% of respondents). While the former has a better perception of GM crops than the latter. The study identifies and characterizes different consumer segments in terms of their knowledge and views on GM foods. Italian law forbids transgenic production. Over the past decades, manufacturers have turned to non-GMO products, and leading food brands' marketing strategies seem increasingly positioned towards natural, biological, and non-GMO products. Information on genetically modified crops is scarce, and the idea of meeting the younger generation is too (Palmieri, N., Simeone, M., Russo, C., & Perito, M. A. ,2020).

In another article, the authors explain in the summary and conclusions that; New plant breeding technologies such as genome editing enable new crop varieties to be developed much faster and more precisely and comprehensively than can be achieved with traditional methods. These developments can help farmers address the challenges of climate change, sustainability and global food security. However, despite their potential, uptake of these new technologies has been slowed by instability regarding the regulation of genome-edited crops. For many European consumers, their outlook on new breeding technologies is influenced by many factors. Those who have never faced a major food crisis may not adequately appreciate the challenges posed by the projected 2 billion increase in human population by 2050. In addition, consumers with regular and abundant food supplies may not need to think about how their food is produced or appreciate the challenges EU farmers are currently facing to meet future demand. Misleading online articles questioning the safety and ethics of these "new" biotech foods can also lead to consumers being reluctant to accept them. As a result, Europe's mixed view of biotech products may also be preventing their espousal in countries that need to gain more from the technology. In this review, we discuss the available data on global and EU GM crop adoption and the potential impact a new wave of crop development could have for agriculture. We think about how the EU looks at GM crops and we think about the future of both genetic modification (GM) and genome editing (GE) in the EU. We explore the lessons learned from the adoption of GM crops and examine the potential impact of the decision not to exempt genome-edited crops from the EU GMO Directive on EU farmers, scientists, consumers, trading countries and the rest of the world. It is clear that the CJEU decision confused many stakeholders and wanted a better understanding of the decision and its consequences. It will

be of great interest to see how the decision impacts Europe's momentum in research and how an already failing regulatory system will undoubtedly keep pace with the volume of products that will come out of countries that choose to adopt new technologies. How the crops developed using these technologies will be tracked in international trade markets when there is no foreign DNA to be detected is another important issue to be resolved. The CJEU decision is far from clearing up some things, and it still leaves many questions unanswered. With the urgent need to address both global food security and sustainability, genome editing offers the potential to make a significant contribution. Given the EU's policy commitment to assist developing countries in solving food security problems, particularly in response to climate change (European Commission, 2010, EU policy framework to assist developing countries in solving food security problems), how can the EU do better? It's time to admit that you can make a good contribution. food security by applying the purposeful provision of improved crops to the long-term goal of the global economy. Regulations need to be aligned with the rest of the world to allow the full potential of these new breeding technologies to be used (Hundleby, P. A., & Harwood, W. A. ,2019).

In another article, the authors explain in the summary and conclusions that; Resistant suppliers can reduce supply chain risk, effectively prevent supply chain disruptions and bring profits to businesses. However, there is no unified system of measurement indexes to evaluate the resilient supplier in the supply chain environment, and appraisal language sets are often net values. Therefore, to fill the research gap, this paper proposes a hybrid method combining triangular fuzzy number, best-worst method (BWM) and modular topsys (GDo-RTOPSIS) in random environments for group decision making. to solve the above problem. First, the decision maker's weight is calculated using fuzzy BWM, which can deal with triangular fuzzy numbers. Second, the triangular fuzzy number is included in the GMO issues, and when combined with the fuzzy BWM, the alternatives are ranked to select the best resistant supply chain partner. Finally, the feasibility and universality of this method has been proven with illustrative examples. In the globalization environment, choosing the right flexible supplier is an important part of supply chain management. This article presents a supplier selection model based on the combination of fuzzy BWM and GMO issues. First, the weights of the decision makers are obtained according to the fuzzy bwm. Second, the GDo-TOPSIS model is used to process the independent decision matrix provided by the decision makers and the alternatives are ranked to select the most suitable system. This article adds background and method to

current research. In the research history of other studies, more emphasis has been placed on supplier selection in a static environment or on increasing resilience by improving the supply chain. This study focuses on supplier selection in the flexible supply chain under the global environment, as globalization creates the supply chain in a dynamic environment and requires immediate dealing with disruptions. Then, according to the related research, a comprehensive index of flexible supplier selection under dynamic environment is proposed. In terms of research methods, a hybrid multi-citation group decision making method is proposed by combining fuzzy BWM and GDo-RTOPSIS. This method can effectively reduce the blurriness, uncertainty and subjectivity in the decision-making process and restore the decision maker's thinking as much as possible. A method for determining weights was developed. The fuzzy BWM can achieve more accurate weights with fewer comparisons. Finally, the combination of triangular fuzzy numbers and GDo-RTOPSIS can handle the independent decision-making evaluation information of multiple decision makers in the dynamic environment. In the weighting and alternative evaluation process, the data is closer to the decision maker's idea and the decision-making process is more scientific. In addition, this article expands on two aspects of highly cited group decision-making research methods. It fully uses the professional knowledge of the decision makers and expresses their ideas more accurately. In the process of determining the weights of the decision makers, triangular fuzzy numbers are introduced to obtain more objective decision maker weights. Hybrid research methods are universal. This research method is suitable for supplier selection and other multi-member group decision-making problems such as supplier segmentation, performance evaluation, and risk assessment. Despite the current work result, there is work to be done in the future. First, this study only focuses on gaining the weights of decision makers, and future studies should focus more on the determination of attribute weights. Second, future studies should use empirical data to further express the plausibility and scientificity of the proposed method. Finally, rough sets, indefinite sets, etc. Future work should be developed to support other language clusters (Gan, J., Zhong, S., Liu, S., & Yang, D. ,2019).

In another article, the authors explain in the summary and conclusions that; It mentions the devices used for the consumption of plant studies. The market for plant meat alternatives has expanded greatly. It can be used in the vicinity of anyone walking around, around those who are fit for health, and around everyone walking around, in health and to visit their vehicles to be processed.

For the production of its analogues, the gastrointestinal fate of meat analogues and their apparent emergence acceptability are thus summarized. Research is driving forces in locking meat analogues. The final material has benefited from modern equipment of plant meat alternatives, but has benefits to improve their appropriate etching, sensory use, and selection. In addition, its admissibility against analogies is insufficient, which should be subject to appropriate scrutiny and scrutiny. At the same time, there is more hope for a better understanding of the gastrointestinal fate of plant meat analogues, their availability to this nutrient source. This will help in the planning of trainings, targeting the future of meat analogues and opening up new research in this field. The issue of plant-based meat analogues has been a major topic of discussion in the food industry for decades, due to growing concern about the health effects and sustainable enhancement of meat alternatives. Therefore, it is important to understand the findings of research focused on the development, improvement, need, sustainability and functionality of meat analogues to design future strategy in this research area. Accordingly, this article provides a detailed discussion on the importance of developing meat analogs, the health and environmental concerns of meat product production, vegetable protein sources for the development of meat analogs, the functionality of the ingredients used for the development of these products, gastrointestinal conduct and consumption attitudes. regarding plant-based meat alternatives. Key considerations for the effective production of sustainable plant-based meat alternatives include the necessity of appropriate processing technologies, improvement of sensory and physico-chemical functionality, safety and quality control, and investigation of gastrointestinal fate. However, while consumer acceptability of meat analogues is not widespread globally, a gradual improvement has been noted recently. Therefore, future strategies should also be devised to increase consumer acceptance of plant-based meat analogs by raising consumer awareness and improving the quality of plant-based meat analogs to improve the functionality of these new food products (Debode, F., Janssen, E., & Berben, G. ,2013).

In another article, the authors explain in the summary that; The issue of plant-based meat analogues has been a major topic of discussion in the food industry for decades, due to growing concern about the health effects and sustainable development of meat alternatives. Therefore, it is important to understand the findings of research focused on the development, improvement, need, sustainability and functionality of meat analogues to design future strategy in this research area. Accordingly, this article provides a detailed discussion

on the importance of developing meat analogs, the health and environmental concerns of meat product production, vegetable protein sources for the development of meat analogs, the functionality of the ingredients used for the development of these products, gastrointestinal behavior and consumption attitudes. regarding plant-based meat alternatives. Key considerations for the effective production of sustainable plant-based meat alternatives include the necessity of appropriate processing technologies, improvement of sensory and physico-chemical functionality, safety and quality control, and investigation of gastrointestinal fate. However, while consumer acceptability of meat analogues is not widespread globally, a gradual improvement has been noted recently. Therefore, future strategies should also be devised to increase consumer acceptance of plant-based meat analogs by raising consumer awareness and improving the quality of plant-based meat analogs to improve the functionality of these new food products (Nadal, A., Esteve, T., & Pla, M. ,2009).

In another article, the authors explain in the summary that; The increase in food demand in Indonesia is one of the consequences of imbalance between population growth and declining food crops. One of the alternative technologies that could be used in herbal breeding programs to increase agricultural production to meet food demands, genetically modified organism (GMO) technology. This technology offers many pros and cons among the public effects to be accepted by consumers. The desing of this study was to determine the level of sustainability between GMO and non-GMO foods. Performance The measurement model for GMO and non-GMO foods evaluated according to seven numbers sustainability, which represents environmental, social and economic aspects. Evaluation The method was performed using Total Price Recovery (TPR) indicators and Adjusted Profit (AP) and Total Factor Efficiency (TFP) using the Data Envelopment Analysis (DEA) Method. Assessments on each procurement chain component included agriculture, processing and shipping. for wholesalers / retailers. Numerical examples of rice production in Indonesia were used in this study. This The research results found that the performance of non-GMO rice chain was better than that of non-GMO rice. HE indicates that non-GMO rice is more sustainable. (Saputri, V. H. L., Sutopo, W., Hisjam, M., & Ma'aram, A. ,2019).

2. Conclusion

A lot of research has been done in the field of biotechnology (KORKMAZ, S. A., & GÜNDOĞDU, S. G.), (KORKMAZ, S. A., & ÇAKIR, V.). However,

in this study, studies in biotechnology on Genetically Modified Organism (gmo) were examined. In an article, the authors explain in the conclusions that; We developed a multiplex PCR-CGE-SC assay for Simultaneous detection of 5 events of GM cotton (Bollgard), Bollgard II, RR, 3006-210-23 and 281-24-236) and cotton endogenous reference gene acp1. This is the first multiplex test this allows specific identification of the most common transgenic cotton events; targets the intersection between inserted sequence and host plant genomic DNA. Attempt fully specific with accurate detection rates >98.3% aim. Has LOD values similar to those obtained for Valide real-time PCR analyzes and therefore complies with EU legislation Requirements for GMO analytical methods. Sensitivity are kept at different relative percentages of targets. Adaptation of validated real-time PCR tests to uniplex PCR-CGE-SC was simple and required multiplexing liners must be compatible and fine-tuned. primer concentrations, we have shown that it is possible 5 corn (12) and 6 cotton targets. This further confirmed Compliance of the multiplex PCR-CGE-SC approach to detect multiple targets simultaneously. Offers many offers advantages such as simultaneous detection of many targets arrays, high precision and efficiency and automation capabilities while maintaining a very simple protocol, e.g., specific amplification and labeling in one step. Our take was this: designed to allow detection of more than half. EU-approved individual or stacked GM cotton events. it's possible can be used as an easy and inexpensive tool for initial screening should be further complemented by the available validated real-time PCR quantitative analyzes targeting specific flanks lineups. Also, various multiplex products PCRs can be solved in a single CGE, which approach more economically. simultaneous detection 5 maize and 6 cotton targets (2 endogenous reference controls and 9 GM events) are particularly interesting for GMO analysis (Nadal, A., Esteve, T., & Pla, M. ,2009).

In another article, the authors explain in the conclusions that; The results show that the performance of non-GMO rice chains is better than GMO chains. This can be seen from the Adjusted Profit results, which consists of TFP and tpr. Perceptible From the differences in AP values, non-GMO rice is more sustainable than non-GMO products. This components that can be used as study material on economic, social and environmental issues is seed use, seed prices, fertilizer and pesticide use, and fuel use in the process? rice production and distribution. The DEA method was used to propose a performance measurement model for sustainable agrifood. Supply chains for GMOs and non-GMOs. Numerical analysis showed that the model can be used for Determine Adjusted

Profit (AP) with aggregate Price Recovery (TPR) indicators and Total Factor Efficiency (TFP). Research results found that non-GMO rice chains performed better than non-GMO rice, which means non-GMO rice is more sustainable. Therefore, to maintain the sustainability of GMO and non-GMO products, consider the following The use of such ingredients in the GMO and non-GMO supply chain process should be given products. In the future, this research is expected to continue to inform relevant policies. Use of GM products to preserve food compared to non-GMO products in Indonesia sustainability in Indonesia (Saputri, V. H. L., Sutopo, W., Hisjam, M., & Ma'aram, A. ,2019).

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CHAPTER VIII

GREEN APPROACHES IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: APPLICATIONS IN PHARMACEUTICAL AND FOOD ANALYSIS

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1. Introduction

In recent years, green chemistry (GC) has become a popular issue in both industry and academia. GC is the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances (Anastas & Warner, 1998; Lancaster, 2020; Welch et al., 2010; EPA, 2022). Green analytical chemistry (GAC) aims to decrease or eliminate the hazardous solvents, reagents, and preparation, pre-treatment techniques and preparation steps of an analysis process (Kaya et al., 2022). There are several primary goals of GAC, including the reduction or elimination of harmful reagents, the design and optimization of analytical methods that use less energy and resources, and the reducing of overall analysis times (Boltia et al., 2022). GAC can be expressed as an approach that aims to ensure that the materials used in sample preparation, pre-treatment and determination steps are environmentally friendly, non-toxic,

biodegradable and safe. GAC minimizes instruments, sensors, and materials and chemicals needed for analysis (Koel & Kaljurand, 2021).

The eco-friendly of an analytical approach is now a crucial consideration, and GAC concepts are taken into account in the development of new processes. Importantly, the selection of eco-friendly solvents is a crucial aspect in evaluating the environment friendliness of an analytical technique. Therefore, toxic solvents must be replaced with green alternatives. Besides, GAC minimizes organic solvents and hazardous reagents and reduces energy, waste, and resources. There are many studies/reviews in the literature about green solvents. (Anastas & Warner, 1998; Byrne et al., 2016; Claux et al., 2021; Montero et al., 2021; Pacheco-Fernández & Pino, 2019; Prat et al., 2016; Santana-Mayor et al., 2021).

One of the challenges in green chemistry is assessing the greenness of chemical processes (Płotka-Wasylka et al., 2021). Various metrics are used to evaluate the greenness (environmental effects of chemical processes etc.) of the applied method (Sajid & Płotka-Wasylka, 2022; Tobiszewski et al., 2015).

The term of GC gained formal recognition with the publication of the 12 principles by Anastas and Warner (Anastas & Warner, 1998). GC principles are shown in **Figure 1.**

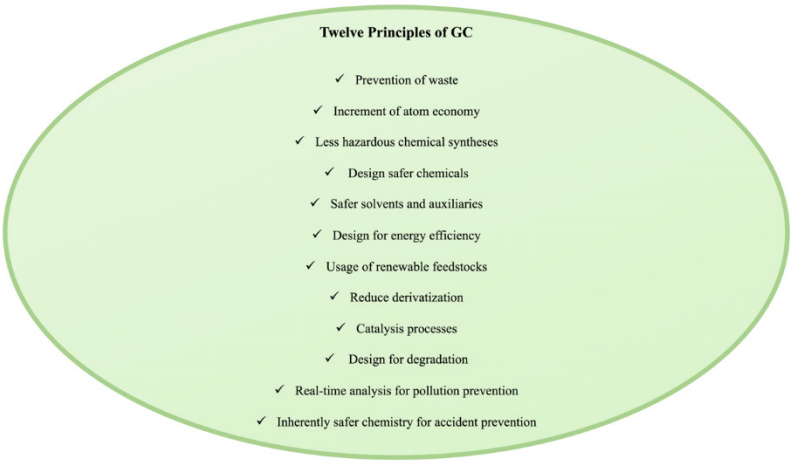

Figure 1. 12 principles of GC (Anastas & Warner, 1998)

Since not all green chemistry concepts are applicable to analytical chemistry, twelve GAC principles were developed. Thus, GAC has its own

purposes and objectives for determining the environmental friendliness of analytical procedures (Gałuszka et al., 2013). 12 principles of GAC have been showed in **Figure 2.**

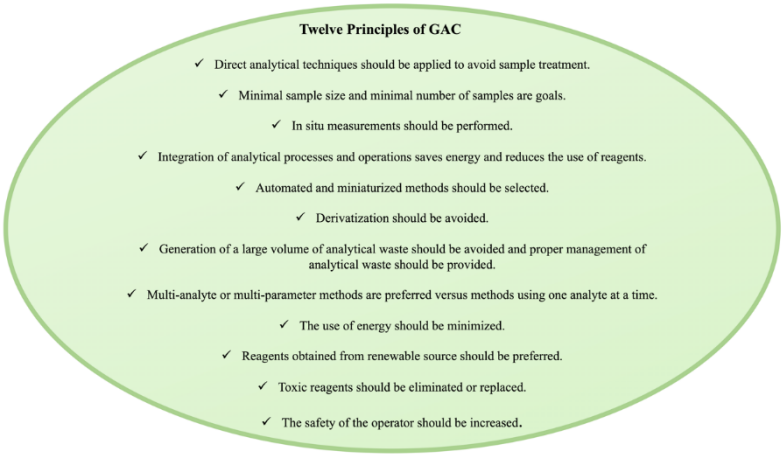

Figure 2. 12 principles of GAC (Gałuszka et al., 2013)

2. Green Chemistry Approaches in HPLC Techniques

Around the world, many analytical HPLC instruments are used, which creates millions of liters of chemical waste, some of which is toxic or harmful to the environment (Fitch et al., 2022; Gaber et al., 2011). Green solvents (such as EtOH, water) are preferred over traditional solvents (such as acetonitrile/water or methanol/water mixtures) in the green chemistry approach in liquid chromatographic applications (Ordoñez et al., 2015).

The application of sustainable chemical approaches to chromatography has become increasingly popular recent years. The objective of GC is to run processes in accordance with the principles of sustainability (Hemdan et al., 2022).

The most crucial GC principles, which are clearly related to analytical chemistry, are waste reduction, cleaner solvents and auxiliaries, energy-efficient design, and safer chemistry to prevent the risk of chemical accidents (Koel & Kaljurand, 2006).

One solution for greening chromatography is to solvent-replacement of conventionally hazardous organic solvents such as acetonitrile and methanol with eco-friendly alternatives such as water, isopropanol, biobased solvents,

deep eutectic solvents and ethanol. Currently, ethanol is one of the most eco-friendly organic solvents due to its low vapour pressure, lower toxicity, and lower disposal costs (Claux et al., 2021; Płotka et al., 2013; Rainville et al., 2012; Smith, 2008). In addition, the polarity of EtOH is relatively similar to ACN and MeOH in the chromatographic system. Also, its performance is satisfactory in the green liquid chromatography (Yabré et al., 2018).

Sampling, sample preparation, analytical measurement, and data evaluation are the steps of a chemical measurement technique. In order to get results that are analytically accurate, it is important to supply a homogeneous and representative sample. As part of the sample preparation process, samples are often converted to a form compatible with the analysis instrument, cleaned of interfering components, or analyte enrichment is applied to meet the sensitivity requirements of the analytical method. Without a doubt, "no solvent" is the best solvent for sample preparation in analytical chemistry. The issue is that the majority of chemical analysis techniques require the introduction of samples in a liquid form. To prepare a sample for analysis, it is not enough to merely dissolve the target chemicals in a solvent. It entails a number of procedures, including sampling, homogenization, extraction, cleanup, and concentration. Preferring to prepare samples in situ; use of safer solvents and reagents; targeting sustainable, reusable and renewable materials; minimizing waste, the amount of samples, chemicals, materials, and energy consumption; maximizing sample throughput; integrating steps and promoting automation; selecting the greenest possible post-preparation configuration for analysis, and ensuring safe procedures for the operator are the basic principles for greening the sample preparation step in green analytical chemistry (López-Lorente et al., 2022; Vian et al., 2017).

Since HPLC and related chromatographic techniques are the most widely preferred analytical tools in pharmaceutical and food industries, minimizing of environmentally harmful chemicals is essential. Various HPLC techniques including green approach in recent years are shown in **Table 1.**

Table 1. Current green approach HPLC methods used in food and pharmaceutical analysis

Chromatographic technique	Analyte	Matrices	References
HPLC - DAD	Deferasirox Deferiprone	Tablets Capsules	(Fares et al., 2022)
HPLC – PDA	Lamivudine Zidovudine Nevirapine	Tablets	(Vieira-Sellaï et al., 2022)
UHPLC - DAD	Metronidazole Dexibuprofen	Tablets	(Farid & Abdelwahab, 2022)
HPLC - UV	Amlodipine Telmisartan Hydrochlorothiazide	Synthetic laboratory mixture	(Tiris et al., 2022)
HPLC-UV	Ribavirin Sofosbuvir Ledipasvir	Tablets	(EL-Shorbagy et al., 2020)
HPLC - UV	Piracetam Levetiracetam Brivaracetam	Tablets	(Mansour et al., 2022)
HPLC-MS/MS	Atropine Scopolamine	Tea Herbal tea	(González-Gómez et al., 2022)
UPLC-MS/MS MLC-UV HPLC - UV	Sulfadiazine Trimethoprim	Bovine meat Chicken muscles	(Mohamed & Fouad, 2020)
HPLC - ELSD	Aspartame	Beverages	(Soyseven et al., 2022)
HPLC - UV	Rhodamine B	Chili powder Gelly powder Tomato ketchup	(Arabi et al., 2020)
HPLC - UV	Ferulic acid Caffeic acid Cinnamic acid	Olive Almond Sesame Cinnamon oil	(Khezeli et al., 2016)

Table 1. *(continued)*

HPLC - UV	Acesulfame-K Butylated Hydroxytoluene Aspartame Phenylalanine	Chewing Gum	(Zamzam et al., 2019)
HPLC - DAD	Oleuropein	Olive oil Olive leaf extracts Nanostructured lipid carriers	(Huguet-Casquero et al., 2020)
HPLC - UV	Sulfanilamide Sulfadiazine Sulfamethazine Sulfamerazine Sulfapyridine Sulfathiazole Sulfamethoxypyridazine Sulfamethoxazole Sulfadimethoxine	Milk Beef	(Duan et al., 2020)
HPLC - UV	Timolol Latanoprost	Eye Drops	(Ibrahim et al., 2019)
HPLC - DAD HPLC - FD	Amlodipine besylate Candesartan cilexetil	Tablet Human plasma	(Boltia et al., 2022)
HPLC - DAD	Adenosine Inosine Guanosine Uridine	Cordyceps	(Zheng-Ming et al., 2021)
HPLC - UV	Silymarin and vitamin E	Capsules Human plasma samples	(Gamal et al., 2019)
HPLC - RID	Narasin	Feed	(Abid et al., 2022)

3. Conclusion

Analytical methods such as HPLC are frequently used in many laboratories for the analysis of substances in the food and pharmaceutical industry. During these studies, a wide variety and high amount of hazardous chemicals are used.

Green chemistry approaches have increased recently in method development and application using HPLC in food and pharmaceutical analysis. Green approaches are discussed from different perspectives. Especially, it is essential to carry out studies to minimize harmful chemicals released during routine analysis. Green approaches can be considered from many different aspects, from solution selection to sample preparation. For sustainable environment the use of eco-friendly techniques should be preferred.

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CHAPTER IX

THE DIFFERENCES BETWEEN GEOMETRIC ARITHMETIC INDEX AND HARMONIC INDEX

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1. Introduction

Graph theory, which started in 1736 with the solution of the mathematical problem called "The seven bridges of Königsberg" by Leonhard Euler, is used in many relational structures from financial and communication systems to social and biological processes in the real world. Graph theory has become useful and functional in maps, navigation, electrical circuits, artificial intelligence, computer networks and in many structures such as the structure of atomic bonds. For this reason, many articles in the literature try to develop mathematical methods using graph theory.

Topological indices are constant numbers that have many applications, especially chemical graphs. These indexes, which were first defined in the early 1940s, have started to get ahead of the previously used methods with the acceleration of calculations in parallel with the developments in processor speeds in recent years. For this purpose, many topological graph indices have been defined. These indices are mostly defined depending on the vertex degrees, the distances between the vertices or the matrices of the graphs. Geometric arithmetic index and harmonic index are among these indexes defined depending on degrees.

In this paper, the some bounds are found about geometric arithmetic index and harmonic index. Different indices, vertices and degrees are used to find these bounds.

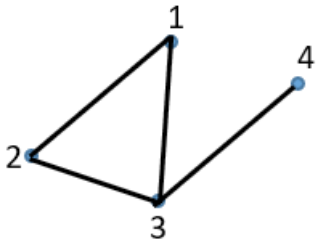
2. Preliminaries

In this section, some definitions and lemmas are given to use in main results:

2.1. Graph:

A graph is a collection of lines called vertices, each of which consists of edges connecting these vertices or just the vertices themselves. It is denoted by G= (V, E); V=V(G) (Set of vertices (Nodes)) and E=E(G) (Set of edges).**[1]**

Example 2.1.1:



Shape 2.1.1

V= {1,2,3,4}

E= {(1,2), (2,3), (1,3), (3,4)}

Example 2.1.2:

Graf Theory Usage Areas:

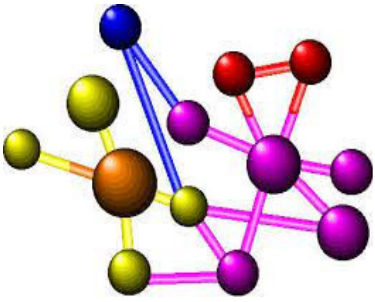
Maps**:**

Shape 2.1.2.1

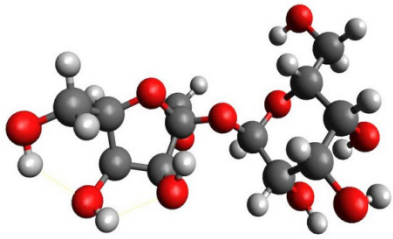
Navigations:

Shape 2.1.2.2

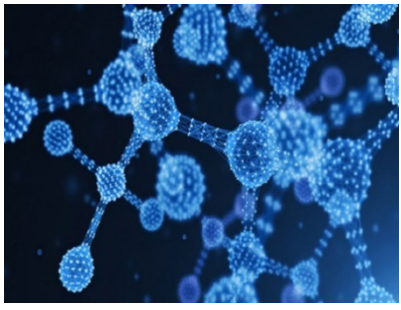
Atomic Bonds:



Shape 2.1.2.3



Shape 2.1.2.4



Shape 2.1.2.5

2.2. Adjancecy:

Let G= (V, E) be a graph. If there is at least one edge between any u and v points of G, the points u and v are said to be adjacent and denoted by u~v.

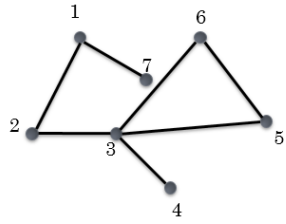
Example 2.2.1

ın the shape 2.1.1,

1~2, 2~3, 1~3, 3~4

2.3. Degree of a Point:

The number of edges emerging from any point u of the graph G= (V, E) is called the degree of u. Degree of u is denoted as .

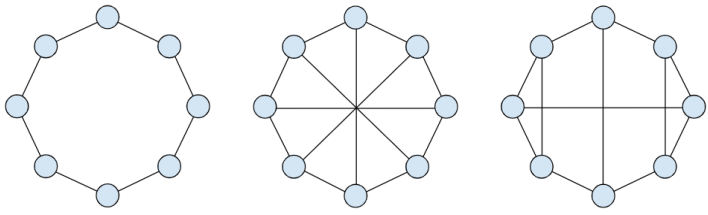


Shape 2.3

$d_1 = 2,\ d_2 = 2,\ d_3 = 4,\ d_4 = 1,\ d_5 = 2,\ d_6 = 2,\ d_7 = 1.$

2.4. Regular Graph:

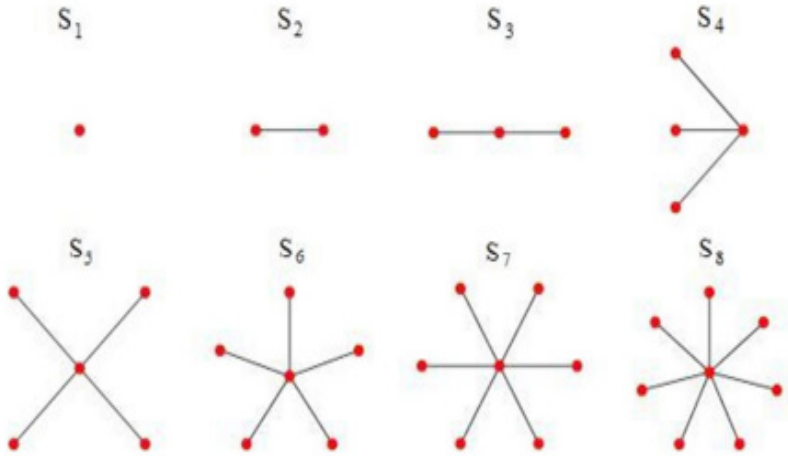
A graph with every point having the same degree is called a regular graph.



Shape 2.6.

2.5. Star Graph:

If the degree of one vertex of n-pointed tree graph is n-1 and the degrees of all the remaining vertices are 1, such graphs are called star graphs and are denoted by S_n *or* $K_{1,n}$.



Shape 2.7.

2.6. Geometric Arithmetic index [6]:

Geometric-Arithmetic index is described by degrees:

$$GA=GA(G)=\sum_{uv\in E(G)}\frac{2\sqrt{d_u d_v}}{d_u+d_v}$$

where and are degrees of u and v, respectively.

2.7. The Harmonic Index [2]:

The Harmonic index is one of the toplological indices in molecular graph theory. It is defined as follows:

$$H(G)=\sum_{uv\in E(G)}\frac{2}{d_u+d_v}$$

2.8. Forgotten Index [3]:

The Forgotten index is defined as the sum of the squares of the degrees:

$$F(G)=\sum_{uv\in E(G)} {d_u}^2+{d_v}^2.$$

2.9. Second Zagreb Index [4]:

The second zagreb index found by Zagreb is also determined by degrees:

$$M_2(G)=\sum_{uv\in E(G)} d_u d_v.$$

2.10. Lemma [5]:

Let a=(a_i) and b=(b_i), i=1, 2,…,n be positive real number sequences. For r≥ 0,

$$\sum_{i=1}^{n} \frac{{a_i}^{r+1}}{{b_i}^{r}} \geq \frac{(\sum_{i=1}^{n} a_i)^{r+1}}{(\sum_{i=1}^{n} b_i)^{r}}.$$

3. Main Results

Topological indices are interesting because they capture some properties of a molecule in a single number. Geometric index and harmonic index are among the popular topological indexes. Here, different bounds are given for geometric index and harmonic index difference in terms of degrees and edges.

Theorem 3.1. Let G be a graph with m edges, then

$$GA-H \leq \frac{\sqrt{m(M_2(G)-2\sqrt{M_2(G)}+m)}}{\delta}.$$

where minimum degree is δ.

Proof. Taking the mathematical difference between geometric index and harmonic index, it is obtained as

$$\text{GA-H}= \sum_{uv\in E(G)} \frac{2\sqrt{d_u d_v}-2}{d_u+d_v}$$

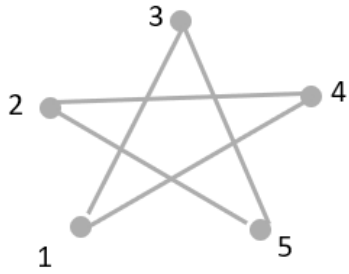
$$\leq \sqrt{4 \sum_{uv\in E(G} (\sqrt{d_u d_v}-1)^2 \sum_{uv\in E(G)} \frac{1}{(d_u+d_v)^2}}$$

$$=2\sqrt{(\sum_{uv\in E(G)} d_u d_v - 2\sum_{uv\in E(G)} \sqrt{d_u d_v} + m)\sum_{uv\in E(G)} \frac{1}{(d_u+d_v)^2}}.$$

By the definition of Second Zagreb index,

$$GA-H \leq \frac{\sqrt{m(M_2(G)-2\sqrt{M_2(G)}+m)}}{\delta}.$$

Example. 3.1.



Shape 3.1

According to Theorem 3.1, m=5, δ=2. Also, (GA-H) =0,5 and . Then, 0,5.

Theorem 3.2. Let G be a graph having m edges, then

$$(GA-H)^2 \geq \frac{4(\delta-1)^2}{F(G)+2M_2(G)}+\frac{2m(m-1)(\delta-1)^2}{\Delta^2}.$$

where the minimum degree δ and maximum degree Δ.

Proof. Let remove some points:

$$(GA-H)^2=\sum_{uv\in E(G)}4\left(\frac{(\sqrt{d_u d_v}-1)}{d_u+d_v}\right)^2+8\sum_{uv\neq rs\in E(G)}\left(\frac{\sqrt{d_u d_v}-1}{d_u+d_v}\right)\left(\frac{\sqrt{d_r d_s}-1}{d_r+d_s}\right)$$

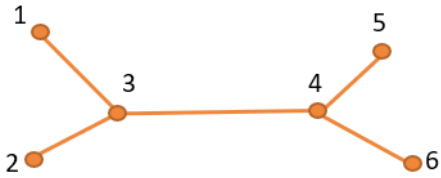
$$\geq \sum_{uv\in E(G)}4\frac{(\delta-1)^2}{{d_u}^2+{d_v}^2+2d_u d_v}+2\sum_{uv\neq rs\in E(G)}\frac{(\delta-1)^2}{\Delta^2}$$

$$\geq 4\frac{(\delta-1)^2}{\sum_{uv\in E(G)}({d_u}^2+{d_v}^2+2d_u d_v)}+\frac{2m(m-1)(\delta-1)^2}{\Delta^2}.$$

Using the Forgotten and Second Zagreb index,

$$(GA-H)^2=\frac{4(\delta-1)^2}{F(G)+2M_2(G)}+\frac{2m(m-1)(\delta-1)^2}{\Delta^2}.$$

Example 3.2.



Shape 3.2

According to Theorem 3.2, m=5, δ=1. Also, (GA-H) =2,12 and**.** Then, 4,5.

Theorem 3.3. Let G be a graph having n$\geq$ 3 vertices, m edges, then

$$\frac{\delta-1}{\Delta} < GA - H < 2m\sqrt{\Delta-1}$$

where Δ is the maximum degree.

Proof. By the help of the definition of Geometric Arithmetic index and Harmonic index, it shows that

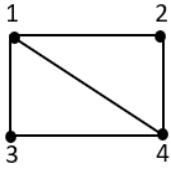
$$\sum_{uv\in E(G)} \frac{2\sqrt{d_u d_v}-2}{d_u+d_v} > \frac{\sum_{uv\in E(G)} 2\sqrt{d_u d_v}-2}{\sum_{uv\in E(G)} d_u+d_v} > \frac{\delta-1}{\Delta}.$$

Also, the upper bound is

$$\sum_{uv\in E(G)} \frac{2\sqrt{d_u d_v}-2}{d_u+d_v} < \sum_{uv\in E(G)} 2\sqrt{d_u d_v}-2$$

$$< 2\sqrt{\sum_{uv\in E(G)} 1 \cdot \sum_{uv\in E(G)} \sqrt{d_u d_v}-1} < 2m\sqrt{\Delta-1}.$$

Example 3.3.



Shape 3.3

Let m=5, $\delta = 2$ then GA(G)$\approx$ 4,91, H(G)$\approx$ 1,93 and Δ= 3.

$\frac{2-1}{3} < 4{,}91 - 1{,}93 < 2.5\sqrt{3-1}$ then 0,33< 2,98 < 14,1.

Theorem 3.4. Let G be a graph having m edges, p pendant vertices, then

$$(m\text{-}p)\,\frac{\delta-1}{\Delta}+2p\,\frac{\sqrt{\delta}-1}{\Delta+1}\le GA-H\le(m\text{-}p)\,\frac{\Delta-1}{\delta}+2p\,\frac{\sqrt{\Delta}-1}{\delta+1}.$$

Proof. Let p be pendant vertices. Then,

$$\text{(GA-H) (G)}=\sum_{\substack{uv\in E(G)\\ d_u d_v\neq 1}}\frac{2\sqrt{d_u d_v}-2}{d_u+d_v}+\sum_{\substack{uv\in E(G)\\ d_u=1}}\frac{2\sqrt{d_u d_v}-2}{d_u+d_v}.$$

If the maximum degree and minimum degree are placed, it has

$$(GA-H)(G)\le\sum_{\substack{uv\in E(G)\\ d_u d_v\neq 1}}\frac{2\Delta-2}{2\delta}+\sum_{\substack{uv\in E(G)\\ d_u=1}}\frac{2\sqrt{d_v}-2}{1+d_v}$$

$$\le\sum_{\substack{uv\in E(G)\\ d_u d_v\neq 1}}\frac{\Delta-1}{\delta}+\sum_{\substack{uv\in E(G)\\ d_u=1}}2\frac{\sqrt{\Delta}-1}{\delta+1}$$

$$=(m\text{-}p)\,\frac{\Delta-1}{\delta}+2p\,\frac{\sqrt{\Delta}-1}{\delta+1}.$$

Similary,

$$\text{(GA-H) (G)}=\sum_{\substack{uv\in E(G)\\ d_u d_v\neq 1}}\frac{2\sqrt{d_u d_v}-2}{d_u+d_v}+\sum_{\substack{uv\in E(G)\\ d_u=1}}\frac{2\sqrt{d_u d_v}-2}{d_u+d_v}$$

$$\ge\sum_{\substack{uv\in E(G)\\ d_u d_v\neq 1}}\frac{2\delta-2}{2\Delta}+\sum_{\substack{uv\in E(G)\\ d_u=1}}\frac{2\sqrt{d_v}-2}{1+d_v}$$

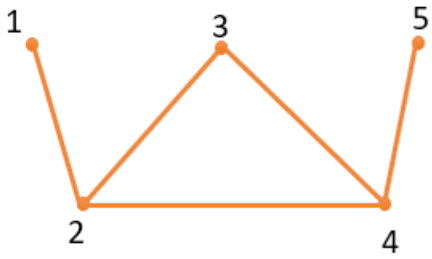
$$\ge\sum_{\substack{uv\in E(G)\\ d_u d_v\neq 1}}\frac{\delta-1}{\Delta}+\sum_{\substack{uv\in E(G)\\ d_u=1}}2\frac{\sqrt{\delta}-1}{\Delta+1}$$

$$=(m\text{-}p)\,\frac{\delta-1}{\Delta}+2p\,\frac{\sqrt{\delta}-1}{\Delta+1}$$

Hence,

$$(m\text{-}p)\,\frac{\delta-1}{\Delta}+2p\,\frac{\sqrt{\delta}-1}{\Delta+1}\le GA-H\le(m\text{-}p)\,\frac{\Delta-1}{\delta}+2p\,\frac{\sqrt{\Delta}-1}{\delta+1}.$$

Example 3.4.



Shape 3.4

Let m=5, p=2. Then, $\delta = 1,\ \Delta = 3$ and (GA-H) (G)$\approx 2{,}54$. By the Theorem 3.4, $0 \leq 2{,}54 \leq 7{,}46$.

Theorem 3.5. Let G be a tree having n vertices. Therefore

$$(\text{GA-H})\ (G) \geq \frac{2}{n}(\sqrt{M_2(G)}\text{-m}).$$

with equality if and only if the star graph G.

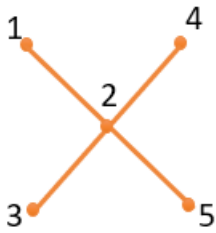
Proof. It is known that $d_u + d_v \leq n,\ uv \in E(G)$. If the geometric index and harmonic index difference is considered from a different perspective, it gets,

(GA-H) (G)=

$$\sum_{uv\in E(G)} \frac{2\sqrt{d_u d_v}-2}{d_u+d_v} \geq \sum_{uv\in E(G)} \frac{2\sqrt{d_u d_v}-2}{n} \geq \frac{2}{n}\left(\sqrt{\sum_{uv\in E(G)} d_u d_v}\text{-}m\right)$$

$$= \frac{2}{n}(\sqrt{M_2(G)}\text{-m}).$$

Example 3.5.



Shape 3.5

Let m=p=4, $\Delta = 4, \delta = 1$ and n=5. Then, (GA-H) (G)=1.6 and $M_2(G)$=16. Using the Theorem 3.5, 1,6≥ $\frac{2}{5}(\sqrt{16}-4)$ and hence, 1,6≥ 0.

Theorem 3.6. Let G be a connected graph having $\delta \geq 2$ edges. Then,

$$\text{(GA-H) (G)} \geq \frac{\delta H(G)}{m\Delta}.$$

Proof. Setting r=1, $a_{uv}=\sqrt{\frac{2}{d_u+d_v}}$ and $b_{uv}=\frac{d_u+d_v}{2\sqrt{d_u d_v}-2}$ for every $uv \in E(G)$. Applying Lemma 2.11, it is obtained by

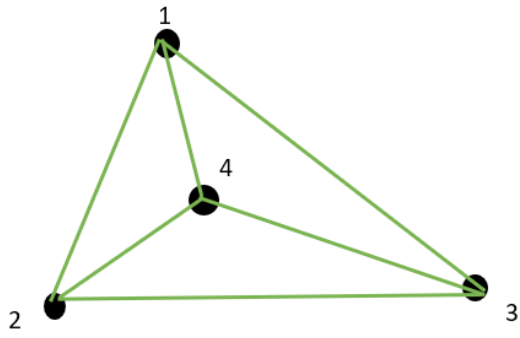
$$\sum_{uv\in E(G)} \frac{\frac{2}{d_u+d_v}}{\frac{d_u+d_v}{2\sqrt{d_u d_v}-2}} \geq \frac{\sum_{uv\in E(G)}\frac{2}{d_u+d_v}}{\sum_{uv\in E(G)}\frac{d_u+d_v}{2\sqrt{d_u d_v}-2}}$$

$$= \frac{H(G)}{\sum_{uv\in E(G)}\frac{d_u+d_v}{2\sqrt{d_u d_v}-2}} \geq \frac{H(G)}{\sum_{uv\in E(G)}\frac{d_u+d_v}{2\sqrt{d_u d_v}}} \geq \frac{H(G)}{\frac{m\Delta}{\delta}}.$$

Hence,

$$\text{(GA-H) (G)} \geq \frac{\delta H(G)}{m\Delta}.$$

Example 3.6.



Shape 3.6

Let m=6 and. Then, GA(G)=4 and H(G)=2. Hence, Theorem 3.6 gives that 40,33.

4. Conclusion

In this paper, two topological indexes, which are defined based on point degrees, are examined. By comparing these two topological indices, the bounds for the difference between them are found. It is thought that these results will shed light on other indices and the chemical graph theory, which is the area of use of these indices.

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CHAPTER X

EXACT, NUMERICAL AND EMPIRICAL METHODS FOR LATERALLY LOADED PILE PROBLEM

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1. Introduction

The objective of this writing is to summarize the formulation and the available solution methods for the laterally loaded pile problem, frequently encountered in civil-geotechnical engineering analysis. The summary is primarily a synthesis of the information available in the published literature.

The mathematical formulation of the laterally loaded pile problem is based either on subgrade reaction approach or elastic continuum approach (Horvath, 1991). The subgrade reaction approach is based on the Winkler hypothesis (1867), and is the most widely used method (Prakash and Sharma, 1990). With this approach, a laterally loaded pile is treated as a beam resting on an elastic subgrade. A series of closely spaced, independent elastic springs replaces the subgrade. The governing differential equation is given as:

$$EI\frac{d^4y}{dx^4} + P_x\frac{d^2y}{dx^2} + k_h y = 0 \qquad \textbf{(1)}$$

Where, EI = flexural stiffness of pile
y = lateral deflection
x = length along pile

P_x = axial load

k_h = spring constant to represent the soil.

The solution of the above differential equation is obtained with appropriate representation of the soil by a spring constant and with the inclusion of the proper boundary conditions. A solution can be obtained by either in closed form (exact), or using approximate solution methods. Although the closed form solutions are desired, they can be time consuming to achieve and limited in use. Approximate solutions are most likely to be used in practice since they provide a satisfactory answer for most of the time. Approximate solutions include:

- Series expansion,
- Finite difference method,
- Finite element method, and
- Other approximate methods.

2. Closed Form Solution

It is usually assumed that the vertical loads and the lateral loads are not coupled. Thus, the laterally loaded pile problem is solved by neglecting the effect of the vertical load. Under this assumption, the governing differential equation becomes:

$$EI\frac{d^4y}{dx^4} + k_h y = 0 \qquad \textbf{(2)}$$

If one assumes that k_h is constant, which is a good approximation for clays but not for granular soils and divide both sides of Equation (2) by EI, the following is obtained

$$\frac{d^4y}{dx^4} + 4\alpha^4 y = 0 \qquad \textbf{(3)}$$

$$y = e^{\alpha x}\left(C_1\cos\alpha x + C_2\sin\alpha x\right) + e^{-\alpha x}\left(C_3\cos\alpha x + C_4\sin\alpha x\right) \qquad \textbf{(4)}$$

In which, $a^4 = k_h/(4EI)$. The solution to Equation (3) is given by Equation (4) as obtained first by Hetenyi (1946).

Where, x is the depth from the pile top, and C_1, C_2, C_3, and C_4 are the constants to be determined from the boundary conditions.

These constants (C1, C2, C3, and C4) for long and short piles are defined separately because the boundary conditions are different for short and long piles (Desai and Christian, 1977). A brief summary is presented below.

2.1. Long Piles

The lateral deflection at the tip (the farthest end from the ground level) of the long piles is negligible. Mathematically, this corresponds to zero deflection when pile length (x) has very large values. For this condition, $C_1 = C_2 = 0$. At the top of the pile (x=0), the second derivative of pile deflection is known as $y'' = M_t/EI$, from which C_4 is obtained as:

$$C_4 = -\frac{M_t}{2EI\alpha^2} \quad \textbf{(5)}$$

Where, M_t is the applied moment at the top of the pile. C_3 is obtained from the third derivative of the pile deflection from $y''' = V_t/EI$ as:

$$C_3 = \frac{V_t}{2EI\,\alpha^3} + \frac{M_t}{2EI\,\alpha^2} \quad \textbf{(6)}$$

In which, V_t is the applied shear force (lateral load) at the top of the pile.

2.2. Short Piles

The boundary conditions for short piles for the second and third derivatives of lateral pile deflection $y'' = M_t/EI$ and $y''' = V_t/EI$ at pile top at x = 0 yield Equations (7) and (8), respectively.

$$C_2 - C_4 = \frac{M_t}{2EI\alpha^2} \quad \textbf{(7)}$$

$$-C_1 + C_2 + C_3 + C_4 = \frac{V_t}{2EI\,\alpha^3} \quad \textbf{(8)}$$

At the tip of the pile (x = L), both the second derivations and the third derivatives become zero (i.e., $EIy'' = 0$ and $EIy''' = 0$). Applying these boundary conditions, the following two equations are obtained:

$$2\alpha^2 e^{\alpha L}\left(C_2\cos\alpha L - C_1\sin\alpha L\right) + 2\alpha^2 e^{-\alpha L}\left(C_3\sin\alpha L - C_4\cos\alpha L\right) = 0 \quad \textbf{(9)}$$

$$2\alpha^3 e^{\alpha L}\left(C_2 \cos\alpha L - C_1 \sin\alpha L - C_2 \sin\alpha L - C_1 \cos\alpha L\right)$$
$$+ 2\alpha^3 e^{-\alpha L}\left(- C_3 \sin\alpha L + C_4 \cos\alpha L + C_3 \cos\alpha L + C_4 \sin\alpha L\right) = 0 \quad \textbf{(10)}$$

Equations (7) through (10) form a linear system with respect to C_1, C_2, C_3, and C_4. The solution of this linear system provides the relations for the constants C_1, C_2, C_3, and C_4.

3. Approximate Solution by Series Expansion

A series solution to Equation (2) can be obtained by using the following series:

$$y = \sum_{i=0}^{\infty} C_i x^i \quad \textbf{(11)}$$

Where, x = depth,

y = lateral displacement, and

C_i = coefficients yet to be determined.

An approximate solution can be obtained by truncating the series. The accuracy of the solution will increase with increasing number of coefficients, but determining the coefficients will get equally difficult. Therefore, the series solution is likely to handle simple geometric and boundary conditions. For complicated geometric and boundary conditions numerical techniques such as the Finite Difference and the Finite Element methods become more advantageous.

4. Approximate Solution by Finite Difference Methods

The finite difference method can be applied to laterally loaded piles if the piles are initially straight and carry no bending moment originated from pile driving. A brief formulation, adopted from Reese and Desai (1977) is presented below. The pile is first divided into a number of small elements. The governing differential equation for each element is the same as Equation (1). Firstly, the fourth derivative is written as:

$$\left[\frac{d^2}{dx^2}\left(E\ \frac{d^2 y}{dx^2}\right)\right] = \left(\frac{d^2 M}{dx^2}\right) \quad \textbf{(12)}$$

Secondly, the terms of the governing differential equation will have the following finite difference forms for the i^{th} element. Based on the equilibrium of forces on a pile element,

$$\left(\frac{d^2M}{dx^2} \right)_i = \frac{\left(\frac{dM}{dx} \right)_{i-1/2} - \left(\frac{dM}{dx} \right)_{i+1/2}}{h} \tag{13}$$

in which h is the length of the i^{th} pile element and M is the bending moment.

The formulation in Equation (13) means that the second derivative of M with respect to x for the i^{th} element is equal to the slope of the first derivative of M, which is numerically calculated with respect to the values of the first derivatives at the midpoints of the $(i-1)^{th}$ and $(i+1)^{th}$ elements. After substituting the finite difference forms for dM/dx and d^2y/dx^2 into Equations (12) and (13), the following is obtained.

$$\left(\frac{d^2M}{dx^2} \right)_i \approx \frac{1}{h^4} \begin{bmatrix} EI_{i-1}y_{i-2} - 2(EI_{i-1} + EI_i)y_{i-1} \\ +(EI_{i-1} + 4EI_i + EI_{i+1})y_i \\ -2(EI_i - EI_{i+1})y_{i+1} + EI_{i+1}y_{i+2} \end{bmatrix} \tag{14}$$

The finite difference for the second term of Equation (1), assuming P_x is constant over the entire length of the pile, is of the form:

$$P_x \frac{d^2y}{dx^2} = \frac{P_x (y_{i-1} - 2y_m + y_{i+1})}{h^2} \tag{15}$$

Finally, the final form of the finite difference form of Equation 1 for the i^{th} pile element is obtained as:

$$\begin{aligned} &E_{i-1}y_{i-2} + (-2E_{i-1} - 2E_i + P_x h^2)y_{i-1} \\ &+ (E_{i-1} + 4E_i + E_{i+1} - 2P_x h^2 + k_h h^4)y_i \\ &+ (-2E_i - 2E_{i+1} + P_x h^2)y_{i+1} + E_{i+1}y_{i+2} = 0 \end{aligned} \tag{16}$$

Equation (16) needs to be written for all pile elements. This will lead to a set of linear equations. After applying the boundary conditions, a linear set of equations is obtained and subsequently solved and therefore, the displacements at the nodes are determined. Other quantities (slope, moment, and shear) are numerically derived from the displacements. The moment at node *i* is calculated as:

$$M_i \approx \frac{EI_i\ (y_{i-1} - 2y_i + y_{i+1})}{h^2} \quad \textbf{(17)}$$

The boundary conditions involving the derivatives of *y* are expressed in the finite difference forms as well. In order to achieve this, one needs to introduce some imaginary nodes called fictitious or phantom points according to Reese and Desai, (1977) at the pile boundaries (i.e., the top and the bottom of the pile).

5. Approximate Solution by Finite Element Method

The true behavior of a laterally loaded pile is a three-dimensional problem. The vertical displacement is not due to the lateral loading; it takes place due to the vertical loading. Therefore, the lateral loading causes lateral displacement on planes perpendicular to the vertical axis of the pile along the pile length. It is usually of greatest interest to know the largest displacement on these planes, particularly at the ground level. The bending moment and the applied torsion are also of interest. Equation (1) provides the two dimensional representation of the laterally loaded pile problem. In most cases, this will lead to a satisfactory solution. However, it should be born in mind that three dimensional representation of the pile is the most correct approach. Equation (1) can be modified as follows:

$$EI\ \frac{d^4y}{dx^4} + P_x\ \frac{d^2y}{dx^2} + p_{(x)} = 0 \quad \textbf{(18)}$$

Where $p(x)$ = soil pressure acting on the pile ($p(x) = k_h\, y$),

P_x = axial force, and

k_h = coefficient of horizontal subgrade reaction, which is expressed as the ratio of applied soil pressure *p* to pile deflection *y*.

The essence of the finite element method lies in obtaining a functional, function of function(s), and minimizing this functional, which is equivalent to solving the governing differential equation. Over the years, mathematical foundations of the method have been perfected and various approaches have been established in obtaining a functional for a given problem. One of the available techniques is to obtain the "weak form" of the differential equation given, using variational methods. If both sides of Equation (18) are multiplied by a slowly varying test function v(x) and integrated over the domain ($0< x <L$), an integral is obtained, which leads to the weak form.

The test function can be any function with one limitation: v(x) is zero whenever the essential boundary terms are specified. This means that the solution is exact at boundaries and approximate elsewhere. The integration is done through integration by parts, which reduces the order of derivatives, thereby gives the name "weak form" because the derivatives are weakened. After partial integration, the function v(x) is assumed to be the same as the solution y(x) and the weak form is obtained. The mathematics involved is too lengthy to fully present for the scope of this chapter. The weak form is given as follows:

$$\pi = \frac{1}{2}\int_{x=0}^{L}\left[\ EI\ (y'')^2 + P_x\ (y')^2 + p_{(x)}y\ \right] dx \tag{19}$$

An approximate solution to Equation (19) can be found by dividing the pile into a finite number of elements and assuming a solution over each element:

$$y_{(x)} = \sum_{j=1}^{m} N_j q_j \tag{20}$$

Where, m = number of unknowns per node,

N_j = interpolating functions called shape functions, and

q_j = nodal unknowns yet to be calculated.

The shape functions N(x) must be twice differentiable as can be understood by examining Equations (19) and (20). The next step is to substitute Equation (20) and the first and the second derivatives from Equation (20) into Equation (19). The stationary values of this new equation after the substitution are obtained by equating the partial derivatives of the new p with respect to q_i to zero. As a result, the desired condition of the equilibrium is provided. This leads to the following equation for a given element:

$$\sum_{j=1}^{m}\left[\ \int \left(EI\, N''_i N''_j + P_x N'_i N'_j\right) dx\ \right] q_j = Q_i \tag{21}$$

In which Qi = nodal forces obtained by assuming a variation for p(x).

The symbolic representation of Equation (21) can be given as: $[K]\{q_j\}=\{Q_i\}$, in which K_{ij} is given in Equation (22) and Q_i is given in Equation (23).

$$K_{ij} = \int \left(EI\, N_i^{''} N_j^{''} + P_x N_i^{'} N_j^{'}\right) dx \tag{22}$$

$$Q_i = \int N_i p_{(x)} dx \tag{23}$$

Using Equations (22) and (23), the stiffness matrix and the load vector, respectively, are determined for each element. These quantities are then assembled together to obtain the global stiffness matrix, and the global load vector. The solution is obtained by solving $[K]\{q_j\}=\{Q_i\}$ for $\{q_j\}$.

The most crucial point of the solution is the proper representation of the soil modulus through p(x). If p(x) is assumed to be linear, then a system of linear equations is obtained. The solution becomes trivial with matrix solvers. It is a well known fact that p(x) is a function of the lateral deflection, which leads to a set of non-linear equations. For nonlinear p(x), the solution is obtained by iterative procedures by assuming deflections for each node and thereby calculating p(x) and solving for q_j (nodal unknowns) until the assumed and the calculated nodal unknowns are the same within a tolerance range. The Newton and the Modified Newton methods are mostly used for the iterations.

6. Empirical Methods

This section summarizes four commonly used empirical methods when analyzing piles under lateral loading. The methods include p-y, SALLOP, Evans and Duncan (1982) and equivalent cantilever approach.

6.1. The Method of p-y Curves

The p-y method is the most widely used empirical method in the subject area. The method considers the fact that the relationship between the soil pressure (p) and the pile deflection (y) is non-linear. The greatest contributors to the development of the p-y method are Matlock (1970), Reese et al. (1974), Reese and Welch (1975), and Bhushan et al. (1979).

The essential of the method is to introduce a series of p-y curves to represent the true behavior of soils by considering the non-linearity of the soil modulus. The main purpose of the method is to obtain a representative value of k_h for the desired depth and deflection values. This is accomplished through an iterative process by assuming a deflection and calculating the value of k_h. The iterations are continued until the assumed and calculated deflections are the same within a tolerance limit. When representative p-y curves are used, the method is capable of reflecting the real deflection behavior of the pile and the moment distribution along the pile. The challenge is to obtain a representative set of p-y curves for each site.

Several procedures are available to estimate the p-y curves (Reese and Wang, 1997; Stevens and Audibert, 1979; Bhushan et al., 1979; Briaud et al., 1982; O'Neill and Gazioglu, 1984; O'Neil and Dunnavant, 1984; Dunnavant and O'Neill, 1985; Reese and Cox, 1968; Reese et al, 1975; Kooijman, 1989; and Brown et al., 1989).

6.2. Evans and Duncan (1982) Method

This method is based on the results of series of computer analyses/ simulations with COM624 using the p-y curves method. The results are intelligently normalized and compiled in chart forms such that the lateral load at the ground level and the maximum moment can be estimated directly for a given deflection at the pile head. The charts can be used for either fixed head or free head piles in either cohesive or cohesionless soils. The normalization process included the definition of a characteristic shear load and a characteristic moment. These definitions are based on empirical equations and can be found in Evans and Duncan (1982). The deflection is normalized by the width or the diameter of the pile.

To find the lateral load and the corresponding moment, one simply needs to assume a tolerable deflection at the pile head and read off the normalized lateral load and the corresponding normalized maximum moment. These normalized load and normalized moment are then multiplied by the characteristic load and the characteristic moment, respectively, to find the lateral load at the top of the pile and the maximum moment in the pile, respectively.

6.3. SALLOP Method

SALLOP stand for Simple Approach for Lateral Loads on Piles and it is a simplification of a method developed at Texas A&M University based on the p-y curves concept. The method is geared towards making use of the pressuremeter limit pressure and the pressuremeter modulus. Briaud (1997) indicates that SALLOP is a semi-empirical and semi-theoretical method. It was developed based on the theory and then the theoretical equations were modified based on 20 full-scale pile tests.

The method assumes uniform Winkler soil and uses the closed-form solution as first developed by Hetenyi (1946). The essential of the method is based on the fact that the shear forces in a laterally loaded pile are negligible at some depth, called zero-shear depth, which is mostly responsible for the

behavior of the pile. The horizontal equilibrium at this depth constitutes the basis of SALLOP. However, the challenge is to find the extent of this depth. Briaud (1997) provides a recommendation about this zero-shear depth along with the details of how one would make use of the pressuremeter test results to estimate the maximum lateral load and the maximum moment acting on the pile.

6.4. Equivalent Cantilever Method

The equivalent cantilever method is proposed for designing piles of integral bridges by Greimann and Wolde-Tinsea (1988) and Abendroth, Greimann and Ebner (1989). This method appears to be widely accepted by the bridge engineers. The method is based on analytical and finite element studies. An equivalent cantilever column is used to replace the actual pile. In other words, the soil-pile system is reduced down to an equivalent cantilever column. Two alternatives are provided, one involving elastic behavior, and the other involving inelastic behavior of the piles. Finite element simulations indicated that both alternatives were conservative. Both alternatives are concerned with the vertical load carrying capacity of piles under lateral displacements induced by temperature changes. A worked-out example on the design of an integral abutment using the equivalent cantilever method is given by Barker et al. (1990). Girton et al. (1991) who evaluated this method experimentally concluded that the equivalent cantilever column model is sufficiently accurate for design purposes. The method does not consider the effects of the abutment/approach fill interactions and the effects of the induced stresses in the superstructure.

7. Conclusions

This writing summarizes the formulation and the available solution methods for the laterally loaded pile problem, frequently encountered in civil-geotechnical engineering analysis. It is observed that the methods for solving laterally loaded pile problems are mostly empirical since the soil modulus is not a unique soil property. Numerical methods such as finite difference and finite element methods provide very accurate results if the soil pressure is appropriately represented. The equivalent cantilever method does not consider the effects of the soil interactions around the piles.

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CHAPTER XI

DIFFERENCE SETS AND CHARACTER SUMS

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1. Introduction

Difference sets first appeared in James Singer's article in 1938 (Singer, 1938). Although it describes difference sets, its main theorem concerns the automorphism of a design. The fully systematic work of difference sets resulted from Marshall Jr Hall in the late 1940s (Hall, 1947).

Difference sets, which have many applications in real-life problems, combine abstract algebra, combinatorics, and geometry fields of mathematics. To follow up on some of these applications, the North Atlantic Treaty Organization (NATO) has supported the institute for advanced study of difference sets and sequences by bringing together experts and students from the fields of electrical engineering, computer science, and mathematics from many NATO countries (Cusick et al., 1998).

Difference sets are used in high-energy optics, in signal noise in communication, in the display of astronomical events, in constructing error correction codes, in facilitating processes in quantum computing, and in application areas such as coding and decoding (Ding, 1994 and 1997).

To understand what a difference set is, we need to know how the difference set is created. These sets have first been defined in commutative additive groups. The difference set, a particular subset of a group, exists only if it satisfies the structural conditions of the symmetric design.

On the other hand, group characters are associated not only with mathematical problems but also with problems related to engineering and

cryptography. Some of the mathematical problems issues as follows; algebraic structures' morphism theorems, solution of equations on finite fields, and exponential sums such as Gauss and Jacobi. In addition, character sums are used in evaluating cyclotomic numbers and finding difference sets. They are also good cryptographic functions used in keystream generators (Anh, 2014).

There is a relationship between group characters and difference sets. Group characters are used to examine difference sets if χ is a non-trivial irreducible character of an abelian (v, k, λ)- difference set D for $z\bar{z} = n$ and $n = k - \lambda$. G satisfies this condition for every non-trivial irreducible character of k (Turyn, 1965).

This work will give information about the necessary definitions and related theorems concerning difference sets and group characters and their relation.

2. Preliminaries

2.1. Groups

Definition 2.1.1. Let G be a non-empty set and a binary operation $*$ on that set.

G1) $a * b \in G$ for all $a, b \in G$ (Closure property),

G2) $a * (b * c) = (a * b) * c$ for each $a, b \in G$ (Association property),

G3) Every $a \in G$, there is an element $e \in G$ such that $a * e = e * a = a$ (Unit),

G4) Every $a \in G$, there is at least $a^{-1} \in G$ such that

$$a * a^{-1} = a^{-1} * a = e,$$

where $e \in G$ is the unit.

If its algebraic structure satisfies the above axioms, then $(G,*)$ is said to be a *group*.

Definition 2.1.2. If for $\forall a, b \in G$, $a * b = b * a$ in a group $(G,*)$, this group is called the *commutative group* or *Abel group*.

The addition (+) on commutative groups is called an *additive group*. Multiplication operation $(.)$ of the group is called the *multiplicative group*. The multiplicative group may not be commutative.

Definition 2.1.3. Let $(G,*)$ and (H,o) be two groups and $f: G \longrightarrow H$a be a function. For every $a,b \in G$, we have $f(a*b) = f(a)of(b)$, then function f is called a *homomorphism* from G to H. If homomorphism f is one-to-one and surjective, it is called *an isomorphism*. Also, $G = H$ and f is an isomorphism, then called an *automorphism*.

2.2 Finite fields

Definition 2.2.1: A *field* $\mathbb{F}$, along with the operations of addition $(+)$ and multiplication of $(.)$, provides the following axioms:

(1) $\mathbb{F}$ is a commutative group under operation $+$ with the unit element 0.

(2) The set of nonzero elements of $\mathbb{F}^* = \mathbb{F} - \{0\}$ is the commutative group according to the multiplication operation, and 1 is the unit element.

(3) Multiplication has the property of distribution over addition.

We describe the field with q element as the Galois field $GF(q)$. If p is prime, the mod p integers form $GF(p)$. Finite fields with the same number of elements are isomorphic to each other.

The basic properties of the finite field $GF(q)$ are given below.

- For some p prime number and an m positive integer such that $q = p^m$, then p is called the *characteristic of* $GF(q)$,
- $GF(q)$ contains the subfield $GF(p)$,
- $GF(q)$ is an m-dimensional vector space over $GF(p)$,
- Every subfield of $GF(q)$ has an order of p^t for t that divides m,
- Multiplicative group $GF(q)^*$ of the set of GF(q) nonzero elements is cyclic.

The *primitive element of* $GF(q)$ is defined by a generator of the multiplicative group $GF(q)^*$.

See (Gilbert & Gilbert 2009) for more algebraic definitions.

2.3 Vector spaces

Definition 2.3.1: Let V be a non-empty set and $\mathbb{F}$ be a field. If the following propositions are true, the set V is said to be a *vector space* on the field $\mathbb{F}$.

(V1) In a set V, an operation $+$ called *summation* is well defined. This process has the following features.

(1) Every $u, v \in V$, $u + v$ is defined, and $u + v \in V$. Finally, set V is closed according to the addition operation.

(2) For all $u, v, w \in V$, $(u + v) + w = u + (v + w)$. The set V has the associative property of addition.

(3) For $\exists 0 \in V$, $\forall u \in V$, we have $u + 0 = u$ and $0 + u = u$. Set V has a unit element according to the addition operation. We have denoted this element by 0.

(4) For each $u \in V$, an element $-u$ in V satisfies their equality $u + (-u) = 0$ and $(-u) + u = 0$. Each element in the set has an inverse concerning the addition. It is shown with $-u$ as an inverse of u.

(5) For each $u, v \in V$, $u + v = v + u$. In set V, the addition operation has a commutative property.

Hence, $(V, +)$ is a *commutative group*.

(V2) A function $\mathbb{F} \times V \to V$ defined by $(a, u) \to au$ is called *scalar multiplication*, and this function confirms the following propositions:

(a) For each $a \in \mathbb{F}$, $u, v \in V$, $a(u + v) = au + av$

(b) For each $a, b \in \mathbb{F}, u \in V$, $(a + b)u = au + bu$

(c) For each $a, b \in \mathbb{F}, u \in V$, $(ab)u = a(bu)$

(d) 1 is the unit element of $\mathbb{F}$ for multiplication, then every element of V, $1u = u$.

Definition 2.3.2. (Trace function) $GF(q^m)$ is an extension of $GF(q)$, and let a be an element of $GF(q^m)$. $a, a^q, \dots, a^{q^{m-1}}$ elements of GF(q) are called *conjugates* to a. Trace function $Tr_{q^m/q}(x) = x + x^q + x^{q^2} + \cdots + x^{q^{m-1}}$ is a function from $GF(q^m)$ to $GF(q)$. When q is a prime, $Tr_{q^m/q}(x)$ is the absolute trace of x.

Theorem 2.3.1. The trace function $Tr_{q^m/q}(x)$ provides the following features.

(a) All $x, y \in GF(q^m)$, $Tr_{q^m/q}(x + y) = Tr_{q^m/q}(x) + Tr_{q^m/q}(y)$.

(b) For all $x \in GF(q^m)$ and all c$\in GF(q)$, $Tr_{q^m/q}(cx) = cTr_{q^m/q}(x)$.

(c) Both $GF(q^m)$ and $GF(q)$ are viewed as vector spaces on $GF(q)$; $Tr_{q^m/q}$ is a *linear transformation* from $GF(q^m)$ to $GF(q)$.

(d) Each linear transformation from $GF(q^m)$ to $GF(q)$ is expressed as $Tr(ax)$ for some $a \in GF(q^m)$.

(e) For all c $\in GF(q)$, $Tr_{q^m/q}(c) = cm$.

(f) For all $x \in GF(q)$, $Tr_{q^m/q}(x^q) = Tr_{q^m/q}$.

Definition 2.3.3. The *norm* $N_{q^m/q}(a)$ of $a \in GF(q^m)$ is described by $N_{q^m/q}(a) = a^{q^m-1/q-1}$. $N_{q^m/q}(a)$ is always an element of $GF(q)$.

Theorem 2.3.2. The norm function $N_{q^m/q}(x)$ provides the following properties.

(i) For each $x, y \in GF(q^m)$, $N_{q^m/q}(xy) = N_{q^m/q}(x)N_{q^m/q}(y)$.

(ii) $N_{q^m/q}$ is onto.

(iii) For each a$\in GF(q)$, $N_{q^m/q}(a) = a^m$.

(iv) For all $x \in GF(q^m)$, $N_{q^m/q}(x^q) = N_{q^m/q}(x)$.

Definition 2.3.4. Let $\{\alpha_1, \alpha_2, \dots, \alpha_m\}$ and $\{\beta_1, \beta_2, \dots, \beta_m\}$ be two bases of $GF(q^m)$ over $GF(q)$. For $0 \le i, j \le m$,

$$Tr_{q^m/q}(\alpha_i, \beta_j) = \begin{cases} 0 & i \neq j \\ 1 & i = j. \end{cases}$$

It can be easily proved that $\{\beta_1, \beta_2, \dots, \beta_m\}$ is a dual basis for any base $\{\alpha_1, \alpha_2, \dots, \alpha_m\}$ of $GF(q^m)$ over $GF(q)$.

Let α be a primitive element of $GF(q^m)$. Then, $\{1, \alpha, \alpha^2, \dots, \alpha^{m-1}\}$ is the *polynomial basis* $GF(q^m)$ over $GF(q)$.

See (Nicholson, 2006) for more details.

3. Difference sets

3.1. Structures and designs

Definition 3.1.1. Let $\mathcal{P}$ be the set of points and $\mathcal{B}$ the set of blocks; an ordered triple $(\mathcal{P}, \mathcal{B}, \mathcal{I})$ is called an *incidence structure* such that $\mathcal{P} \cap \mathcal{B} = \emptyset$ and $\mathcal{I} \subseteq \mathcal{P} \times \mathcal{B}$ is an incidence relation between $\mathcal{P}$ and $\mathcal{B}$. For $p \in \mathcal{P}$ and $B \in \mathcal{B}$, $(p, B) \in \mathcal{I}$ means that p and B are *incident*s.

Definition 3.1.2. ((v, k, λ) Symmetric design) A (v, k, λ) parameterized symmetric design including incidence structure $(\mathcal{P}, \mathcal{B}, \mathcal{I})$ with $0 < k < v$ has the following features:

1. There are v points ($|\mathcal{P}| = v$).
2. There are v blocks ($|\mathcal{B}| = v$).
3. Each point is contained in k blocks.
4. Each block has k points.
5. Any two blocks have λ common points.
6. Any two points coexist in λ blocks.

Definition 3.1.3. Let $\mathcal{P} = \{p_1, p_2, \dots, p_v\}$ the set of points and $\mathcal{B} = \{B_1, B_2, \dots, B_v\}$ the set of blocks with $i, j = \{1,2, \dots, v\}$ its elements defined as

$$m_{ij} = \begin{cases} 1 & p_j = B_i \\ 0 & otherwise. \end{cases}$$

$M = [m_{ij}]_{v \times v}$ is called the *incidence matrix of symmetric design*.

Example 3.1.1. In the Euclidean plane, we can think of $\mathcal{P}$ as the set of points and $\mathcal{B}$ as the set of lines.

Example 3.1.2. Let us take $\mathcal{P} = \{a, b, c\}$ as the set of points with three elements and $\mathcal{B} = \{\{a\}, \{a, b\}, \{a, c\}, \{a, b, c\}\}$ as the set of blocks, where the element a is in every four blocks, the element b is in the 2nd and 4th blocks, and the element c is in the 3rd and 4th blocks. The incidence matrix of this incidence structure is in the form of the following.

$$M = \begin{bmatrix} 1 & 0 & 0 \\ 1 & 1 & 0 \\ 1 & 0 & 1 \\ 1 & 1 & 1 \end{bmatrix}.$$

Definition 3.1.4. Let $(\mathcal{P}, \mathcal{B}, \mathcal{I},)$ and $(\mathcal{P}', \mathcal{B}, ' \mathcal{I}')$ be two incidence structures. If there is a one-to-one and covering function that maps $\mathcal{P}$ to $\mathcal{P}'$ and $\mathcal{B}$ to $\mathcal{B}$,' these structures are called *isomorphic*.

3.2. Definitions of difference set

We will begin our analysis of difference sets by considering the finite group G. If G is cyclic, we will use the additive group represented by $\mathbb{Z}_v = \{0,1,2, \dots, v-1\}$.

Definition 3.2.1. Let $D = \{d_1, d_2, \dots, d_k\}$ be a non-empty subset of $(G, +)$ with order v and be composed of k elements of the remaining classes in mod v. Congruence

$$d_i - d_j \equiv a(mod v)$$

with $d_i, d_j \in D,\ d_i \neq d_j$ and $a \not\equiv 0(mod\ v)$ has λ ordered (d_i, d_j) solutions. The subset D here is called a (v, k, λ) *parameterized difference set of the group* G.

We can also define difference sets by any operation $*$. If we say $|G| = v$ and $|D| = k$ two different elements from a non-empty D subset of G are defined as

$$d_i * {d_j}^{-1} \equiv a(mod\ v)$$

Which is called *the multiple set* Δ formed by the elements contained in D. The multiple set Δ includes each of the elements G λ times; the set D of G is then called a *difference set* with (v, k, λ) parameters.

Example 3.2.1. The set $D = \{0,1,3,9\}$ in group $(\mathbb{Z}_{13}, +)$ is a $(13,4,1)$ parameterized difference set.

$$1 - 0 \equiv 1\ (mod\ 13) \qquad 0 - 1 \equiv 12\ (mod\ 13)$$
$$3 - 0 \equiv 3\ (mod\ 13) \qquad 0 - 3 \equiv 10\ (mod\ 13)$$
$$9 - 0 \equiv 9\ (mod\ 13) \qquad 0 - 9 \equiv 4\ (mod\ 13)$$
$$3 - 1 \equiv 2\ (mod\ 13) \qquad 1 - 3 \equiv 11\ (mod\ 13)$$
$$9 - 1 \equiv 8\ (mod\ 13) \qquad 1 - 9 \equiv 5\ (mod\ 13)$$
$$3 - 9 \equiv 7\ (mod\ 13) \qquad 9 - 3 \equiv 6\ (mod\ 13).$$

All the elements of $\mathbb{Z}_{13}$ except zero are represented once in the multiple set Δ, and set D is a $(13,4,1)$ parameterized difference set.

Example 3.2.2. D={(0,1),(0,2),(0,3),(0,4),(0,5),(1,0),(2,0),(3,0), (4,0), (5,0),(1,1),(2,2),(3,3),(4.4),(5.5)} in the group $(\mathbb{Z}_6 \times \mathbb{Z}_6, +)$ is the difference set with parameter $(36,15,6)$.

	(0,1)	(0,2)	(0,3)	(0,4)	(0,5)	(1,0)	(2,0)	(3,0)	(4,0)	(5,0)
(0,1)	–	(0,5)	(0,4)	(0,3)	(0,2)	(5,1)	(4,1)	(3,1)	(2,1)	(1,1)
(0,2)	(0,1)	–	(0,5)	(0,4)	(0,3)	(5,2)	(4,2)	(3,2)	(2,2)	(1,2)
(0,3)	(0,2)	(0,1)	–	(0,5)	(0,4)	(5,3)	(4,3)	(3,3)	(2,3)	(1,3)
(0,4)	(0,3)	(0,2)	(0,1)	–	(0,5)	(5,4)	(4,4)	(3,4)	(2,4)	(1,4)
(0,5)	(0,4)	(0,3)	(0,2)	(0,1)	–	(5,5)	(4,5)	(3,5)	(2,5)	(1,5)
(1,0)	(1,5)	(1,4)	(1,3)	(1,2)	(1,1)	–	(5,0)	(4,0)	(3,0)	(2,0)
(2,0)	(2,5)	(2,4)	(2,3)	(2,2)	(2,1)	(1,0)	–	(5,0)	(4,0)	(3,0)
(3,0)	(3,5)	(3,4)	(3,3)	(3,2)	(3,1)	(2,0)	(1,0)	–	(5,0)	(4,0)
(4,0)	(4,5)	(4,4)	(4,3)	(4,2)	(4,1)	(3,0)	(2,0)	(1,0)	–	(5,0)
(5,0)	(5,5)	(5,4)	(5,3)	(5,2)	(5,1)	(4,0)	(3,0)	(2,0)	(1,0)	–
(1,1)	(1,0)	(1,5)	(1,4)	(1,3)	(1,2)	(0,1)	(5,1)	(4,1)	(3,1)	(2,1)
(2,2)	(2,1)	(2,0)	(2,5)	(2,4)	(2,3)	(1,2)	(0,2)	(5,2)	(4,2)	(3,2)
(3,3)	(3,2)	(3,1)	(3,0)	(3,5)	(3,4)	(2,3)	(1,3)	(0,3)	(5,3)	(4,3)
(4,4)	(4,3)	(4,2)	(4,1)	(4,0)	(4,5)	(3,4)	(2,4)	(1,4)	(0,4)	(5,4)
(5,5)	(5,4)	(5,3)	(5,2)	(5,1)	(5,0)	(4,5)	(3,5)	(2,5)	(1,5)	(0,5)

	(1,1)	(2,2)	(3,3)	(4,4)	(5,5)
(0,1)	(5,0)	(4,5)	(3,4)	(2,3)	(1,2)
(0,2)	(5,1)	(4,0)	(3,5)	(2,4)	(1,3)
(0,3)	(5,2)	(4,1)	(3,0)	(2,5)	(1,4)
(0,4)	(5,3)	(4,2)	(3,1)	(2,0)	(1,5)
(0,5)	(5,4)	(4,3)	(3,2)	(2,1)	(1,0)
(1,0)	(0,5)	(5,4)	(4,3)	(3,2)	(2,1)
(2,0)	(1,5)	(0,4)	(5,3)	(4,2)	(3,1)
(3,0)	(2,5)	(1,4)	(0,3)	(5,2)	(4,1)
(4,0)	(3,5)	(2,4)	(1,3)	(0,2)	(5,1)
(5,0)	(4,5)	(3,4)	(2,3)	(1,2)	(0,1)
(1,1)	–	(5,5)	(4,4)	(3,3)	(2,2)
(2,2)	(1,1)	–	(5,5)	(4,4)	(3,3)
(3,3)	(2,2)	(1,1)	–	(5,5)	(4,4)
(4,4)	(3,3)	(2,2)	(1,1)	–	(5,5)
(5,5)	(4,4)	(3,3)	(2,2)	(1,1)	–

(36,15,6) difference set D, representing all elements of $\mathbb{Z}_6 \times \mathbb{Z}_6$ without the element (0,0) six times in its multiple set Δ.

Theorem 3.2.1. Let D be (v, k, λ) parameterized difference set of G. Then, the relationship between parameters (v, k, λ) is in the form of

$$k(k-1) = \lambda(v-1).$$

We then obtain $n = k^2 - \lambda v$.

Proof 3.2.1. If D is a difference set, the number of elements of it is k. The number of each ordered pair (d_i, d_j) of difference set D is $k(k-1)$. The number of the elements in the multiple set Δ is the number of differences of $d_i - d_j$. Hence, it is $k(k-1)$. $v-1$ non-empty element of G is repeated λ times among desired elements in the multi-set Δ. The number of elements of the multi-set Δ is then $\lambda(v-1)$. From here, the equation

$$k(k-1) = \lambda(v-1)$$

is obtained. If the equation is arranged, we attain

$$k^2 - k = \lambda v - \lambda$$
$$k^2 - \lambda v = k - \lambda.$$

Therefore, $n = k^2 - \lambda v$ is obtained.

You can see more properties for difference sets and their applications in (Moore et al., 2013) and (Ding, 2014).

4. Group characters

Group characters mainly exploit the irreducible representations of a group. We investigate a more general set of functions that contains the characters.

Since group representations are homomorphisms that map conjugate group elements to similar matrices, as similar matrices have equal traces, group characters can be seen as constant functions on conjugacy classes.

Basic definitions and properties are given below.

4.1. Additive character

Definition 4.1.1. Let $(G, +)$ be a finite abelian group. Let us define a homomorphism $\psi: G \to \mathbb{S}^1$ as an *additive character of* G. Thus, for every $x, y \in G$, we get $\psi(x+y) = \psi(x).\psi(y)$. Here $\mathbb{S}^1 = \{z \in \mathbb{C}: |z| = 1\}$ is the unit circle.

Let p be prime. We will examine the cases when $G = \mathbb{Z}_p$, $G = \mathbb{Z}_p \, x \, \mathbb{Z}_p$, and $G = \mathbb{Z}_{p-1} \, x \, \mathbb{Z}_{p-1} \, x \, \mathbb{Z}_{p-1}$.

If $G = \mathbb{Z}_p$, all additive characters of G are as follows:

For $r \in \{0,1,2, \dots, p-1\}$ and $\forall x \in G$,

$$\psi_r(x) = e\left(\frac{rx}{p}\right)$$

where $e(x) = e^{2i\pi x}$. When $= 0, \psi_0(x) = 1$.

If $G = \mathbb{Z}_p \, x \, \mathbb{Z}_p$, all additive characters of G are as follows:

For every $(x, y) \in G$ and $r, s \in \{0,1,2, \dots, p-1\}$, we get

$$\psi_{r,s}((x,y)) = e\left(\frac{rx + sy}{p}\right).$$

If $G = \mathbb{Z}_{p-1} \, x \, \mathbb{Z}_{p-1} \, x \, \mathbb{Z}_{p-1}$, all additive characters of G are as follows:

For all $(x, y, z) \in G$ and $r, s, t \in \{0,1,2, \dots, p-2\}$ we this time get

$$\psi_{r,s,t}((x,y,z)) = e\left(\frac{rx + sy + tz}{p-1}\right).$$

We will now define the fundamental propositions of additive characters.

Proposition 4.1.1. For $x \in G$,

$$\sum_{\psi} \psi(x) = \begin{cases} |G|, & x = 0 \\ 0, & x \neq 0. \end{cases}$$

Proposition 4.1.2. Let ψ be an additive character of G.

$$\sum_{x \epsilon G} \psi(x) = \begin{cases} |G|, & \psi = \psi_0 \\ 0, & \psi \neq \psi_0. \end{cases}$$

4.2. Multiplicative character

Definition 4.2.1. $\chi: \mathbb{Z} \to \mathbb{C}$ is also a *multiplicative character* over mod n when it provides the following features:

i. For all integers k, $\chi(k + n) = \chi(k)$.

ii. $\chi(k) = 0 \Leftrightarrow (k, n) > 1$.

iii. For all integers k and h, $\chi(kh) = \chi(k)\chi(h)$.

Warning 4.2.1.

i. Multiplicative character χ_0 is trivial (simple) on a mod n and $\chi_0(k) = 1$. For all integers, k with $(k, n) = 1$, $\chi(k) = 1$ and, for all integers k with $(k, n) > 1, \chi(k) = 0$.

ii. The smallest positive integer d is the *order of a multiplicative character* χ such that $\chi^d = \chi_0$. Then the order d is written by $ord(\chi) = d$.

iii. $\bar{\chi}(k) = \overline{\chi(k)}$.

We can write $n = 2^{\propto} p_1^{\propto_1} p_2^{\propto_2} \dots p_r^{\propto_r}$ with a positive integer n as a product of primes. Let p_i be different odd primes. Then, every multiplicative character in mod n is in form $\chi = \chi' \chi_1 \chi_2 \dots \chi_r$. Here χ' is a character of mod $2^{\propto}$, and for $i = 1,2, \dots, r$, χ_i is a character of mod $p_i^{\propto_i}$.

Note: The number of positive integers $a < n$, and $(a, n) = 1$ is called the *Euler Function* and is denoted by $\phi(n)$.

Note: If $g(mod\ p)$ is a primitive root, then $0 \le i \le p - 1$ can be found for each $p \nmid a$ such that $a \equiv g^i\ (mod\ p)$. i is called the *index of* an of $mod\ p$ in base g, and it is denoted by $ind_g a \equiv i$.

When p is an odd prime and $\alpha \ge 1$ or, $p = 2$, $\alpha \in \{1,2\}$, then all multiplicative characters in the modp^{α} are in the form of

$$\chi(k) = \begin{cases} e\left(\dfrac{ind_g(k) l}{\phi(p^{\alpha})}\right), & p \nmid k \\ 0, & p\ I\ k. \end{cases}$$

where g is also a primitive root in mod $p^{\propto}$ and $l = 0,1,2, \dots, \phi(p^{\propto}) - 1$.

This case is different when $\alpha \ge 3$ in mod $2^{\propto}$. For every odd integer k, we denote $b(k)$ as the odd integer; hence $1 \le b(k) \le \frac{\phi(p^{\alpha})}{2}$ and $k \equiv (-1)^{\frac{k-1}{2}} 5^{b(k)}\ (mod\ 2^{\propto})$.

All multiplicative characters in mode $2^{\propto}$ are as follows:

$$\chi'(k) = \begin{cases} (-1)^{\frac{k-1}{2}\alpha} e\left(\dfrac{b(k)}{2^{\alpha-2}} c\right), & b(k)\ is\ odd \\ 0\ , & otherwise \end{cases}$$

where $\alpha = 1,2\ and\ c = 1,2, \dots, \frac{\phi(2^{\alpha})}{2}$.

We have defined all multiplicative characters only in mod n so far. For example, another multiplicative character definition is as follows.

Let T be the linear transformation of the vector space V. Let us take the matrix M consisting of the bases of V in T. We know from linear algebra that there $Tr(M)$ is a trace of T. If V is an m -dimensional vector space on $\mathbb{F}$, we define $GL(V)$ to be the set of invertible linear transformations from V to V.

Let G be a finite group and $\rho: G \to GL(V)$ be the representation of G on finite-dimensional complex vector space. $\chi_{\rho}: G \to \mathbb{C}$ is the character of ρ,

and for $\forall g \in G$, the function $\chi_\rho(g) = Tr(\rho(g))$ is defined. The degree of ρ is the degree of χ_ρ, that is, the dimension of the vector space V. If ρ is irreducible, χ_ρ consists of irreducible characters. If ρ is a trivial representation (matching each group element to 1), χ_ρ is a trivial character.

When the degree of χ_ρ is 1, $\chi_\rho = \rho$ (trace is a and it is 1×1 $[a]$ matrix), and $\chi_\rho$is a homomorphism from G to the multiplicative group $\mathbb{C}^*$. However, characters do not become homomorphic when degrees are greater than 1. Therefore, when any ρ representation with degree m is given, $\rho(1_G) = I_m$, in this case, we take the degree of χ_ρ as $\chi_\rho(1_G)$.

Example 4.2.1. Let ρ be the natural representation of $G = S_3$. $\rho(g)$ is a permutation matrix for $\forall g \in G$, and $\chi_\rho(g)$ counts the elements whose trace is fixed by the permutation. We show this as follows;

$\chi_\rho(1_G) = 3$ $\quad\quad$ $\chi_\rho((23)) = 1$
$\chi_\rho((12)) = 1$ $\quad\quad$ $\chi_\rho((123)) = 0$
$\chi_\rho((13)) = 1$ $\quad\quad$ $\chi_\rho((132)) = 0.$

In this case, the values of the characters are integers.

Example 4.2.2. Let us remember the group representation $G =< a|a^5 = 1 >$ with $\rho(a) = e^{2\pi i/5}$. Representations coincide with the character representation with a degree 1. The character, in this case, is a homomorphism, and its values are the fifth root of the unit.

Example 4.2.3. We know that the bivariate group $G =<$ a, b $| a^5 = b^2 = 1, bab^{-1} = a^{-1} >$ is represented by ρ

$$\rho(a)= \begin{bmatrix} \eta & 0 \\ 0 & \eta^{-1} \end{bmatrix} \text{ and } \rho(b)= \begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix}$$

where η= $e^{2\pi i/5}$.

Let χ be a character. Let us find that $\chi(\ a^j b) = 0$ for $.j = 0,1,2, \ldots 4.$ What are the values χ in the a nnotations? Let us take $\chi\ (\ a^j)$= $\eta^j + \eta^{-j}$as, the sum of the roots of the unit. We will see that $\chi\ (\ a^j)$ is a real number when the inverse of unit roots is a complex conjugate. Then, of course, $\chi($ 1_G)=2, but other values of $\chi(\ a^j)$ are not integers.

$$\sum_{g\in G} x(g) = (1+1) + (\eta+\eta^4) + (\eta^2+\eta^3) + (\eta^3+\eta^2) + (\eta^4+\eta) = 0$$

where $1+\eta+\eta^2+\cdots+\eta^4=(\eta^5-1)/(\eta-1)=0$.

Example 4.2.4. Let G be any group, and ρ_{reg}be the regular representation of G in the complex vector space V. If G also $has\ m$ elements, the dimension of V is m. $\rho_{reg}\ (g)$ is mxm permutation matrix forming by the bases of $\{\ e_h\ |h \in H\}$. Every element is formed in the form of 's. The trace of the permutation matrix is the number of fixed points, and therefore, $\chi_{reg}(g) = 0$ for each $g \neq 1_G$ and $\chi_{reg}(\ 1_G) = m$.

We will now give the fundamental propositions of multiplicative characters.

Proposition 4.2.1. We have

$$\sum_{\chi\ (mod\ n)} \chi(x) = \begin{cases}\phi(n), & x \equiv 1\ (mod\ n)\\ 0, & x \not\equiv 1\ (mod\ n).\end{cases}$$

Proposition 4.2.2. We have

$$\sum_{x=1}^{n} \chi(x) = \begin{cases}\phi(n), & \chi = \chi_0\\ 0, & \chi \neq \chi_0.\end{cases}$$

Proposition 4.2.3. Let x be an integer and n be a positive integer such that $(x,n) = 1$. For integers $m_1, m_2, \dots, m_r$, we have

$$\{i: m_i \equiv x\ (mod\ n)\} = \frac{1}{\phi(n)} \sum_{\chi\ (mod\ n)} \chi(x) \sum_{i=1}^{r} \bar{\chi}\ (m_i).$$

Proposition 4.2.4. Let the character χ with degree d of the mod n be given by

$$1 + \chi(x) + \chi(x)^2 + \cdots + \chi(x)^{d-1} = \begin{cases}d, & if\ \ x = y^d for\ some\ \ y\epsilon\mathbb{Z}_n^*\ ,\\ 1, & if\ \ \ (x,n) > 1\ ,\\ 0, & otherwise.\end{cases}$$

4.3. Character sums

Let ψ be the additive and χ be the multiplicative character of $GF(q)$. *Then, the Gaussian sum* is defined as follows.

$$G(\chi,\psi) = \sum_{x\in GF(q)^*} \chi(x)\psi(x).$$

Theorem 4.3.1. Let ψ be the additive and χ be a multiplicative character of $GF(q)$. Then, the Gaussian sum is defined as:

$$G(\chi,\psi)=\begin{cases} q-1, & \chi=\chi_0,\ \psi=\psi_0 \\ -1, & \chi=\chi_0,\ \psi\neq\psi_0 \\ 0, & \chi\neq\chi_0,\ \psi=\psi_0 \end{cases}$$

where χ_0 and ψ_0 are trivial multiplicative and trivial additive characters of $GF(q)$, respectively.

If $\psi \neq \psi_0$ and $\chi \neq \chi_0$, then $|G(\chi,\psi)| = \sqrt{q}$.

Theorem 4.3.2. Take $q = p^s$. Let p be an odd prime number, and s be a positive integer. Let η be the quadratic character of $GF(q)$ and ψ_1 be the standard additive character of $GF(q)$. Then,

$$G(\eta,\psi_1)=\begin{cases} (-1)^{s-1}\sqrt{q}, & p\equiv 1(mod\ 4) \\ (-1)^{s-1}(\sqrt{-1})^s\sqrt{q}, & p\equiv 3\ (mod\ 4). \end{cases}$$

When $G(\chi,\psi_b) = \bar{\chi}(b).G(\chi,\psi_1)$, we use $G(\chi)$ instead of $G(\chi,\psi_1)$ for short.

If $\chi \neq \chi_0$, it is possible to say

$$|G(\chi)| = \sqrt{q}.$$

Theorem 4.3.3. Let q be an odd number, and ψ be the non-trivial additive character of $GF(q)$, and $f(x) = a_2x_2 + a_1x + a_0 \in GF[x]$ with $a_2 \neq 0$, then

$$\sum_{c\in GF(q)} \psi\big(f(c)\big) = \psi(a_0 - a_1^2(4a_2)^{-1})\eta(a_2)G(\eta,\psi)$$

where η is the $quadratic$ character of $GF(q)$.

Theorem 4.3.4. Let us take $\psi_b(x) = \psi_1(bx)$. Let q be an even number, and for $b \in GF(q)^*$, ψ_1 is the standard additive

character of $GF(q)$. f $(x) = a_2x_2 + a_1x + a_0 \in GF[x]$ is given. Then, we have

$$\sum_{c\in GF(q)} \psi_b\big(f(c)\big) = \begin{cases} \psi_b(a_0)\,, & a_2 = ba_1^2 \\ 0\,, & otherwise. \end{cases}$$

In general, it is not very easy to evaluate character sums. In such cases, it is to widen narrow boundaries by taking the absolute value of character sums. The Weil Bound given in the following theorem is an example of such a cases.

Theorem 4.3.5. (Weil bound) Let $e \geq 1$ be the degree of $f \in GF(q)[x]$ and $(e, q) = 1$. ψ is the non-trivial additive character of $GF(q)$. We have

$$\left|\sum_{x\in GF(q)} \psi\big(f(x)\big)\right| \leq (e-1)\sqrt{q}.$$

We can also give the following theorem regarding the multiplicative character.

Theorem 4.3.6. Let χ be a multiplicative character of $GF(q)$ with degree $t > 1$. Let $f \in GF(q)[x]$ be a positive degree polynomial, and e is the number of different roots of the splitting field of polynomial f over $GF(q)$.

For each $a \in GF(q)$, we get

$$\left|\sum_{x\in GF(q)} \chi(af(x))\right| \leq (e-1)\sqrt{q}.$$

Kloosterman sums, other character sums, are also defined as follows.

$$K(\psi;\, a, b) = \sum_{x\in GF(q)^*} \psi(ax + bx^{-1})$$

where ψ is the non-trivial additive character of $GF(q)$ and $a, b \in GF(q)$.

Kloosterman sums are related to problems in many fields of mathematics and engineering. Kloosterman sums are complicated to evaluate. We will give the Kloosterman sum in narrow limits in the following theorem.

Theorem 4.3.7. Let ψ be the non-trivial additive character of $GF(q)$ and $a, b \in GF(q)$, $(a, b) \neq (0,0)$. Then,

$$\left|K\big(\psi;(a,b)\big)\right| \leq 2\sqrt{q}.$$

For detailed information about exponential sums, see (Berndt et al., 1998).

5. Relation between difference sets and group characters

This section explores the connection between difference sets and group characters. Let us have a complex representation ρ of G and transformations $\rho(G)$. We define a function $\chi_p\,(g)\colon G \to C$ as a character of ρ and its trace given by $\chi_p\,(g) = Tr(\rho(g))$ for $g \in G$. Instead of determining representation to calculate its character, it is much better to do the contrary, finding the character to determine representation. Hence, we easily recognize it by using characters' properties to find whether it is reducible.

Smith's construction about (100, 45, 20)-difference set uses the character of non-abelian groups (Smith, 1995). This is the first work that gives the relation between group characters and abelian difference sets. It is already known that each non-trivial irreducible character χ of the group G must satisfy $z = \tilde{\chi}\,(D),\ z\bar{z} = n$, and $n = k - \lambda$. This already limits constructing a difference set. Smith's work for this specific example, Davis & Jedwab (1997) turned this idea into a general strategy for constructing difference sets.

The following theorem exploits that a subset of group G with k elements getting thorough this finding process for all non-trivial characters of the group is a difference set.

Theorem 5.1. Let G be an abelian group with size v and G^* be the set of irreducible characters of G. Then, D is a (v, k, λ) difference set in G if and only if for every irreducible character χ

$$\tilde{\chi}(D)\,\overline{\tilde{\chi}(D)} = \begin{cases} n, & \chi = \chi_0 \\ k^2, & \chi \neq \chi_0. \end{cases}$$

In order to extend Theorem 5.1 to non-abelian difference sets, we can look at the below theorem due to Liebler (Liebler, 1999). This generalization depends mainly on the structure of group integral $\mathbb{C}G$ (Davis & Smith, 1994).

Theorem 5.2. Let us assume $\varphi_1, \varphi_2, \dots, \varphi_t$ are the non-trivial irreducible representations of G, with $m_j = \deg \varphi_j$. If for $j = 1,2, \dots t$,

$$\widetilde{\varphi_j}(D)\widetilde{\varphi_j}(D^{-1}) = n\, I_{m_j}$$

then D is a (v, k, λ) difference set in G.

6. Conclusion

The origin of group characters in a finite group goes back to Frobenius. He was foresighted that they would be the main tools for studying group

theory. It was correct. They have been used not only by algebraists but also chemists and physicists.

In Smith's work, he detailed the use of group characters in the search for difference sets. (Smith, 1995) Later, Liebler theorized using characters in a more general setting for searching difference sets. (Liebler, 1993, 1999)

Difference sets, which are increasingly used in the application areas mentioned above, are significant. There are many different methods of constructing difference sets. One of them is group characters. In order to determine whether set D is a difference set, each non-trivial irreducible character χ of the group G must satisfy $z = \tilde{\chi}(D)$, $z\bar{z} = n$, and $n = k - \lambda$. The set with k elements satisfying these conditions is a difference set with parameters (v, k, λ). Therefore, group characters are used in studying difference sets.

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CHAPTER XII

LIFE EXPECTANCY AT BIRTH IN THE CONTEXT OF SOCIAL ECONOMIC AND HEALTH INDICATORS: A STRUCTURAL MODEL

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1. Introduction

Life expectancy at birth (LEB) is the average number of years that people who reach a certain age have not yet lived (Dudley and Bouvier, 2010). It is one of the leading indicators widely used to assess the health status in developed and developing countries. It is also closely related to the socio-economic development level of a country (Delavari et al., 2016). Estimate of LEB is influenced by low and high mortality rates (Preston et al., 2001).

Many scientists have studied the factors affecting LEB. Grosse (1980) stated a relationship between income per capita and life expectancy of a zero-year-old individual; It determined no relationship between per capita income and the increase in LEB. With the rise in health investment in developed

countries, feeding and cleaning conditions would improve, and infant mortality will decrease. In their study, Pendleton and Yang (1985) divided 92 countries into two as the beginning and after the demographic process. At the beginning of the demographic period, the living standards variable did not affect the expected life expectancy, but after the demographic period, it concluded that the living standards variable significantly affected life expectancy. Anand and Ravallion (1993) found a significant positive correlation between the birth expectancy of the gross domestic product per capita. Hertz (1994) suggested a strong negative relationship between life expectancy at birth and infant mortality rate. Shimouchi et al. (1996) stated that gross domestic product and infant mortality were not significantly associated with the fertility rate. Wilkinson (1996), on the other hand, examined the relationship between increases in gross domestic product per capita and high life expectancy and did not find a significant relationship. He argued that the reason for this was that countries reached a certain income level. On the other hand, Husain (2002) accepted a predictable relationship between the country's economy and life expectancy and infant mortality rate variables. Monsef and Mehrjardi (2015), on the other hand, stated that countries with high gross national income have a longer life expectancy than others.

Kabir (2008) could not say that the variables affecting LEB differ between countries depending on the age range and that no variable entirely affects the life expectancy at birth. In their study, Balan and Jaba (2011) stated that the number of doctors, the number of beds in the hospital, and the net income positively affect Romania's LEB. Teker et al. (2012), old-age dependency ratio, number of beds, number of doctors, and health expenditures to national income; revealed that it affects the life span of men and women significantly and in the same direction. Delavari et al. (2016) concluded that while the number of doctors per thousand people, urbanization, literacy, gross domestic product, and carbon dioxide emissions affect LEB positively, fertility rate and inflation affect negatively.

Shah (2009) stated that while there is no relationship between suicide rate and gross domestic product, life expectancy has a positive relationship. On the other hand, Oblander et al. (2016) investigated the reason for high suicide rates in high-income countries and found a relationship with LEB.

In this study, meaningful structural models were created for 2010, and 2015 with the variables thought to affect the life expectancy at birth. In the literature review, no similar study was found using the structural equation modeling method in which the variables affecting the life expectancy were examined.

2. Material And Method

In this study, data sets consist of 78 countries from 33 variables for 2010 and 2015, which are thought to affect LEB, are created from the "World Development Indicators" tab in the World Bank database with extensive literature review. The data set was chosen to show the countries' socioeconomic status, living standards, development level, and health status. The recommended models have been estimated on 24 variables that do not contain missing data. According to the factor analysis results applied to the data sets, both data sets have the same factor structure. With confirmatory factor analysis and structural equation modeling, two different structural models are obtained according to years, and their statistical significance is tested.

LEB is highly affected by the values of the years before the calculated year. Accordingly, creating LEB data for the analysis according to the consecutive years will enable more reliable results. Accordingly, the variable of LEB to be included in the model is taken to cover the last five years before the year in which it is calculated. In other words, while the life expectancy factor at birth consists of the values for 2005-2009 for the year 2010. For the year 2015, it consists of the values of 2010-2014.

Population structures contain essential information about the past, future, and present of the countries. LEB is an important indicator for understanding population structures. It differs in developed, developing, and undeveloped countries. In general, it is expected that the life expectancy of developed countries will be high; that is, the number of people reaching a certain age will be high. In other words, the elderly population is high in developed countries. This means that old-age dependency ratio, which is the population ratio over the age of 65 to the working population, is high. On the other hand, the youth dependency ratio, which is the ratio of the young population under the age of 15 to the working population, is expected to be low. The reason for this is generally low fertility and mortality rates in developed countries (Barlow and Vissandjée, 1999; Mahdian et al., 2016). Among the causes of high mortality in some countries, the prevalence of malnutrition, the small population using at least basic sanitation services, inadequate health facilities, and diseases such as tuberculosis can give examples (Murray and Lopez, 1997; Barlow and Vissandjée, 1999). The high rates of newborn death and infant mortality are interpreted as inadequate health and economic conditions of the country. Infant mortality rates differ by gender. Girl babies are more resilient at birth than boy babies. If the infant mortality rate is high in a country, this indicates the resource inequality in that country (WDI, 2012). Variables such as the number

of doctors per hundred thousand people and the number of beds in the hospital used to understand health services. One of the variables indicating a long life expectancy at birth is the suicide death rate. This is because suicides are a variable that should be examined based on age, not income. (Shah, 2009). Because suicide rates are higher with advancing age (Oblander et al., 2016). One of the phenomena affecting life expectancy at birth is the social and economic levels of countries. Economic development is an essential phenomenon for health development. It is affected by variables such as domestic and foreign economic investments made by countries, armed forces personnel, inflation, and labor force (Hanada, 1982).

In addition to this, airline passengers and annual tourist earnings also show the country's tourism income. One of the socio-economic development indicators is the gross domestic product. The social, economic, and health variables used in the study are given in Table 1.

Table 1. Variables Used for Both Model

LEB_05-LEB_14	Life expectancy at birth in 2005-2014
UNDEV1	Mortality rate, infant, female (per 1,000 live births)
UNDEV3	Mortality rate, neonatal (per 1,000 live births)
UNDEV4	Age dependency ratio, young
UNDEV6	Prevalence of undernourishment (% of population)
UNDEV9	Fertility rate
LIFESTD2	Electric power consumption (kWh per capita)
LIFESTD4	GNI per capita, (current US$)
LIFESTD7	GDP per capita (current US$)
HEALTH1	Death rate, crude (per 1,000 people)
HEALTH 2	Age dependency ratio, old
HEALTH 4	Suicide mortality rate (per 100,000 population)
HEALTH 5	Hospital beds (per 1,000 people)
ECODEV1	Air transport, passengers carried
ECODEV 2	Merchandise exports (current US$)
ECODEV 3	Merchandise imports (current US$)
ECODEV 4	International tourism, receipts (current US$)
ECODEV 5	Labor force, total
ECODEV 6	Armed forces personnel, total
ECODEV 7	Gross national expenditure (current US$)

Structural equation modeling predicts models that reveal relationships between multiple dependent and independent variables (Hoyle, 1995). At the same time, this method tests the hypotheses about the statistical significance of the relations, preserves the covariance structure of the variables, minimizes the information loss due to factorization, and proposes new models for the relations (Tabachnick & Fidell, 2013). The estimation processes for the structural parameters generate from the relationships of the covariance matrix of the observed variables. The model's hypothesis is given such that Σ is equal to Σ(θ) (Bollen, 1989).

This method is generally used in data sets consisting of discrete variables with the help of scales developed to measure a single phenomenon. The data set in this study consists of continuous variables. For this reason, to reduce skewness and get accurate results, the data has been adapted to the standard normal distribution with the standardization transformation. Then, the new variable values obtained are converted into a five-point Likert scale type according to the unit standard deviation distances from their mean (considering the variable values at 1, 2, 3 standard deviations from the mean), and two models estimated for both time sections with structural equation modeling.

A multivariate regression model is used with a causal theory to describe the entire structure of the relationships between independent and dependent variables in a complex data structure (Ho, 2006). In order to interpret this situation, path analysis output, showing causal and non-causal relationships, can use. Path analysis includes indirect, direct, and total effects. In the study, the mediator variable effect is taken into account to measure the indirect effects. In structural equation modeling, mediator variables, when independent variables are considered, depending on dependent variables; When dependent variables are considered, it is defined as the independent variable (Sümer, 2000).

The Sobel test is used to test the statistical significance of the mediator variable in the model. When examining the mediation effect in the Sobel test, three different regression equations are established by Equations (1), (2), and (3).

$$\text{Mode1}: Y_{dep} = \alpha_1 + \tau X_{Ind} + \varepsilon_1 \quad (1)$$

$$\text{Model2}: X_{med} = \alpha_2 + aX_{Ind} + \varepsilon_2 \quad (2)$$

$$\text{Model3}: Y_{dep} = \alpha_3 + \tau' X_{Ind} + b\, X_{med} + \varepsilon_3 \quad (3)$$

The mediating variable effect is calculated with (τ- τ´) and the equation ab=(τ - τ´) is determined. "The mediating effect of the variables is significant." hypothesis is tested. The Z test statistic, obtained by dividing the mediator variable effect by the standard errors of the measurement, calculates the magnitude of the indirect effects in the model. If the null hypothesis is rejected, it will decide that the mediating effect of the variable is statistically significant (Sobel, 1982; Sobel, 1986).

3. Results and Discussion

This study evaluated the proposed structural models in terms of two different periods (2010 and 2015) to observe the differentiation and changes in the variables that affect the life expectancy at birth.

The 5-dimensional factor structure obtained for 2010 was confirmed by confirmatory factor analysis. The existence of 4 factors affecting the dependent variable of life expectancy at birth in the resulting structure was tested. These factors are; undeveloped (UNDEV), standards of life (LIFESTD), health, and economic development (ECODEV). To evaluate whether the observed variables defined under the latent variables included the related structures, the latent factors' reliability and explained variance criteria were calculated. The construct validity coefficient of the life expectancy factor at birth was 0.99, and the construct validity of the other factors was obtained as 0.91, 0.88, 0.64, and 0.89, respectively. The construct validity coefficient of the life expectancy factor at birth was 0.99, and the construct validity of the other factors was obtained at 0.91, 0.88, 0.64, and 0.89, respectively.

In the structural equation model created for 2010, the variable that most explains LEB is life expectancy at birth for 2007 (LEB_07). The variable that most affected the undeveloped factor was the female infant mortality rate (UNDEV1) with 0.87, the gross national income (LIFESTD4) with 0.95 in standards of life factor, old-age dependency ratio (HEALTH2) in the health factor, and air transported passenger (ECODEV1) in economic development factor. Since using the chi-square value of the proposed model alone would be misleading, the ratio of $\chi 2$ to degrees of freedom is 1.27 (good fit, 304.96 / 239). The covariance structure in the estimated model is similar to the initial covariance structure ($p = 0.0025$). The goodness-of-fit indices -RMSEA (0.06), NFI (0.88), NNFI (0.94), CFI (0.95) - show that the model has an acceptable fit. The sample size for the parameters estimated in the model were limited to 78 countries due to data availability. However, due to the sample size, the criteria

values for explaining the dependent variable in the model by the independent variables, GFI and AGFI, are 0.75 and 0.69, respectively (Schermelleh-Engel ve Moosbrugger, 2003). The path diagram showing all the relationships between the observed and latent variables of the recommend model simultaneously with standard predictive values is given in Figure 1.

Fig.1. Path diagram of the recommend model (year 2010)

According to the path diagram, the t values of all predicted parameters are statistically significant ($p < 0.05$), their standard errors are small, and the independent variables explained 78% of LEB change. In the structural model of 2010, when the undeveloped factor is used as a mediating variable, economic development and standards of life do not directly or indirectly affect the LEB; but it is undeveloped, and the health factor directly affects it. At the same time, the statistically significant total effect of the health factor is 55.72% (0.2 + 0.47 * 0.76 =0.5572) undeveloped factor.

The structural model created for 2015 using the 2010-2014 LEB is a 4-factor structure formed with the same character as 2010. Latent structures in the model are; undeveloped, standards of life, health, and economic development factors. The construct validity of life expectancy at birth is 0.99, and the other factors are 0.91, 0.86, 0.69, and 0.91, respectively. The explained variances are 0.97 for life expectancy at birth and related factors (0.38-0.69) separately and are sufficient (Nunally, 1978; Hair et al., 1998).

In the model, the variables that most explain LEB for 2015 are 2010 and 2013.The variable that most affected the undeveloped factor is the female infant mortality rate (UNDEV1) with 0.91, the gross national income per capita (LIFESTD4) with 0.91 for standards of life, the suicide mortality rate (HEALTH4) for the health factor, and air transported passenger (ECODEV1) in economic development factor.

The ratio of the chi-square value to the degrees of freedom of the model is 1.004. The covariance structure in the estimated model is similar to the first covariance structure (p=0.469). Goodness-of-fit indices -RMSEA (0.0074), NFI (0.91), NNFI (0.98), CFI (0.98) - indicate an acceptable fit of the model. However, due to the sample size, the model's GFI and AGFI explanatory criteria are 0.80 and 0.74, respectively (Schermelleh-Engel & Moosbrugger, 2003). The path diagram showing all the relationships of the recommended model is given in Figure 2.

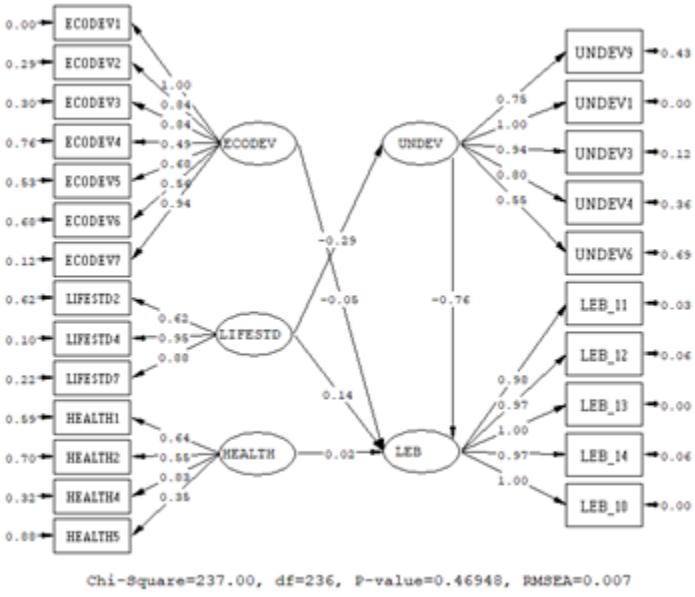

Fig. 2. Path diagram of the recommend model (2015)

According to the path diagram, the t values of all predicted parameters are statistically significant ($p < 0.05$), their standard errors are small, and the independent variables explained 71% of LEB change. Economic development, standards of life, and health factors do not directly affect LEB ($p > 0.05$). However, the undeveloped factor direct inverse affects LEB ($p < 0.05$). Although standards of life factor does not directly affect LEB, it has a statistically significant total effect of 36.04% (0.14+0.29*0.76=0.3604), mediated by the undeveloped factor.

The significant investments and new developments in health by the countries whose LEB was examined from 2010 to 2015 removed the effect of the health factor on the LEB and replaced it with standards of life. This shows the importance of the necessity of taking precautions by predicting such natural disasters in advance that rich countries with strong economies foresee epidemics such as covid-19.

4. Conclusions

In this study, data from 78 countries with LEB above the world average were used to prevent deviations in the data set and create the most accurate model. Variables affecting LEB were included in the study, taking into account the availability of data by the literature review. The data set used in the analysis was obtained from the 'World Development Indicators' tab with the same variables to observe the change in the variables affecting LEB between 2010 and 2015. As a result, it determined that the undeveloped factor has an inverse, direct, statistically significant effect on LEB in both year models. In this case, the increase in the variable values of the undeveloped factor adversely affects LEB. The variables included are female infant mortality rate, neonatal mortality rate, youth dependency rate, prevalence of undernourishment, and fertility rate. In other words, while the values of these variables increase, LEB decreases. The female infant mortality rate in this factor is the variable that most affects the life expectancy at birth. The high female infant mortality rate is interpreted as inequality in resource access. The high fertility rate leads to a high youth population. This situation is increased the youth dependency rate in the country and creates an unhealthy environment in the country. Moreover, it causes the prevalence of undernourishment and neonatal mortality rate to be high, preventing the individual from reaching a certain age. As a result, all these relationships lead to a shorter LEB.

It was observed that the economic development factor did not have a statistically significant effect on life expectancy at birth in both years. The high economic level does not affect the life expectancy at birth.

The health factor has a statistically significant effect on LEB, both directly and indirectly, in 2010. Here, the undeveloped factor acted as a mediator variable and revealed the indirect effect of the health variable. In this case, the decrease in the variable values of the undeveloped factor causes an increase in both LEB and the variable values in the health factor. The variables that make up the health variable are the mortality rate, old-age dependency ratio, suicide rate, and hospital

beds. Among these variables, the most affected LEB is the old-age dependency ratio. A high old-age dependency ratio means a large elderly population. In addition, the fact that the suicide death rate is higher at later ages supports the high probability of living longer and, therefore, the higher life expectancy from birth.

The high number of hospital beds, which is an indicator of health opportunities in the country, also positively affects LEB. The health factor did not have any direct or indirect effect on LEB in 2015. Because the variables that make up the health factor are approximately the same in every country, as there is no significant difference between countries in terms of health factors in 2015; In the period from 2010 to 2015, it can be shown that countries with undeveloped health systems improved their health systems more.

While the standards of life factor has no significant effect on LEB in 2010, it has a significant and indirect effect on the undeveloped factor in the 2015 model. The change in standards of life factor does not directly affect LEB. While the increase in standards of life affects the undeveloped factor in the opposite direction; On the other hand, the decrease in the undeveloped factor directly and significantly affects LEB and causes an increase in the same direction.

One of the features that make this study unique is the application of structural equation modeling by transforming the data set into a standard normal distribution, arranging the variable values in the regions created according to the unit standard deviation distance values from the mean five-point Likert scale type.

In the structural models proposed for 2010 and 2015, the variables that constitute the undeveloped factor affecting LEB did not differ.

As a result, according to the proposed model of 2010, the increase in the variable values constituting the health factor caused a decrease in the variables included in the undeveloped factor. Thus, LEB has also increased. In this case, the health factor has a statistically significant total effect of 55.72% on LEB through the undeveloped factor.

The model recommended for 2015, on the other hand, increases the decrease in the undeveloped factor standards of life, and LEB. Standards of life factor does not have a direct effect on LEB. However, it has a statistically significant total effect of 36.04%, mediated by the undeveloped factor.

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