



MUHAMMADIYAH COLLEGE
UNIVERSITAS AHMAD DAHLAN
INSTITUTE OF RESEARCH AND COMMUNITY SERVICE

Address: Campus 2 unit B UAD, Pramuka st. 5F. Pandeyan, Umbulhajo, Yogyakarta 55161. Email: lppm@uad.ac.id

RESEARCH IMPLEMENTATION AGREEMENT

Number: 26/RIA/LPPM-UAD/VI/2023

On this day, **Saturday**, tenth of June of Two thousand and twenty three (10-06-2023), we the undersigned:

1. Name : **Anton Yudhana , M.T., Ph.D.**
Position : The Head Institution of Research and Community Service at Universitas Ahmad Dahlan (LPPM UAD), furthermore called as **THE FIRST PARTY**.
2. Name : **Pramudita Budiastuti, S.Pd., M.Pd.**
Position : Lecturer / Researcher on Study Program **Pendidikan Vokasional Teknik Elektronika** Ahmad Dahlan University (UAD), as Chairman Researcher, next called **THE SECOND PARTY**.

THE FIRST PARTY and **THE SECOND PARTY** next called **THE PARTIES**.

THE PARTIES stated that they agreed and agreed to enter into a research implementation agreement hereinafter referred to as the Research Implementation Agreement (RIA) with the following terms and conditions.

Clause 1

LEGAL BASIS

- (1) The results of the proposal *review assessment conducted by the UAD Internal Research Reviewer Team*.
- (2) International Research Decision Meeting on June 8, 2023 about Determination Results Internasional Research 2023.

Clause 2

SCOPE AND DURATION OF THE RESEARCH

- (1) The **FIRST PARTY** grants work to the **SECOND PARTY** and the **SECOND PARTY** declares to have received work from the **FIRST PARTY** in the form of research activities as follows.

Scheme	:	International Research Collaboration
Title study	:	Making tocopherol from corn oil requires column distillation fractionation, H-NMR analysis, and HPLC analysis
Type Research	:	Fundamental Research
Partner Study	:	State University of New York New Paltz
Outside Required	:	International reputable journals
- (2) The execution time the research in point (1) begins since the signing of this Research Implementation Agreement (RIA) until with limit end upload Report End Study on date **February 10, 2024**.



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Clause 6

TYPES OF RESEARCH REPORT

- (1) The SECOND PARTY is obliged to prepare and upload research reports through the Research portal UAD which consists of:
 - a. Progress report
 - b. Final report
- (2) **The Progress Report** file is used as material for internal monitoring and evaluation (monev), uploaded no later than date **October 30, 2023**.
- (3) **Final Report** file used as reference disbursement of Phase II funds and happy consideration continue or whether or not contract study year plural (*multiyears*), uploaded no later than date **February 10, 2024**.

Clause 7

RESEARCH MANDATORY OUTCOMES

- (1) The FIRST PARTY is obliged to realize the mandatory research output as promised in the proposal.
- (2) The **minimum** status of the mandatory output that must be achieved by the SECOND PARTY is as follows.
 - (i) *accepted* for journal/seminar/conference article output types, or
 - (ii) has been published for the type of book output (print version or *online version*), or
 - (iii) accepted or discussed by the user agency for the type of academic manuscript output, or
 - (iv) has issued a Certificate for the type of output Copyright, or
 - (v) has been registered or registered for the type of output of Industrial Design, Patent, or Simple Patent, or
 - (vi) has been realized or laboratory tests have been carried out for the type of output prototype (prototype), and the like.
- (3) The status of the obligatory outputs will be evaluated and become a consideration for the disbursement of the obligatory output funds of 15% of the total research funds.

Clause 8

MONITORING AND EVALUATION

- (1) THE FIRST PARTY has the right to carry out monitoring and evaluation (monev) on the implementation of research, both administratively and in substance.
- (2) Monitoring of research progress is carried out by the Monev Team formed by the FIRST PARTY.
- (3) Internal Monev is carried out to document Report Uploaded progress by the SECOND PARTY.
- (4) THE FIRST PARTY has the right to determine the continuation or termination of a multi-year research contract *based* on the results of the second stage of the monev on the Final Report and the output achievements study year walk uploaded by the SECOND PARTY.

Clause 9

RESEARCH RESPONSIBILITY AND RESEARCH OUTCOMES

- (1) Researcher declared have **dependents study** if until at the time of acceptance of research proposals period next not yet complete obligation upload Report End Research.
- (2) Researchers who have dependents study as meant on paragraph (1) no allowed submit a research proposal on period that.



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- (3) Researcher declared have **dependents outside study** if until at the end upload Report End Research, output Required not yet achieved with minimal status like mentioned on Clause 7 paragraph (2).
- (4) Researchers who have dependents outside study as meant on paragraph (3) still allowed submit a research proposal on period closest.
- (5) Researchers who have not Fulfilled outside required until on acceptance of research proposals on period year next no allowed submit a proposal to period that.
- (6) Dependents study and/or outside required study apply for Chairman and Member researcher from Ahmad Dahlan University.

Clause 10

SANCTIONS AND TERMINATION OF RESEARCH AGREEMENTS

- (1) THE FIRST PARTY has the right to give warnings and or reprimands for negligence and or violations committed by the SECOND PARTY which resulted in the non-fulfillment of this research contract.
- (2) THE FIRST PARTY has the right to terminate the research agreement, if the SECOND PARTY does not heed the warning given by the FIRST PARTY.
- (3) Any material or financial loss caused by the negligence of the SECOND PARTY shall be fully the responsibility of the SECOND PARTY.
- (4) Types of sanctions given can be in the form of:
 - (a) is not allowed to submit a research proposal as meant on Clause 9 paragraph (5) until the obligation is completed; and or
 - (b) Unable to disburse Phase II funds; and or
 - (c) Return the funds received by the SECOND PARTY.

Clause 11

FORCE MAJEUR

The provisions in Clause 10 above do not apply in the following circumstances:

- a. Force Majeure
- b. The FIRST PARTY agrees on the occurrence of delays based on prior notification by the SECOND PARTY to the FIRST PARTY with a Notification Letter regarding the possibility of delays in the completion of research activities as referred to in Clause 2; and conversely the SECOND PARTY agrees to the delay in payment as a result of the delay in the completion of the research agreement.

Clause 12

- (1) Force *majeure* as referred to in Clause 11 paragraph (1) are events that directly affect the implementation of the agreement and occur beyond the power and ability of the SECOND PARTY or FIRST PARTY.
- (2) Events that are classified as *force majeure* include, among others, natural disasters, strikes, disease outbreaks, riots, rebellions, wars, shortened working hours by the government, fires and/or government regulations concerning dangerous conditions and other matters, other equivalents, so that the SECOND PARTY or the FIRST PARTY are forced to be unable to fulfill their obligations.
- (3) The incident as referred to in paragraph (2) above, must be justified by the fourth set of authorities and notified by a notification letter by the SECOND PARTY to the FIRST PARTY or the FIRST PARTY to the SECOND PARTY stating that an event categorized as a *force has occurred*.



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- (4) THE FIRST PARTY provides the SECOND PARTY with the opportunity to complete this contract agreement within the time limit agreed by the PARTIES, if the *force majeure situation* is declared to have been completed.

Clause 13

DISPUTE RESOLUTION

- (1) If in the implementation of the agreement and all of its consequences, a difference of opinion or dispute arises, the FIRST PARTY and the SECOND PARTY agree to settle it by deliberation to reach consensus.
- (2) If the settlement as referred to in paragraph (1) above is not reached, then the FIRST PARTY and SECOND PARTY agree to submit the dispute through mediation with the Chancellor as the direct supervisor of the FIRST PARTY whose decision is final and binding.

Clause 14

RESIGNATION

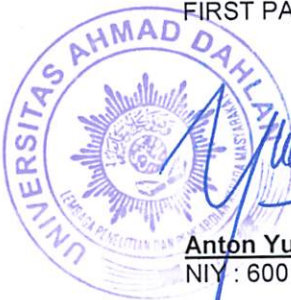
- (1) If the SECOND PARTY resigns or cancels this SP3, then the SECOND PARTY shall submit a Letter of Resignation addressed to the FIRST PARTY.
- (2) The resignation letter as referred to in paragraph (1) must be ratified by the dean of the faculty, the head of the researcher concerned.
- (3) THE SECOND PARTY is obliged to return the funds received to the FIRST PARTY

Clause 15

Et Cetera

- (1) Matters deemed insufficient and amendments to the agreement will be regulated later on the basis of mutual agreement of both parties which will be set forth in the form of a Letter or Additional Agreement (*addendum*), which is an integral and inseparable part of the original agreement.
- (2) This Research Implementation Agreement (RIA) is effective since it is signed and approved by the PARTIES.

FIRST PARTY,



Anton Yudhana, MT, Ph.D.
NIY : 60010383

SECOND PARTY,



Pramudita Budiastuti, S.Pd., M.Pd.
NIY. 60201252



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Anton Yudhana, MT, Ph.D.
NIY : 60010383

SECOND PARTY,

Pramudita Budiastuti, S.Pd., M.Pd.
NIY. 60201252

RESEARCH REPORT

Research summary maximum 500 words consisting of research background, research objectives, stages of research methods, and targeted outputs. In this section, the researcher, should describe the proposed research TKT.

SUMMARY

One of the most extensively cultivated food crops worldwide is corn. According to previous research, corn is mostly used for human and animal use in industrial needs. Diverse corn types have vastly diverse nutrient profiles, price ranges, and applications. The objective of the research is to make tocopherol form corn oil by fractionation method, then it identified by Spectrophotometry UV, NMR, HPLC and LC-MS analysis. The part of corn that is used is seeds which contains oil. This research uses one-kilogram dry corn. It was extracted by maceration method. The EtOH 70% is used as solvent. Thirty-grams yellow oil-shaped extract was obtained after this maceration method. The extract was fractionated and purified to get tocopherol compound using Radial Chromatography (the thickness of stationary phase plate is 4 mm). This fraction eluted using n-hexane:ethyl acetate (8:2) to get pure isolate. The chemical structure was determined using several methods: Spectrophotometry UV, NMR, HPLC and LC-MS. The result showed that the pure isolate is β -tocopherol. This research is basic research with a level of technological readiness level 3. The targeted output of this research is published journal in the Food Research Journal (Q3, SJR: 0,24; journal link: <https://www.myfoodresearch.com/>).

Keywords: maximum 5 keywords. Use semicolon punctuation (;) as a separator and written in alphabetical order.

Keyword 1; Corn Oil; Chromatography; Tocopherol; Isolate.

Results and Discussion (1000-1500 words) containing: (i) the recent progress of research and the achievement, (ii) the recent data obtained, (iii) the results of data analysis, (iv) result discussion, and (v) the recent outputs achieved. The **data** and **research results** can be presented in figures, tables, graphs, etc., that are supported by relevant and up-to-date references. All reported results or achievements must be related to the research phase planned in the proposal.

RESEARCH RESULTS AND DISCUSSIONS

Indonesia has a high level of corn demand, which is rising yearly. The volume of grain exports, which tend to rise. This research objective is making tocopherol form corn oil by fractionation method, then it identified by Spectrophotometry UV, H-NMR, HPLC dan LC-MS analysis. Examining the anti-aging test using the Tyrosinase enzyme was the secondary goal of this study. One of the most extensively cultivated food crops worldwide is corn. Relevant research-reveal that corn is mostly used for human and animal sustenance in industrial needs. Diverse corn types have vastly diverse nutrient profiles, price ranges, and applications. The advancement of hybridization technology creates many hybrid corn seeds are difficult to distinguish. Consumers suffer significant financial losses as a result of the market phenomena of poor corn, which frequently

occurs. Therefore, it is crucial to boost the production, quality, and development of agricultural and animal husbandry by introducing effective corn varieties [1]. Natural antioxidants known as tocopherols (α , β , γ , and δ isomers) prevent lipid oxidation in biological systems by stifling free radicals like hydroperoxyl [2]. According to Martinez de la Cuesta et al. (1995) and Bramley et al. (2000), tocopherols' antioxidant action promotes oil stability. Humans require tocopherols, and research has linked them to a delay in the ageing process of cells [3] [4].

In this study, one kilogramme of dry maize is used. Using the maceration process, it was extracted. The solvent is 70% ethyl alcohol. Following this maceration process, thirty grammes of a yellow oil-shaped extract were recovered. Radial chromatography was used to fractionate and purify the extract in order to obtain the tocopherol component (the stationary phase plate has a thickness of 4 mm). To get pure isolate, this fraction was eluted using an n-hexane:ethyl acetate (8:2) ratio (100 mL volume=3 times fractionations). The results appeared in Figure 1(a)(b)(c), Figure 2 and Figure 3.



(a) (b) (c)
Figure 1. (a) Cord Seeds; (b) Blended Corn; (c) Weighing Corn



Figure 2. Maceration of Blended Corn



Figure 3. Corn Oil Extract

Several spectroscopy techniques, including Spectrophotometry UV, HPLC, LC-MS, and NMR, were used to determine the chemical structure. The data results could be seen in Figure 4, Figure 5, Figure 6 and Figure 7.

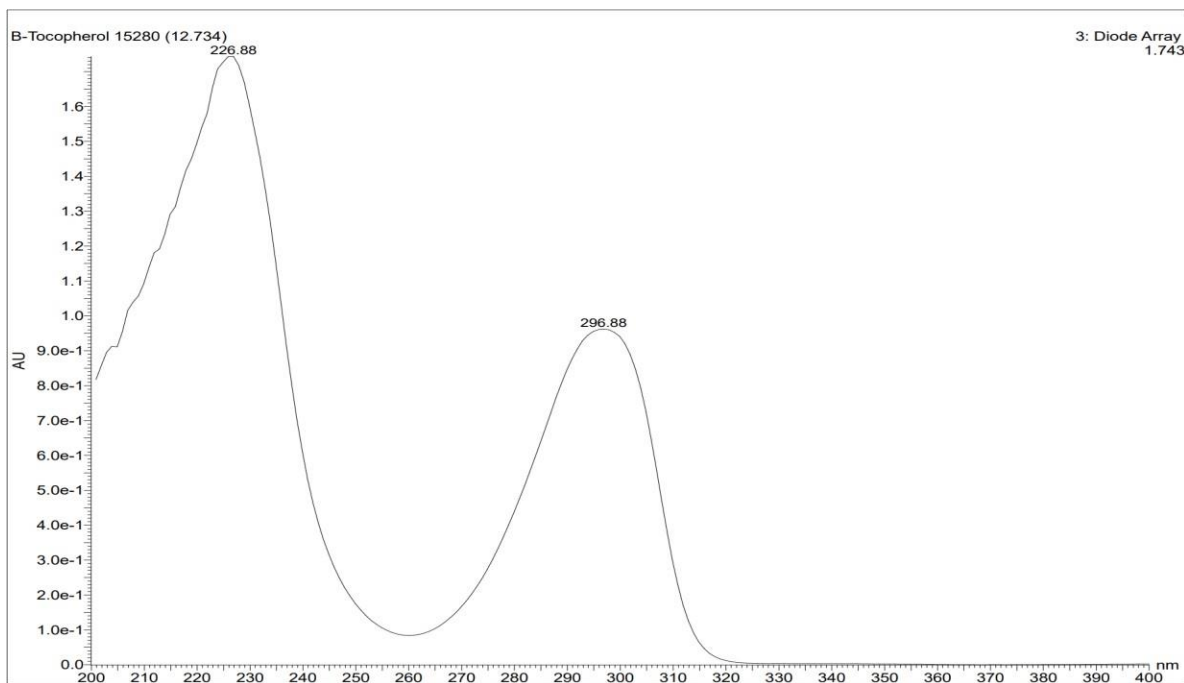


Figure 4. Spectrophotometry Profile

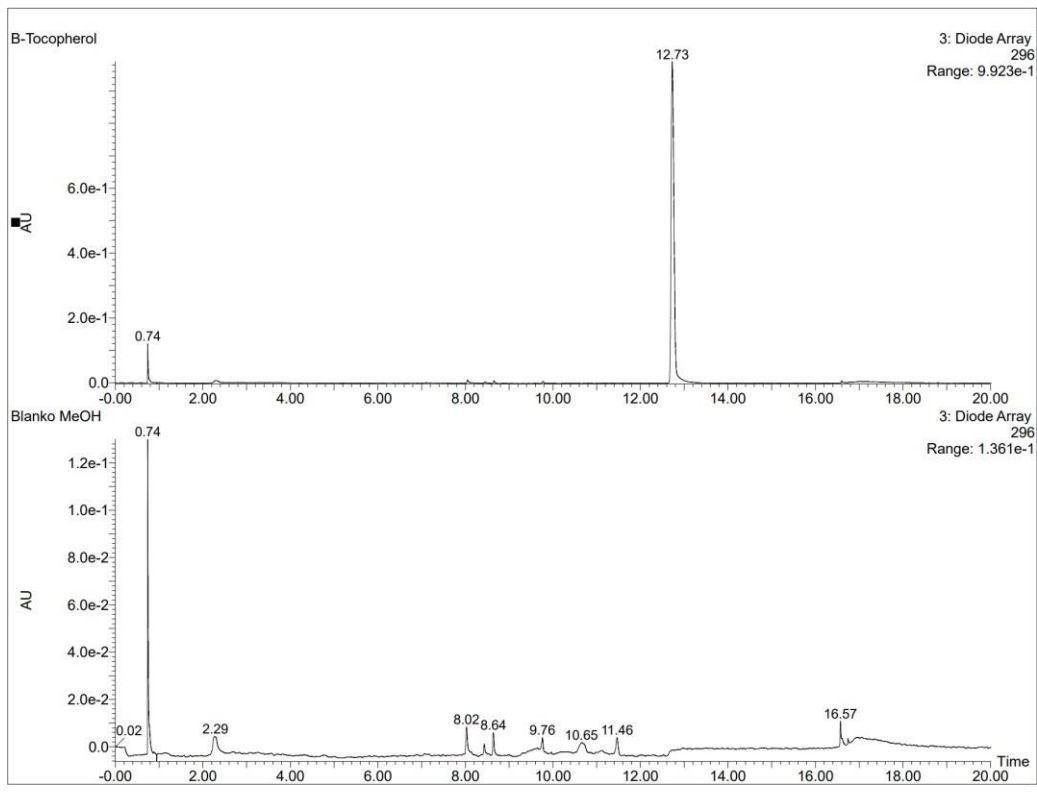


Figure 5. HPLC Profile of Isolate

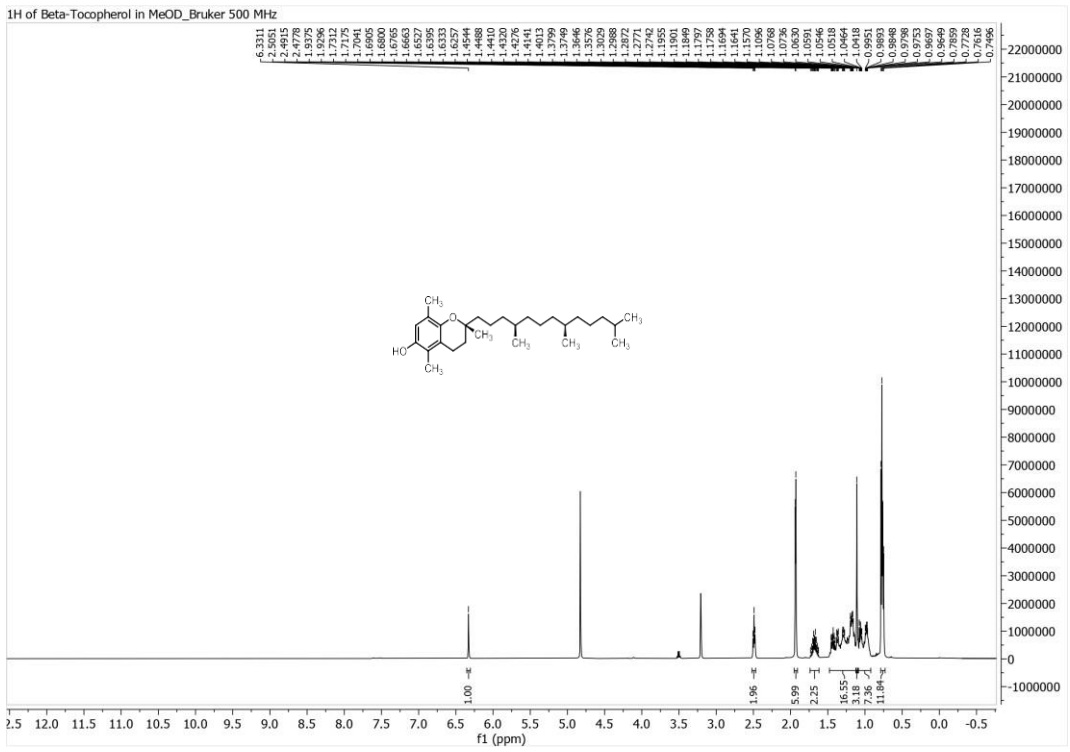


Figure 6. H-NMR Profile of Isolate

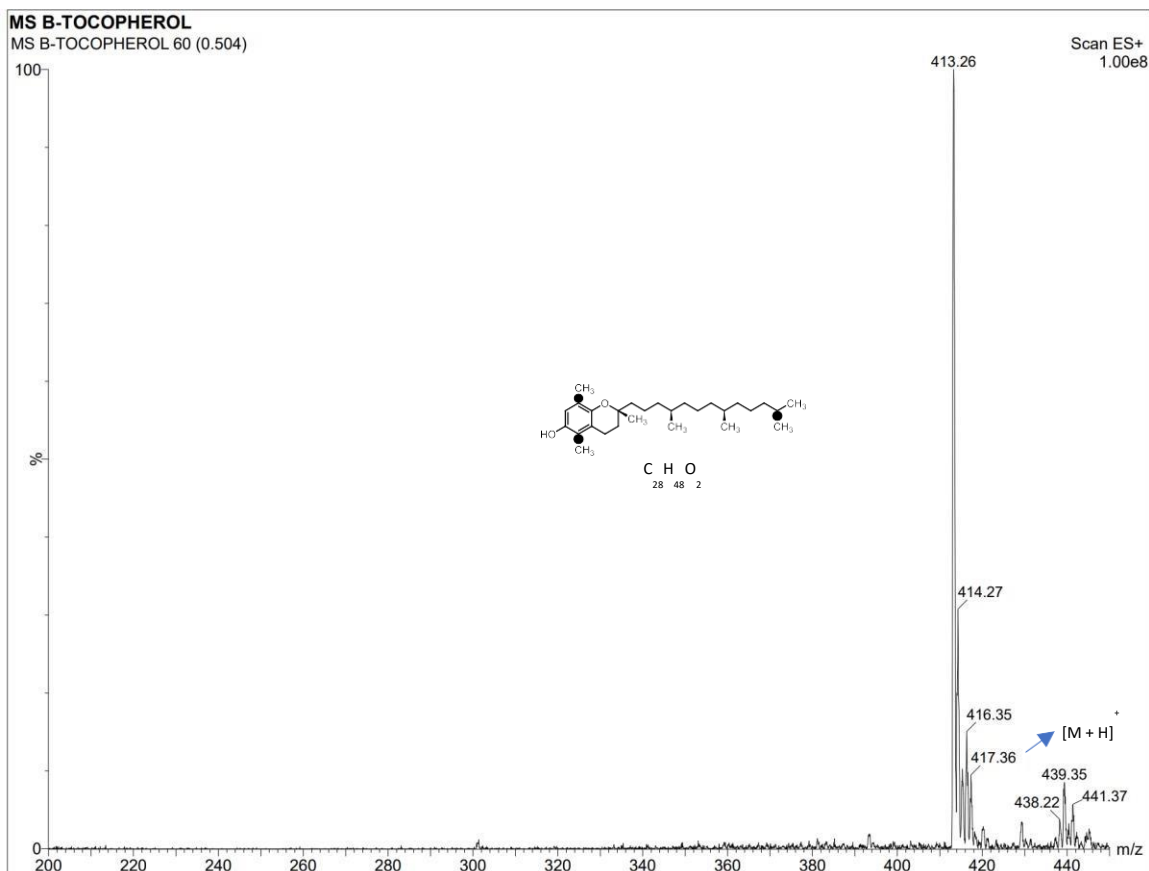


Figure 7. Mass Spectrogram Profile of Isolate

Based on Figure 4, it can be seen that there are 2 peaks, with the peak area in 226.88 and 296.88. Then, based on figure 5, there is peak in 12.73. Figure 6 indicates C₂₈H₄₈O₂. Figure 7 formula C₂₈H₄₈O₂, and its revealed the characteristic fragment m/z 417.36 that is specific to β-tocopherol.

Output status, containing the **type**, **identity**, and **the achievement of each mandatory output and additional output** as stated in the proposal. The researcher should attach the document indicating the current status of the output, such as publication, intellectual property (HKI), experiment results, etc., as stated in the proposal. Scientific papers, books, etc., should attach similarity test results with a maximum of 25%.

OUTPUT STATUS

This research is basic research with a level of technological readiness level 3. The output of this research is **draft journal in the Food Research Journal (Q3, SJR: 0,24; journal link: <https://www.myfoodresearch.com/>)**.

The researcher should describe **the role** of partners in the context of **cooperation realization and partner contributions** both *in-kind* and *in-cash* (for Applied Research and Development/

Penelitian Terapan dan Pengembangan). Supporting evidence/document of this cooperation realization and contribution based on the actual conditions should be attached.

PARTNER ROLE

This research is in partnership with State University of New York New Paltz and EBM Scitech. Both partners taking part in the orientation to find the right method for isolating specific β -tocopherol and they collaborate in providing laboratory equipment during this research (in kind).

Research Implementation Obstacles contain difficulties or obstacles encountered during conducting research and achieving the promised outcomes.

OBSTACLES DURING THE RESEARCH

1. This research has low yield: we need 1 kg dry corn to make 30-gram extract.
2. We need much time to look for good isolation method to get pure isolate (specific isolate) of β -tocopherol.

The Next Plan contains the researcher planning to complete the research as well as to achieve the promised output

NEXT PLAN

1. We are going to measure the beta-tocopherol content in the extract.
2. We are going to do the in vitro test of anti-aging test using Tyrosinase enzyme.

REFERENCES

- [1] Balaikrishnan shammugasamy, Yogeshini Ramakrishan, Hasanah M Ghazali, Kharidah Muhammad. 2020. Tocopherol and tocotrienol contents of different varieties of in Malaysia. Journal Of the Science food and Agriculture: Vol 95-Issue
- [2] White, P.J., and Y. Xing., 1954, Antioxidants from Cereals and Legumes dalam Foreidoon Shahidi: Natural Antioxidants, Chemistry, Health Effect and Applications. AOCS Press, Champaign, Illinois.
- [3] Bramley, P.M. 2000. Is Lycopene Beneficial to Human Health?. Phytochemistry. Vol. 54 : 233-236.
- [4] Martinez de La Cuesta, P.J., Martinez E.R., Chaparro M.G. 1996. Alpha-tocopherol antioxidant role in rancidity of edible oils from vegetable source. An, Quimica Int. Ed. 92; 370-374.
- [5] JY Kim, MJ Kim, BR Yi, S Oh, JH Lee. 2015. Effects of relative humidity on the antioxidant properties α -tocopherol in stripped corn oil. Journal of Food Chemistry.
- [6] D Barrera-Arellano. 2019. Corn oil: composition, processing, and Utilization. Ap Badam-Ribberio
- [7] EC. Grillo, PN Costa, CSS Gurgel. 2014. Alpha-Tocopherol and gamma-tocopherol concentration in vegetable oils. Food Science and Chemistry.










- [8] Y Wen, L XU, C Xue, X Jiang, Z Wei. 2020. Assessing the impact oil types and grades on tocopherol and tocotrienol contents in vegetable oils with chemometric methods. *Journal of Food Chemistry*.
- [9] Abu-Fayyad A, Behery F, Sallam AA, Alqahtani S, Ebrahim H, El Sayed KA, Kaddoumi A, Sylvester PW, Carroll JL, Cardelli JA, Nazzal S (2015) PEGylated γ -tocotrienol isomer of vitamin E: synthesis, characterization, in vitro cytotoxicity, and oral bioavailability. *Eur J Pharm Biopharm* 96 :185–195
- [10] PA Ngoc Doan, TH Tah, Lf Slow, BT Tey. 2021. Dry fractionation approach in concentrating tocopherol from fatty acid distillate: a green pretreatment process for Vitamin E extraction. *Journal Of Food Chemistry*
- [11] Jon Alberdi-Cadeno, Maria L. Ibargotia, Maria D. Guillen. 2020, Effects of the Enrichment of Corn Oil With alpha-of gamma- tocopherol on its in Vitro Digestion Studied by 1H-NMR and SPME- GC/MS; Formation of Hydroproxy-, Hydroxy-, Keto-Dienes and Keto-E-Proxy-E-Monoenes int the More alpha - Tocopherol Enriched Samples. *Journal Of Food Technology*.
- [12] Sajad Pirsā, Elham Banafshechin, Saber Amiri, Amir Rahimirad, Jalal Ghafarzadeh. 2020. Detection of fraud of palm, sunflower, and corn oil in butter using HPLC profile of tocopherol and tocotrienols and by response surface method. *Journal of the Iraninan Chemical Society*.
- [13] J Jin, L Jie, L Jheng, M Cheng, D Xie. 2018. Charecteristic of palm mid-fractions produced from different fractionations paths and their potential usages. *International Journal of Food Chemistry*.

APPENDICES:

- a. Document indicating the mandatory research outputs and their achievement status
- b. Document indicating the additional research outputs and the status of their achievements (if any)
- c. Plagiarism test result indicating 25% similarity (for article or book)
- d. *Logbook* (inputted and downloaded from the portal)
- e. Document showing supervising process (PDP scheme only)
- f. Document showing the cooperation realization with partners for applied research and development research (Riset Terapan dan Pengembangan)

APPENDICES:

Logbook (inputted and downloaded from the portal)

No	Tanggal	Kegiatan	Catatan	File Bukti	Aksi
1	20 Oktober 2023 - 11:00:00	Pure Isolate	Pure Isolate: Determine the chemical structure using several spectroscopic methods, namely NMR, MS, UV Result: B-Tocopherol		
2	05 Oktober 2023 - 08:00:00	Extract in the form of yellow oil	Yellow Oil Extract: Eluted using n-Hexane solvent: Ethyl Acetate (8:2) V = 100 ml x 3		
3	29 September 2023 - 09:00:00	Extract in the form of yellow oil	Yellow Oil Extract: Fractionated and Purified Tocopherol compounds Using Radial Chromatography with a plate thickness of 4 mm) (stationary phase)		
4	14 September 2023 - 10:30:00	Corn oil yield from 70% ethanol extraction process			
5	01 September 2023 - 09:30:00	Checking the readiness of the corn kernel oil extraction process			
6	07 Agustus 2023 - 08:00:00	Coordination of research implementation with partners			

Pure Isolate: Determine the chemical structure using several spectroscopic methods, namely NMR, MS, UV Result: B-Tocopherol



Yellow Oil Extract: Eluted using n-Hexane solvent: Ethyl Acetate (8:2) V = 100 ml x 3. Yellow Oil Extract: Fractionated and Purified Tocopherol compounds Using Radial Chromatography with a plate thickness of 4 mm) (stationary phase)



Corn oil yield from 70% ethanol extraction process



Checking the readiness of the corn kernel oil extraction process








Coordination of research implementation with partners



Document showing supervising process (PDP scheme only)

FORM PEMBIMBINGAN
SKEMA PENELITIAN DOSEN PEMULA (PDP)
PENELITIAN DANA INTERNAL UAD TAHUN AKADEMIK 2021/2022

Nama Peneliti : Pramudita Budiastuti
Judul Penelitian : Making tocopherol from corn oil requires column distillation fractionation, H-NMR analysis, and HPLC analysis
Pembimbing : Dr. Ir. Siti Jamilatun, M.T.

No	Tanggal	Materi dan Uraian Ringkas Pembimbingan	Tanda Tangan Pembimbing
1	04 -09 - 2023	Pengecekan kesiapan proses pengambilan minyak biji jagung (sudah oke)	
2	18 -09 - 2023	Hasil minyak jagung dari proses ekstraksi etanol 70 % (sudah Oke, bisa dioptimalkan lagi dengan konsentrasi etanol lainnya atau pelarut lainnya untuk buat artikel lainnya sebagai alternatif)	
3	02 - 10 - 2023	Ekstrak Berbentuk Minyak Berwarna Kuning: Difraksinasi dan Dimurnikan senyawa Tocopherol Menggunakan Radial Chromatography dengan ketebalan Plat 4 mm) (stationary phase) (sudah oke , silakan dilanjutkan)	
4	12 -10 - 2023	Ekstrak Berbentuk Minyak Berwarna Kuning: Dielusi menggunakan pelarut n-Heksana : Etil Asetat (8:2) V = 100 ml x 3 (OK)	
5	24 -10 - 2023	Isolat Murni: Ditentukan struktur kimianya menggunakan beberapa metode spektroskopi yaitu NMR, MS, UV Hasil: β -Tocopherol (sudah baik, lanjutkan)	

Pembimbing,



Prof. Dr. Ir. Siti Jamilatun, M.T.



Yogyakarta, 26 Oktober 2023

Peneliti,



Pramudita Budiastuti, M.Pd.

Document showing the cooperation realization with partners for applied research and development research (Riset Terapan dan Pengembangan)

	
Letter of Commitment for the Research Collaboration between Universitas Ahmad Dahlan and State University of New York New Paltz	
Whereas the representatives of:	
1. Universitas Ahmad Dahlan , a university located at Jalan Kapas 9, Semaki, Umbulharjo, Yogyakarta 55166, Indonesia	
2. State University of New York New Paltz , a university located at SUNY New Paltz, 1 Hawk Drive New Paltz, New York 12561, United States	
Have committed to conduct collaborative research and development activities which are hereby particularized in this Letter of Commitment as follows:	
1. The collaboration between the Parties shall be undertaken with the objective of promoting research and development endeavour to increase international recognition.	
2. The research and development activities shall be entitled "Making tocopherol from corn oil requires column distillation fractionation, H-NMR analysis, and HPLC analysis"	
3. The Project shall be conducted over a period of eight (8) months commencing 6 June 2023 and ending 6 February 2024 (hereinafter referred to as the "Project Duration").	
4. The responsibilities of the Parties shall be as follows:	
a) UAD shall be responsible for:	
i. Providing as raw materials.	
ii. Conducting test.	
iii. Visiting for sample testing and data analysis.	
b) State University of New York New Paltz, shall be responsible for:	
i. Conducting computer modeling and simulation	
5. The funding support for this Project is provided from UAD at \$ 2.343,33 and from State University of New York New Paltz at \$ 2.343,33 in kind	
6. Pramudita Budiastuti, M.Pd. (UAD) and Rachmadian Wulandana Associate Professor, Mechanical Engineering Program (State University of New York New Paltz) shall act as the Liaison Officer who will develop and coordinate the various activities agreed upon. Through these officers, either party may initiate proposals for additional cooperative activities.	
7. The outcome of this Project would be in the form of:	
a) Basic Research (Penelitian Dasar): publication in International Journal of Public Health Science (IJPHS) (https://ijphs.iaescore.com/index.php/IJPHS)	

by declaring the Grant Number from Institute of Research and Community Service (LPPM) Universitas Ahmad Dahlan in the Acknowledgement.

8. If there is no existing MoU and/or MoA, both Parties committed to draft Memorandum of Understanding (MoU) and Memorandum of Agreement (MoA) by the end of Project Duration.

IN WITNESS WHEREOF the Parties hereto, have caused this commitment to be executed in their names by their duly authorized representatives, on the day and year written below.

Signed by and for and on behalf of]
UNIVERSITAS AHMAD DAHLAN



[Handwritten signature]

Muhammad Sayuti, M.Pd., M.Ed., Ph.D.
Dean of Faculty of teacher training and education science
Date: 02 June 2023

In the presence of]

... (name)
Principal Investigator
Date: ... (insert date month year)
Universitas Ahmad Dahlan

Signed by and for and on behalf of]
State University of New York New Paltz

Rachmadian W
06/02/23

Rachmadian Wulandana Associate Professor,
Mechanical Engineering Program
Date: 02 June 2023

In the presence of:]

... (name)
Principal Investigator
Date: ... (insert date month year)
Nama Mitra Internasional

**Plagiarism test result
indicating 25%
similarity (for article
or book)**

Making tocopherol from corn oil requires column distillation fractionation, H-NMR analysis, and HPLC analysis

by Pramudita Budiastuti

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Making tocopherol from corn oil requires column distillation fractionation, H-NMR analysis, and HPLC analysis

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Abstract

One of the most extensively cultivated food crops worldwide is corn. According to previous research, corn is mostly used for human and animal use in industrial needs. Diverse corn types have vastly diverse nutrient profiles, price ranges, and applications. The objective of the research is to make tocopherol from corn oil by fractionation method, then it identified by Spectrophotometry UV, NMR, HPLC and LC-MS analysis. The part of corn that is used is seeds which contains oil. This research uses one-kilogram dry corn. It was extracted by maceration method. The EtOH 70% is used as solvent. Thirty-grams yellow oil-shaped extract was obtained after this maceration method. The extract was fractionated and purified to get tocopherol compound using Radial Chromatography (the thickness of stationary phase plate is 4 mm). This fraction eluted using n-hexane:ethyl acetate (8:2) to get pure isolate. The chemical structure was determined using several methods: Spectrophotometry UV, NMR, HPLC and LC-MS. The result showed that the pure isolate is β -tocopherol. This research is basic research with a level of technological readiness level 3. The targeted output of this research is published journal in the Food Research Journal (Q3, SJR: 0,24; journal link: <https://www.myfoodresearch.com/>).

Keywords: Corn Oil, Chromatography, Tocopherol, Isolate.

1. Introduction

Glycerol and fatty acids make up the triglyceride that makes up corn oil. Glycerides make up about 98.6% of the mixture; the remainder is made up of non-oil substances like wax, pigments, and ash. Saturated and unsaturated fatty acids are the two types of fatty acids that make up corn oil. About

64 kg of granulated flour and 3 kg of corn oil will be produced from 100 kg of maize with a 16% water content. Corn oil can be obtained from dry milling by pressing or hexane extraction. 18% of maize flour is made up of oil. For wet milling, institutions can be separated first, and oil extraction is then carried out. The average extractable oil content in institutions is 52% [5].

Less than 1.2% of the oil is extracted. Phosphatides, free fatty acids, pigments, waxes, and trace levels of flavorings and odorants are among the dissolved substances that are still present in crude oil. Triacylglycerols (TAGs), which make up around 95% of the lipids in corn kernels, are also present along with waxes, phospholipids, glycolipids, hydrocarbons, phytosterols (sterols and stanols), free fatty acids, carotenoids (vitamin A), and tocol (vitamin E). Linoleic acid (59.7%), oleic acid (25.2%), palmitic acid (11.6%), stearic acid (1.8%), and linolenic acid (0.8%) are among the fatty acids found in corn oil. Table 1 lists the qualities of pure corn oil [6].

Table 1. Composition table for pure corn oil

Chemical Characteristic	(%)	Physical Characteristic	Score
Triglycerida	98,8	Indeks	1,47
Saturation		refraction	125-128
- Saturates	12,9	Iod number	-20 s/d -10
- Mono-unsaturates	24,8	Solid Point	-16 s/d -11
- Polyunsaturation	61,1	Melting point	221-260
- Ratio	4,8	Smoke point	302-338
Profile Fatty Acid		Flash point	310-371
triglycerida	11,1-12,8	Fire point	0,918-0,925
- Palmitat (16:0)	1,4-2,2	Fire point	0,92
- Stearat (18:0)	22,5-36,1	Specific Gravity	15,6
- Oleat (18:1)	49,0-61,9	Particular Gravity	20-35
- Linoleat (18:2)	0,4-1,6	(kg/l)	2,5-5,0
- Linolenat (18:3)	0,0-0,2	Viscosity (cp)	9,42
- Arasidat (20:0)	0,04	Color	-
Fosfolipid	0,02-0,03	- Yellow	-
Free Fatty Acid	0	- Red	-
Waxes	0	Combustion Heat	-
Kolesterol	1,1	(cal/g)	-
Fitosterol	0,09	-	-
Tocopherol	Td	-	-
Karotenoid	12,5-13,9	-	-

Vegetable oils are the richest dietary sources of vitamin E. Vitamin E determination levels in foods are of great importance to adjust the ingestion of nutrients by the population. The purpose of this paper is to determine the concentration of alpha-tocopherol and gamma-tocopherol in vegetable oils and compare the alpha-tocopherol value to the nutritional requirement of vitamin E. The analysis was performed using High Performance Liquid Chromatography. The values expressed as mg/kg for alpha and gamma-tocopherol were, respectively, 120.3±4.2 and 122.0±7.9 in canola oil; 432.3±86.6 and 92.3±9.5 in sunflower oil; 173.0±82.3 and 259.7±43.8 in corn oil; 71.3±6.4 and 273.3±11.1 in soybean oil. A significant difference was encountered between the alpha-tocopherol concentrations in vegetable oils. Similar results were found for gamma-tocopherol, except for corn and soybean oils. It was concluded that the soybean oil was not

considered a source of vitamin E. The canola and corn oils were considered sources, and the sunflower oil was considered an excellent source [7].

Horizontal inner lines in the boxes are the mean values. As depicted in Figure 1, the average contents of the total tocopherols were compared and listed in the following order: soybean oil > cottonseed oil > corn oil > sunflower oil > rapeseed oil > rice bran oil > peanut oil > sesame oil > camellia oil. The average content of total tocopherols in the first-grade traditional edible oils ranged from 65.7 mg/kg of the camellia oil samples (n = 6) to 1052.6 mg/kg of the soybean oil samples (n = 23). Different rice bran oil samples showed large differences in the total tocopherol content, with the coefficient of variation (CV) presenting as 95.3%, and the highest total tocopherol content of the rice bran oil sample was 13.3 times higher than that of the lowest. The probable reason for the variation in the tocopherol contents may be the different cultivars of crops, growing environments, methods for oil extraction, or analytical methods used to detect the tocopherols [8].

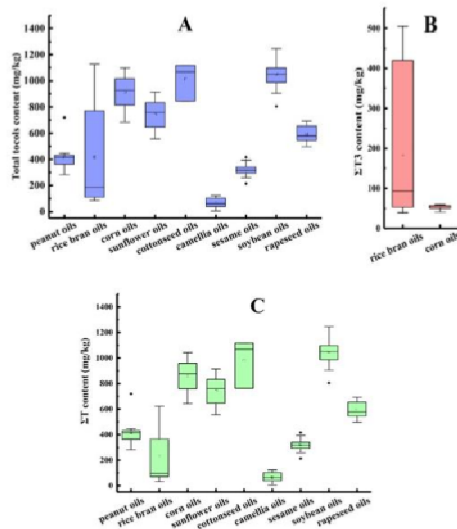


Figure 1. shows a comparison of tocopherol levels in various plant species.

1.1 Tocopherols

Tocopherols (T₃) are two subfamilies of the vitamin E family, which consists of eight related, fat-soluble molecules. Each subgroup has four isomers α , β , γ , and δ , which vary in the quantity of methyl substitutions made to the chromanol group and the extent of phytyl side chain saturation. The first isomer of tocopherol discovered to be crucial for reproduction is tocopherol (-T). Later, these chemicals' antioxidant capabilities were discovered. Different tocopherol isomers of vitamin E have different health and therapeutic effects, according to later research into the anticancer activity of tocopherols in the 1990s. Documented variations in the therapeutic and biological advantages of vitamin E's tocopherol isomers [9][10].



Figure 2. Displays goods made with tocopherols.

1.2 Tocopherol Fractionation

The process of tocopherol fractionation involves dividing and purifying tocopherol compounds into various fractions or components according to variations in their physical and chemical characteristics. Different antioxidant activity may be present in these fractions. Chromatographic methods, such as high-liquid chromatography or column chromatography, are frequently used in the tocopherol fractionation process. The tocopherol mixture is divided into fractions in column chromatography by being passed through a column that is loaded with a specific separating agent. In contrast, the tocopherol combination is broken down in high-liquid chromatography using the differential in solubility between the liquid phase and the solid phase [11].

1.3 Tocopherol Crystallization

One method of processing corn oil that can be used to separate saturated from unsaturated fatty acids is corn oil crystallization. The tocopherol crystallization process typically yields crystals of tocopherol in modest amounts and with high purity. In the business, tocopherol is crystallized using the Forced Circulating Liquid Evaporator Crystallizer type shown in Figure 3 [12].

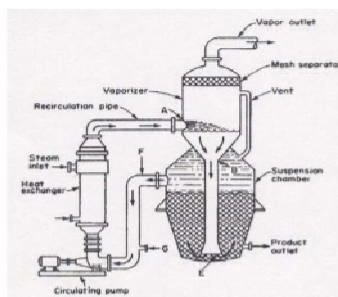


Figure 3. Forced Calculating Liquid Evaporator Crystallizer

1.4 Tocopherol Analysis

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H-NMR Analysis: Modern chemistry uses Nuclear Magnetic Resonance (NMR), one of the simplest analytical techniques. NMR is employed to ascertain the purity of constituents, the direction of chemical processes, the interaction between constituents in solutions that can undergo chemical reactions, and the structure of new natural and manufactured components. **HPLC Analysis:** The acronym for HPLC analysis is The analytical method of high-performance liquid chromatography, also known as high-pressure liquid chromatography (HPLC), is used to separate and quantify the amount of different chemicals and compounds

in a mixed solution.

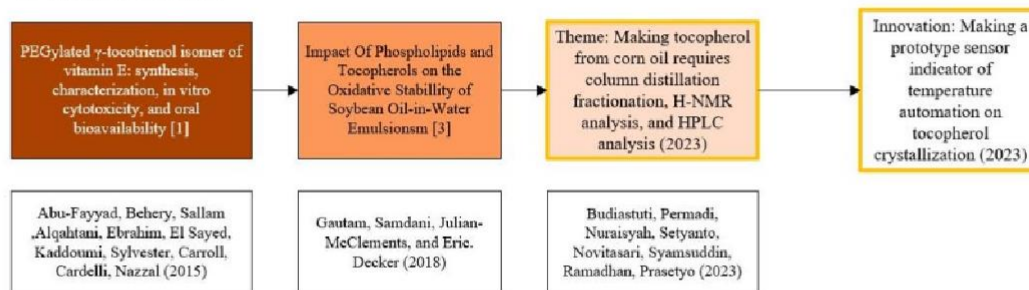


Figure 4. Road map in the field studied

2. Materials and methods

2.1 Sampling Methods

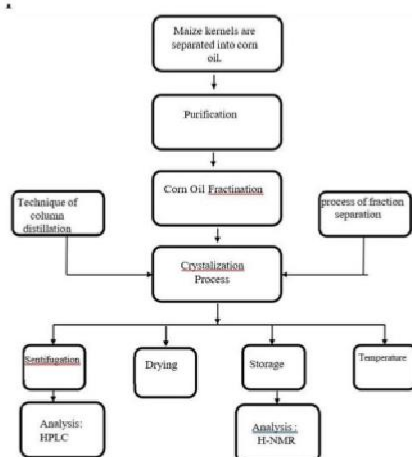


Figure 5. Flowchart of Research

2.2 Data Collection Methods

The study was carried out with refined corn oil C, acquired in a local supermarket. Its composition in molar percentages of linolenic (Ln), linoleic (L), oleic (O) and saturated (S) acyl groups is, $0.6 \pm 0.0\%$, $49.2 \pm 0.5\%$, $34.1 \pm 0.3\%$ and $16.1 \pm 0.1\%$ respectively. This was determined from ^1H NMR spectral data as in previous studies [18,19]. The tocopherols used were alpha-tocopherol (αT) (purity of 98.2%) purchased from Sigma-Aldrich (St. Louis, MO, USA), and gamma-tocopherol (γT) (purity of $\geq 90\%$) provided by Eisai Food & Chemical Co. Ltd. (Tokyo, Japan). Aliquots of the oil were enriched with alpha-tocopherol or gamma-tocopherol at 0.2%, 2% and 5% by weight in each case. The samples submitted to in vitro digestion were the original oil C, and all samples enriched in αT (C0.2 αT , C2 αT and C5 αT) and in γT (C0.2 γT , C2 γT and C5 γT) [13].

2.3 Research Design

In this research, mixing of palm oil, sunflower oil, and corn oil in the butter (as a fraud) was investigated by studying the HPLC profiles of the tocopherols and tocotrienols. The statistical D-Optimal mixture design (D-OMD) was used to design experiments. Based on D-OMD, 20 fraud butter samples with different percent of palm, sunflower, and corn oil (0, 10, and 20%) were prepared. Oil samples were dissolved in n-hexane, and tocopherols/tocotrienols profiles were analyzed by HPLC/fluorescence detector [14].

The identification of the components present in the original oil, in the oil samples enriched with tocopherol and in the lipid extracts of their digestates, was carried out on the basis of the assignments of the $^1\text{H-NMR}$ signals, present in Figure 5; Figure 6, to the different kinds of hydrogen atoms, and to the different compounds.

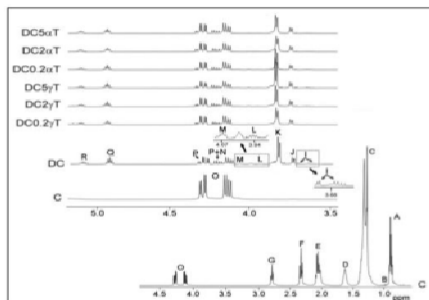


Figure 6. Region comprised H-NMR

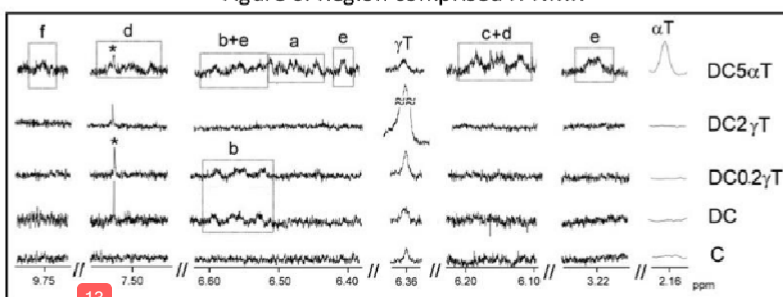


Figure 7. Some regions of the ^1H NMR spectra of oil C and of the lipids extract

2.4 Research Procedures

The soaking and pressing procedure was employed to create the corn oil that was used in this investigation. The purifying procedure is used to carry out the outcome of the extraction of corn oil. Corn oil samples are refined via centrifugal filtering, settling, and extraction. Tocopherol samples to be fractionated must first be isolated from the source, for example corn oil or olive oil [15]. The sample must also be cleaned of other contaminants that can affect the fractionation results. As seen in Figure 3.1, the tocopherol extraction process from corn oil is as follows.

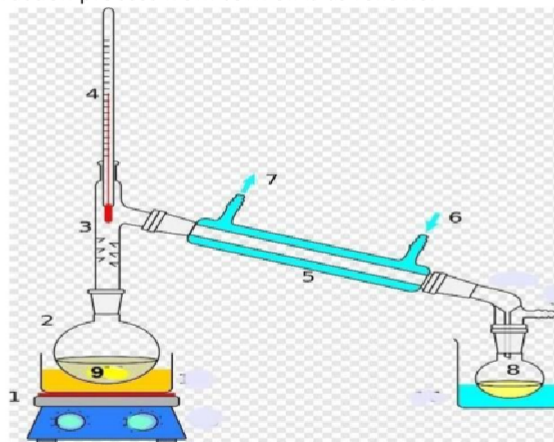


Figure 8. a number of corn oil fractionation tools

Noted of Figure 8:

1. Stove
2. Distillation Flask
3. Precondensation Fraction
4. Thermometer
5. Condenser
6. Colling Water In
7. Cooling Water Out
8. Accumulator
9. Mixture

2.5 Data Analysis

Trial sample data and outcomes were examined by:

1. Trial Sample: HPLC Analysis dan H-NMR Analysis
2. Sample used in experiment: HPLC Analysis dan H-NMR Analysis

3. Results and discussion/Results

Indonesia has a high level of corn demand, which is rising yearly. The volume of grain exports, which tend to rise. This research objective is making tocopherol form corn oil by fractionation method, then it identified by Spectrophotometry UV, H-NMR, HPLC dan LC-MS analysis. Examining the anti-aging test using the Tyrosinase enzyme was the secondary goal of this study. One of the most extensively cultivated food crops worldwide is corn. Relevant research-reveal that corn is mostly used for human and animal sustenance in industrial needs. Diverse corn types have vastly diverse nutrient profiles, price ranges, and applications. The advancement of hybridization technology creates many hybrid corn seeds are difficult to distinguish. Consumers suffer significant financial losses as a result of the market phenomena of poor corn, which frequently occurs. Therefore, it is crucial to boost the production, quality, and development of agriculture and animal husbandry by introducing effective corn varieties [1]. Natural antioxidants known as tocopherols (α , β , γ , and δ isomers) prevent lipid oxidation in biological systems by stifling free radicals like hydroperoxyl [2]. According to Martinez de la Cuesta et al. (1995) and Bramley et al. (2000), tocopherols' antioxidant action promotes oil stability. Humans require tocopherols, and research has linked them to a delay in the ageing process of cells [3] [4].



Figure 9. corn cobs



Figure 10. corn oil

In this study, one kilogramme of dry maize is used. Using the maceration process, it was extracted. The solvent is 70% ethyl alcohol. Following this maceration process, thirty grammes of a yellow oil-shaped extract were recovered. Radial chromatography was used to fractionate and purify the extract in order to obtain the tocopherol component (the stationary phase plate has a thickness of 4 mm). To get pure isolate, this fraction was eluted using an n-hexane:ethyl acetate (8:2) ratio. Several spectroscopy techniques, including Spectrophotometry, HPLC, LC-MS, and NMR, were used to determine the chemical structure. The data results could be seen in Figure 1, Figure 2, Figure 3 and Figure 4.

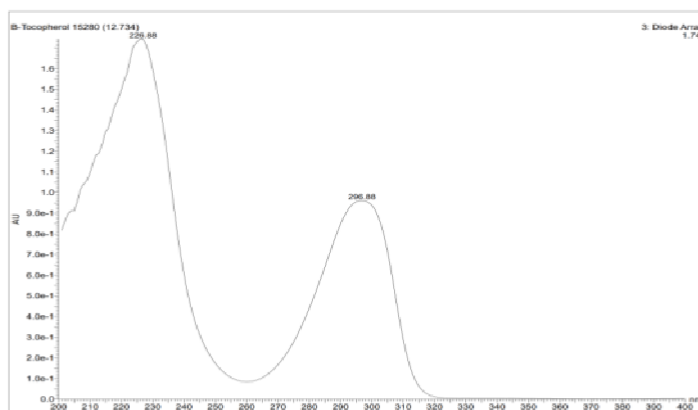


Figure 11. Spectrophotometry Profile

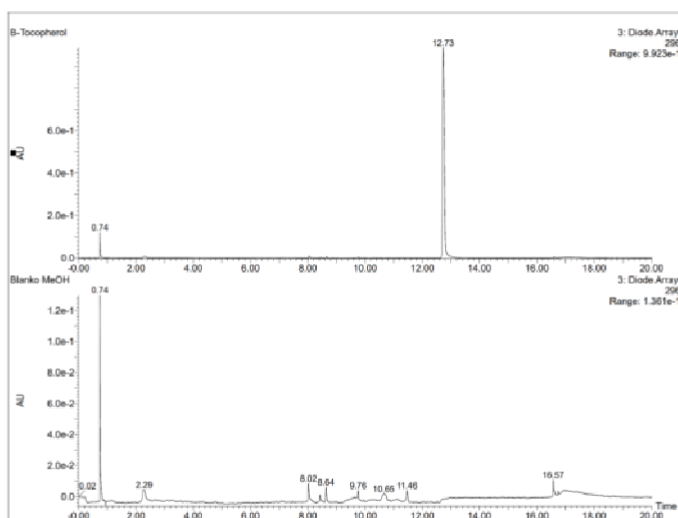


Figure 12. HPLC Profile of Isolate

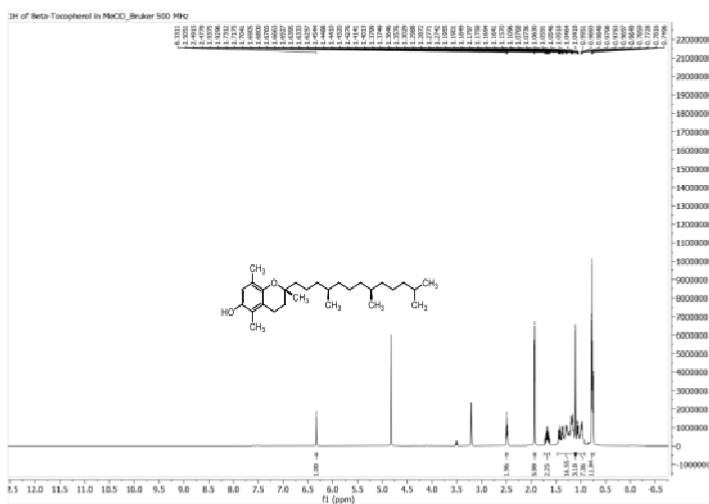


Figure 13. H-NMR Profile of Isolate

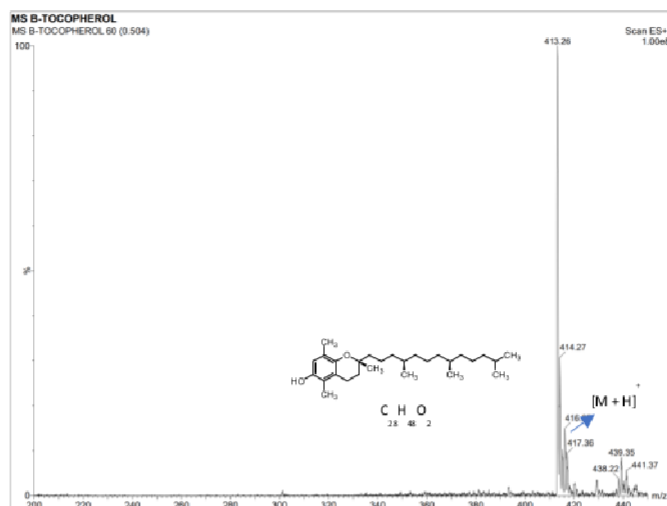


Figure 14. Mass Spectrograph Profile of Isolate

Based on Figure 11, it can be seen that there are 2 peaks, with the peak area in 226.88 and 296.88. Then, based on figure 12, there is peak in 12.73. Figure 13 indicates C₂₈H₄₈O₂. Figure 14 formula C₂₈H₄₈O₂, and it's revealed the characteristic fragment m/z 417.36 that is specific to β-tocopherol.

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