#### 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  $\beta$ -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 ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  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.

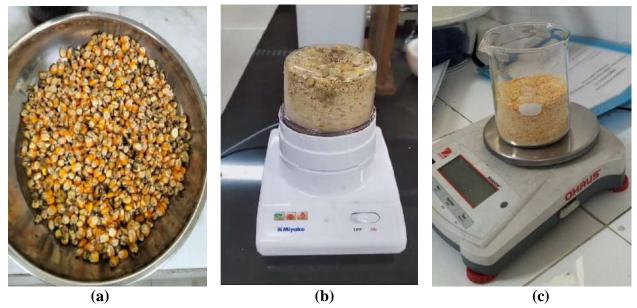


Figure 1. (a) Cord Seeds; (b) Blended Corn; (c) Weighing Corn



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.

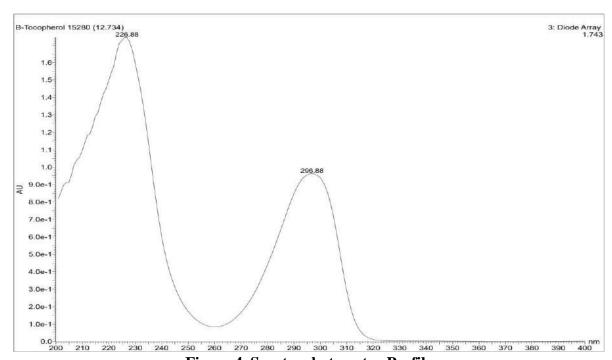


Figure 4. Spectrophotometry Profile

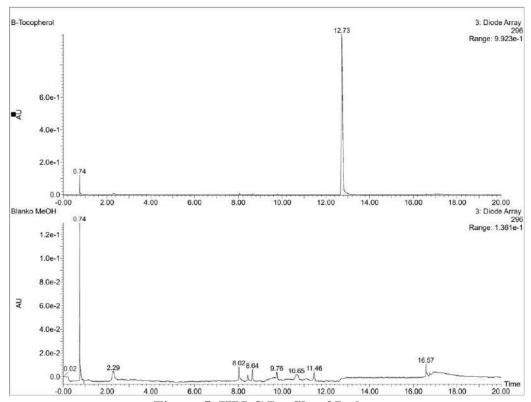


Figure 5. HPLC Profile of Isolate

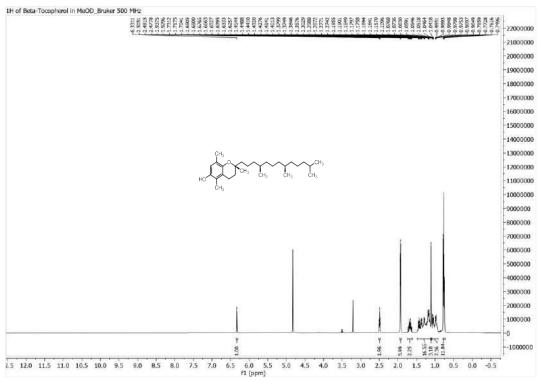


Figure 6. H-NMR Profile of Isolate

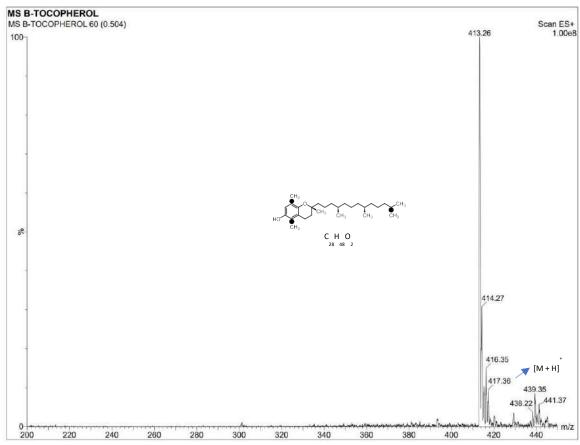


Figure 7. Mass Spectrogram Profile of Isolate

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  $C_{28}H_{48}O_2$ . Figure 7 formula  $C_{28}H_{48}O_2$ , and its revealed the characteristic fragment m/z 417.36 that is specific to  $\beta$ -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  $\beta$ -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  $\beta$ -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.

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#### **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		7
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 $$		1
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			2
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



### 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.

Tanggal	Materi dan Uraian Ringkas Pembimbingan	Tanda Tangan Pembimbing
04 -09 - 2023	Pengecekan kesiapan proses pengambilan minyak biji jagung ( sudah oke )	#
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)	#
02 - 10 - 2023	Ekstrak Berbentuk Minyak Berwarna Kuning: Difraksinasi dan Dimurnikan senyawa Tocopherol Menggunakan Radial Chromatography dengan ketebalan Plat 4 mm) (stationary phase) (sudah oek , silakan dilanjutkan)	#
12 -10 - 2023	Ekstrak Berbentuk Minyak Berwarna Kuning: Dielusi menggunakan pelarut n-Heksana : Etil Asetat (8:2) V = 100 ml x 3  (OK)	#
24 -10 - 2023	Isolat Murni: Ditentukan struktur kimianya menggunakan beberapa metode spektroskopi yaitu NMR, MS, UV Hasil: β-Tocopherol (sudah baik, lanjutkan)	#
	04 -09 - 2023 18 -09 - 2023 02 - 10 - 2023 12 -10 - 2023 24 -10 -	Pengecekan kesiapan proses pengambilan minyak biji jagung  ( sudah oke )  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)  Ekstrak Berbentuk Minyak Berwarna Kuning: Difraksinasi dan Dimurnikan senyawa Tocopherol Menggunakan Radial Chromatography dengan ketebalan Plat 4 mm) (stationary phase)  (sudah oek , silakan dilanjutkan)  Ekstrak Berbentuk Minyak Berwarna Kuning: Dielusi menggunakan pelarut n-Heksana : Etil Asetat (8:2) V = 100 ml x 3  (OK)  Isolat Murni: Ditentukan struktur kimianya menggunakan beberapa metode spektroskopi yaitu NMR, MS, UV  Hasil: β-Tocopherol

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:

- 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
- 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
- 4. The responsibilities of the Parties shall be as follows:
  - a) UAD shall be responsible for:
    - Providing as raw materials.
  - Conducting test.
     Visiting for sample testing and data analysis.
     State University of New York New Paltz, shall be responsible for:
     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:
  - Basic Research (Penelitian Dasar): publication in International Journal of Public Health Science (IJPHS) (https://ijphs.iaescore.com/index.php/IJPHS)

		m Institute of Research and Community Dahlan in the Acknowledgement.
	tanding (MoU) a	MoA, both Parties committed to draft nd Memorandum of Agreement (MoA) by
		o, have caused this commitment to be zed representatives, on the day and year
Signed by and for and on bel UNIVERSITAS AHMAD DAH		19 Jahren
Muhammad Sayuti, M.Pd., M Dean of Faculty of teacher tra Date: 02 June 2023		ation science
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(name) Principal Investigator Date: (insert date month ye	ear)	

# 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

**Submission date:** 30-Oct-2023 10:47AM (UTC+0700)

**Submission ID: 2211438270** 

File name: fr - full paper template 1.docx (1.95M)

Word count: 2697

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

<sup>1</sup>Adi Permadi, <sup>2</sup>Pramudita Budiastuti, <sup>1</sup>M. Kaisar, <sup>3</sup>Fatma Nuraisyah, <sup>4</sup>Putri Rachmah Novitasari, <sup>5</sup>Arief Syamsuddin, <sup>2</sup>Barry Nur Setyanto, <sup>1</sup>Nurmustaqimah, <sup>1</sup>Stradivary Maulida Firdaus]

<sup>1</sup> Program Magister Teknik Kimia, Fakultas Teknologi Industri, Universitas Ahmad Dahlan, Jl. Ringroad Selatan, Kragilan, Tamanan, Kec. Banguntapan, Kabupaten Bantul, Daerah Istimewa Yogyakarta 55191

<sup>2</sup>Program Studi Pendidikan Vokasional Teknik Elektronika, Jl. Pramuka No.42, Pandeyan, Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta 55161

<sup>3</sup>Program Studi Ilmu Kesehatan Masyarakat, Fakultas Kesehatan Masyarakat, Universitas Ahmad Dahlan, Jl. Prof. DR. Soepomo Sh, Warungboto, Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta 55164

<sup>4</sup>Program Strudi Farmasi, Fakultas Farmasi, Universitas Ahmad Dahlan, Jl. Prof. DR. Soepomo Sh, Warungboto, Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta 55164

<sup>5</sup>Magister Pendidikan Vokasi, <mark>Jl. Pramuka No.42, Pandeyan, Kec. Umbulharjo, Kota Yogyakarta, Daerah Istimewa Yogyakarta 55161</mark>

\*Corresponding author: Adi.permadi@che.uad.ac.id

Author No.1: Adi Permadi

Author No. 2: Pramudita Budiastuti

#### 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 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 together the 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 cornoil. About

64 kg of granulated flour and 3 kg of corn oil will be produced from 100 kg of maizewith 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 (TA35), which make up around 95% of the lipids in corn kernels, are also present along with waxes, phospholipids, glycolipids, hydrocarbons, phytosterols (sterols and stanols), gee fatty acids, carotenoids (vitamin A), and tocol (vitamin E). Linoleic acid (59.7% 17 leic 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. Table1 lists the qualities of pure corn oil [6].

Table 1. Composition table for pure corn oil

Chemical Charecteristic	(%)	Physical Charecteristic	Score
Trigliserida	98,8	Indeks	1,47
Saturation		refraction	125-128
- Saturates	12,9	Iod number	-20 s/d -10
- Saturates	70		-16 s/d -11
<ul> <li>Mono-unsaturates</li> </ul>	24,8	Solid Point	221-260
- Polyunsatration	61,1 4,8	Melting point	302-338
- Ratio	4,0	Smoke point	310-371
		Flash point	0,918-0,925
Profile Fatty Acid	11,1-12,8		0.92
trigliserida	1,4-2,2	Fire point	15,6
- Palmitat (16:0)	22,5-36,1	Spesific Gravity	
- Stearat (18:0)	49,0-61,9	Particullar Gravity	20-35
Olere (18:1)	0,4-1,6	(kg/l)	2,5-5,0
- Oleat (18:1)	0,0-0,2	Viscocity (cp)	
- Linoleat (18:2)	0.04	SHOOD CITY	9,42
<ul> <li>Linolenat (18:3)</li> </ul>	0,02-0,03	Color	_
- Arasidat (20:0)	0	- Yellow	
Fosfolipid	ő	- Red	=
	1.1	Combustion Heat	-
Free Fatty Acid			=
Waxes	0,09 Td	(cal/g)	-
Kolesterol	14	-	
Fitosterol			
***************************************		-	
Tocopherol	12,5-13,9	-	
Karotenoid		1.5	

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 vegrable 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 tocols 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 tocols 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 tocol content, with the coefficient of variation (CV) presenting as 95.3%, and the highest total tocol 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 tocol contents may be the different cultivars of crops, growing environments, methods for oil extraction, or analytical methods used to detect the tocols [8].

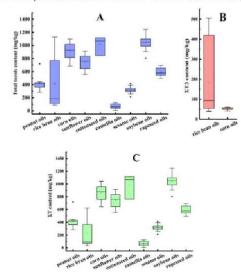


Figure 1. shows a comparison of tocopherol levels in various plant species.

#### 1.1 Tocopherols

Tocopherols ( $T_8$ ) are two subfamilies of the vitamin E family, which consists of eight related, fatsoluble molecules. Each subgroup has four isomers  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ . which vary in the quantity of methyl substitutions made to the chromana 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 1990. 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 solidphase [11].

#### 1.3 Tocopherol Crystallization

One method of processing corn oil that can be used to separate saturated from unsaturated fattyacids is corn oil crystallization. The tocopherol crystallization process typically yields crystalsoftocopherol in modest amounts and with high purity. In the business, tocopherol is crystalli zed using the Forced Cilculating Liquid Evaporator Crystalizer type shown in Figure 3 [12].

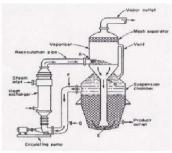


Figure 3. Forced Calculating Liquid Evaporator Crystalizer

#### 1.4 Tocopherol Analysis

16

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 shatural 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.

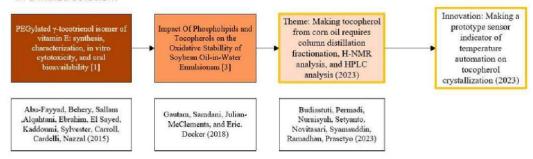


Figure 4. Road map in the field studied

#### 2. Materials and methods

#### 2.1 Sampling Methods

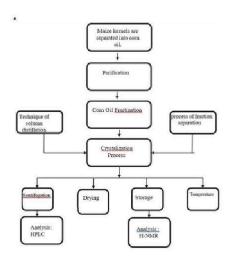


Figure 5. Flowchart of Research

#### 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 ta as in previous studies [18,19]. The tocopherols used were alpha-tocopherol ( $\alpha$ T) (purity of 98.2%) purchased from Sigma-Aldrich (St. Louis, MO, USA), and gamma-tocopherol ( $\gamma$ T) (purity of  $\gamma$  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  $\alpha$ T (C0.2 $\alpha$ T, C2 $\alpha$ T and C5 $\alpha$ T) and in  $\gamma$ T (C0.2 $\gamma$ T, C2 $\gamma$ T and C5 $\gamma$ T) [13].

#### 2.3 esearch 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 <sup>1</sup>H-NMR signals, present in Figure 5; Figure 6, to the different kinds of hydrogen atoms, and to the different compounds.

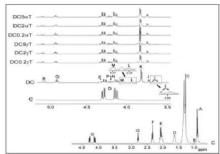


Figure 6. Region comprised H-NMR

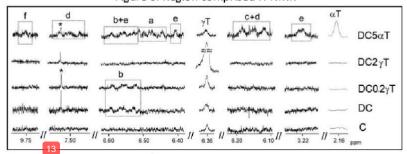


Figure 7. Some regions of the <sup>1</sup>H NMR spectra of oil C and of the lipids extrac

#### 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.

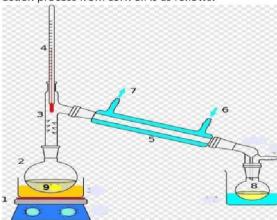


Figure 8. a number of corn oil fractionation tools

#### Noted of Figure 8:

- 1. Stove
- 2. Distilation Flask
- Precondensation Fraction
- 4. Thermometer
- Condenser
- 6. Colling Water In
- 7. Cooling Water Out
- 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 antiaging 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 ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  isomers) provent 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 carnels



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 U 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.

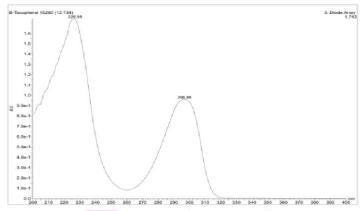


Figure 11. Spectrophotometry Profile

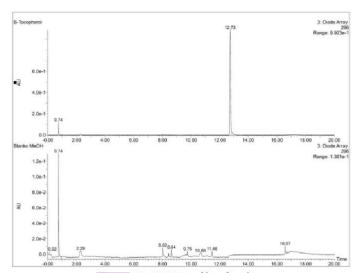


Figure 12. HPLC Profile of Isolate

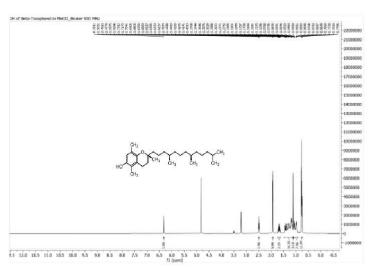


Figure 13. H-NMR Profile of Isolate

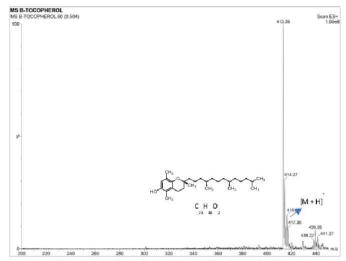


Figure 14. Mass Spectrogram Profile of Isolate

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  $C_{28}H_{48}O_2$ . Figure 14 formula  $C_{28}H_{48}O_2$ , and it's revealed the characteristic fragment m/z 417.36 that is specific to  $\beta$ -tocopherol.

#### Acknowledgements

We are so thankfull 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  $\beta$ -tocopherol and they collaborate in providing laboratory equipment during this research (in kind).

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