

Liquid Gold: Quantification of Vitamin E in Mustard Seed Oil

Or liquide: quantification de la vitamine E dans l'huile de graine de moutarde

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Abstract | Résumé

In addition to its popularity in the culinary world, the mustard plant, a member of the *Brassicaceae* family, has a variety of medicinal applications. The plant is well known for its potent antioxidant and anti-inflammatory properties, often derived from its golden seed oil. Existing studies acknowledge the presence of vitamin E in mustard oil; however, extensive research has not been done to quantify vitamin E in mustard seed oil. This study aimed to examine the vitamin E content in yellow (*Brassica alba*) and black (*Brassica nigra*) mustard seed to determine the best source. Analysis and quantification, carried out by gas chromatography-mass spectrometry (GC-MS), revealed that a sample of black mustard oil contained a total vitamin E content of 8.0 $\mu\text{L}/\text{mL}$; 6.8 μL of γ -tocopherol and 1.2 μL of α -tocopherol. Alternatively, yellow mustard oil contained 0.43 $\mu\text{L}/\text{mL}$ in only the g -tocopherol form. These results suggest that black mustard seed oil is a better source of vitamin E, as it contains an overall higher concentration and various forms of tocopherol, in contrast to the singular form found in yellow mustard at a lower concentration.

En plus de sa popularité dans le monde culinaire, la plante de moutarde, membre de la famille des Brassicacées, a une variété d'applications médicales. Cette plante est bien connue pour ses puissantes propriétés antioxydantes et anti-inflammatoires, souvent issues de l'huile dorée de ses graines. Les études existantes reconnaissent la présence de la vitamine E dans l'huile de moutarde, cependant, des recherches approfondies n'ont pas encore été effectuées pour quantifier la vitamine E dans l'huile de graine de moutarde. Cette étude visait à examiner la quantité de vitamine E dans la graine de moutarde jaune (*Brassica alba*) et noire (*Brassica nigra*) pour en déterminer la meilleure source. L'analyse et la quantification, réalisées par la chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG-SM), ont révélé que le contenu en vitamine E d'un échantillon d'huile de graine de moutarde noire était de 8.0 $\mu\text{L}/\text{mL}$; 6.8 μL étant du γ -tocophérol et 1.2 μL étant du α -tocophérol. D'une autre part, l'huile de graine de moutarde jaune contenait 0,43 $\mu\text{L}/\text{mL}$ uniquement sous forme de g -tocophérol. Ces résultats suggèrent que l'huile de graine de moutarde noire est une meilleure source de vitamine E puisqu'elle possède une concentration globalement plus élevée et des formes variées de tocophérol, en contraste avec l'huile de graine de moutarde jaune qui en possédait seulement une forme et ce, à une concentration plus faible.

Keywords: mustard seed oil; vitamin E; tocopherols; *Brassica nigra*; *Brassica alba*; GC-MS; antioxidant compounds; γ -tocopherol; α -tocopherol; phytochemical analysis

Introduction

The mustard plant, a member of the *Brassicaceae* family, is cultivated worldwide for its oil-rich seeds. Human use of mustard dates to 3000 BCE, with ancient civilizations such as those in India, Greece, and Rome using it as both a spice and a medicine (1). Modern research has confirmed that the golden seed oil has potent antioxidant and anti-inflammatory effects stemming from metabolic compounds found within it. Various phenolic compounds have been linked to antioxidant benefits while anti-inflammatory properties are attributed to glucosinolates, sulfur-containing secondary metabolites responsible for mustards distinctive bitter taste and pungent smell (2). When hydrolyzed by the enzyme myrosinase, glucosinolates are converted into

isothiocyanates, their biologically active form (3). This transformation is triggered by damage to the plant tissues, which occurs when seeds are crushed during the oil extraction process (4). As a result, mustard oil is enriched with isothiocyanates. Different mustard species can offer slightly different degrees of therapeutic benefits due to their varying chemical compositions (1).

Yellow mustard (*Brassica alba*) is a species native to the Mediterranean (Figure 1). Its predominant glucosinolate is sinalbin, which is converted to 4-hydroxybenzyl isothiocyanate. Its phenolic compounds include sinapine, sinapic acid, and vitamin E (3,5). Yellow mustard seeds are especially acknowledged for their high content of omega-3 fatty acids such as alpha-linolenic acid (2).



Figure 1. Seeds of black mustard (left) (*Brassica nigra*) and yellow mustard (right) (*Brassica alba*). Original image created by Veganbaking.net, licensed under CC-BY-SA-2.0 and cropped.

Black mustard (*Brassica nigra*) is native to regions of Africa, Asia, and Europe (Figure 1). These seeds contain a high level of anthocyanins, water-soluble pigments that give them their characteristic dark color. Like yellow mustard, black mustard is rich in sinapine, sinapic acid, and vitamin E, but also contains p-coumaric acid (3,5). The predominant glucosinolate in black mustard is sinigrin, which is converted to allyl-isothiocyanate (2). Studies suggest that sinigrin is the glucosinolate most strongly associated with anti-inflammatory response, making black mustard especially valuable for medicinal applications related to treating inflammation (6). With their higher oil content, black mustard seeds also offer a stronger flavor profile.

Despite the many beneficial components of these seed oils, mustard is also recognized for a less highly praised counterpart: a monounsaturated fatty acid known as erucic acid. Studies conducted in animal models have suggested that prolonged consumption of this fatty acid may result in the development of myocardial lipidosis, a condition involving abnormal accumulation of fats in the heart muscle (7). Erucic acid can potentially make up 50% of mustard oil's fatty acid content (8). Given this high percentage, North American regulatory bodies such as the Food and Drug Administration and Health Canada have issued warnings and placed restrictions on its use in cooking to prevent negative impacts on cardiac health. Regardless of its culinary limitations, mustard seed oil remains widely available for external use and is instead commonly incorporated into skincare routines as a result. In the cosmetic industry, many brands have begun incorporating vitamin E into products to obtain benefits mirroring those provided by mustard seed oil. This potent and well-studied fat-soluble antioxidant has been used in dermatology for over 50 years (9). Vitamin E's mechanism of action involves quenching reactive oxygen species, reducing oxidative stress (10). The human body cannot produce vitamin E on its own, and as a result, it must be obtained from an external source, such as food, or in the case of cosmetics, applied directly on the skin.

It is important to note that vitamin E exists in eight chemical forms referred to as tocopherols and tocotrienols (11). These molecules are differentiated based on 1) the arrangement and number of

methyl groups on the main chromanol ring and 2) the saturation of the carbon side chain (Figure 2).

Although all forms are proven to be antioxidants, they have varying degrees of biological activity and potency due to their slight structural differences. While gamma (γ) and delta (δ) forms are more potent than antioxidants *in vitro* (as they do not have as many methyl groups blocking the OH (hydroxyl group) on the main ring), they are less bioavailable in the human body than alpha (α) tocopherol (12-16). Thus, the α form is considered the most potent and is usually used in supplements and skincare products. Previous studies have confirmed the natural presence of vitamin E in its tocopherol forms in both yellow and black mustard oil (1, 4-5, 9). These tocopherols are recognized as the main contributors to the antioxidant effects provided by the oils. Mustard plants produce Vitamin E as a protective mechanism against UV and oxidative stress induced by the environment (4).

With mustard seed oil being restricted to topical applications in Canada, it is important to further assess its effectiveness in delivering benefits through this route of administration. Although the presence of vitamin E in mustard oil has been confirmed, extensive research has not been done specifically on the tocopherol content of the seed oil from various species. This study aims to examine and quantify vitamin E tocopherols in different species of mustard seed and determine the best source.

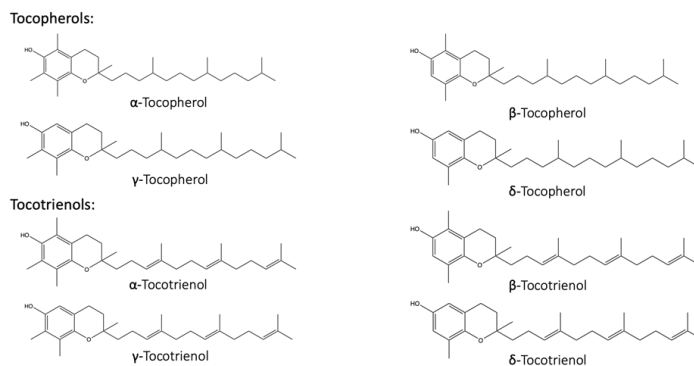


Figure 2. Eight different structural isomers of vitamin E

Methods

Yellow and black mustard seeds from the brand Suraj were obtained from Real Canadian Superstore. Approximately 50 grams of yellow and black mustard seeds were separately weighed out and crushed using a hand-operated seed oil press. Before operation, the hand press was preheated to approximately 40°C with a heat gun for 10 minutes. 5 mL of oil from each species was kept for testing. 10 μ L of black mustard seed oil was then diluted to 1 mL in ethyl acetate solvent and filtered using a chromatography syringe filter. The same was done for 70 μ L of yellow mustard seed oil.

1 μL of each of the diluted oil samples was injected into the gas chromatography-mass spectrometry (GC-MS) for analysis. The GC used was the Agilent 6890 with RXI-5SIL MS column from ResTech (30 m in length, 0.25 mm internal diameter, 0.25 micrometer coating - DB-5-MS 25 column) with a Mass Spec 5975C detector and 7683B Series injector autosampler. Helium gas was used as the mobile phase, and a (5%-phenyl)-methylpolysiloxane crosslinked column was used as the stationary phase. 1 μL of vitamin E oil standard containing mixed tocopherols (New Directions Aromatics) was diluted in 1 mL ethyl acetate and 1 μL of this solution was injected into the GC-MS for analysis. Reference mass spectra for the four tocopherol types were obtained from this standard for the four different tocopherol types. An external calibration curve was constructed using 100% pure vitamin E oil from the brand Cliganic as a standard (Figure 3). Compounds were identified using the National Institute of Standards and Technology's mass spectral library.

Results and Discussion

Mixed Tocopherols in Vitamin E Oil Standard

Naturally sourced vitamin E oil contains the four types of tocopherols. As tocopherols all have the same structural backbone, the number of methyl groups on the rings serves as their differentiating factor. The varying numbers of these side chains give each tocopherol a slightly different retention time on the gas chromatogram (Figure 4) and a characteristic mass spectrum (Figure 5).

Yellow Mustard Oil

The chromatogram and subsequent compound identifications can be found in Figure 6 and Table 1. The chromatogram identified peaks 1–3 as fatty acids (Table 1). A single γ -tocopherol peak appeared at a retention time of 12.81 minutes. Peaks 4 and 5 were identified as long-chain non-polar metabolites, and peaks 6–8 were identified as sterols (Table 1). The calibration curve yielded a vitamin E concentration of 0.03 $\mu\text{L}/\text{mL}$ for the injected yellow mustard sample. These values correspond to a vitamin E concentration of 0.43 μL per mL of pure yellow mustard oil. This concentration is attributed completely to γ -tocopherol.

Black Mustard Oil

The chromatogram and subsequent compound identifications can be found in Figure 7 and Table 2. The chromatogram revealed two forms of vitamin E in the black mustard sample: a peak at 9.48 minutes corresponding to γ -tocopherol and one at 9.88 minutes matched with α -tocopherol. Peaks 1–3 correspond to sterols (Table 2).

The black mustard oil exhibited no detectable fatty acids. While the literature identifies yellow mustard as being more abundant in fatty acids compared to black mustard, black mustard is still confirmed to contain several fatty acids. Their lack of appearance on the chromatogram indicates that they may not have been completely removed from the cell membrane of the seed shells during extraction and mixed with the oil, or they exist in concentrations that are too low for the GC to detect. Some of these compounds may need to be derivatized to be properly vaporized and visualized by a GC, especially at lower concentrations. The calibration curve yielded a total tocopherol concentration of 0.08 $\mu\text{L}/\text{mL}$ for the injected black mustard sample; 0.068 $\mu\text{L}/\text{mL}$ belonging to γ -tocopherol and 0.012 $\mu\text{L}/\text{mL}$ belonging to α -tocopherol. These values correspond to a total vitamin E concentration of 8.0 μL per mL of pure black mustard oil perol and 1.2 μL of α -tocopherol.

Conclusions

This study successfully utilized GC-MS to analyze and quantify various vitamin E tocopherols in two distinct species of mustard. Results revealed measurable concentrations of γ -tocopherol in both species, as well as α -tocopherol in black mustard alone. Noticeable variations in concentrations between the seed species indicated the possible influence of genetic and environmental factors on tocopherol content. Black mustard's higher overall

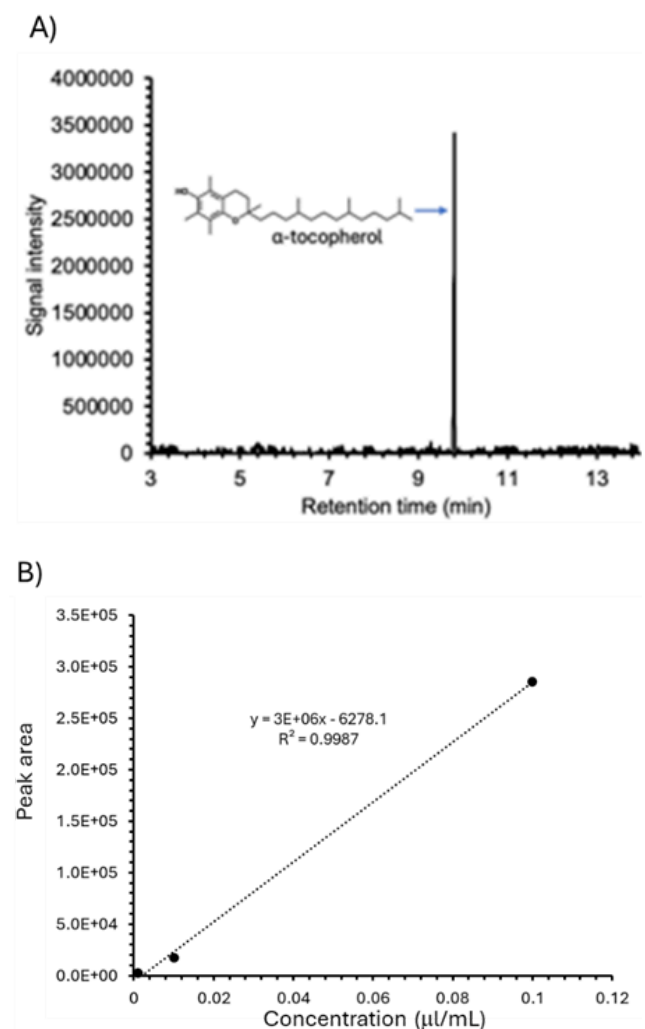


Figure 3. A) Gas chromatogram of pure α -tocopherol standard used for calibration curve (1 $\mu\text{L}/\text{mL}$) and B) Calibration curve generated using pure α -tocopherol standard.

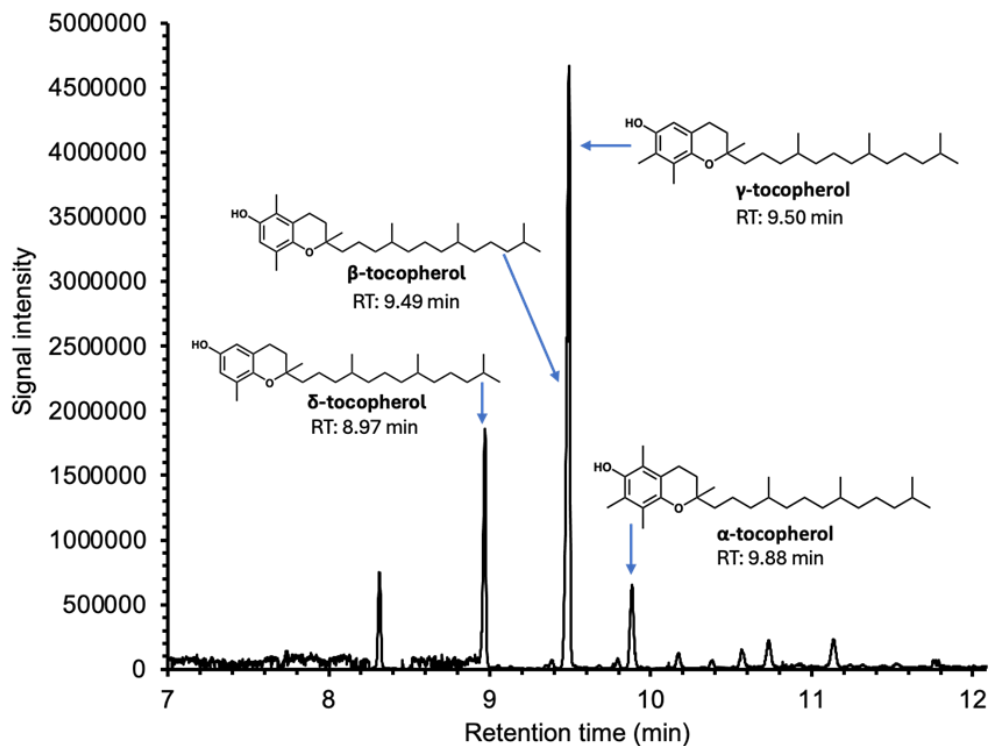


Figure 4. Gas chromatogram of vitamin E oil standards dissolved in ethyl acetate (1 μL/mL) containing all four forms of tocopherol.

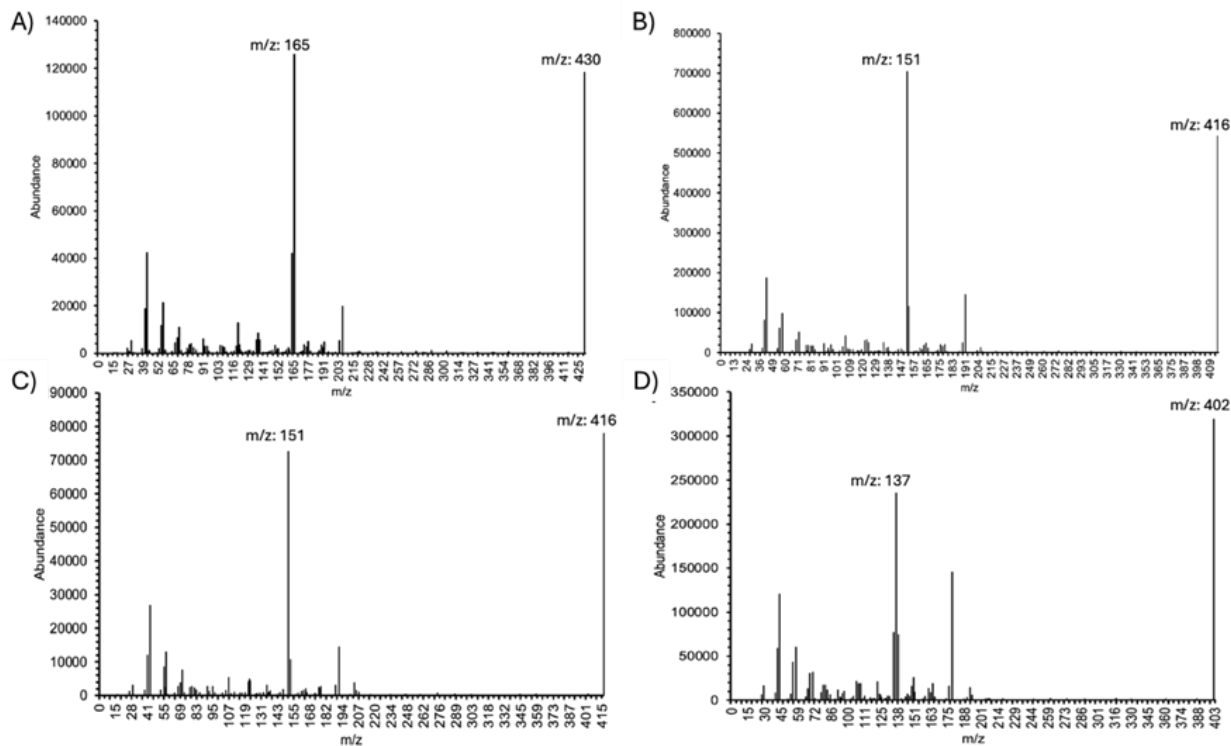


Figure 5. Characteristic mass spectra of A) α-tocopherol B) β-tocopherol C) γ-tocopherol and D) δ-tocopherol.

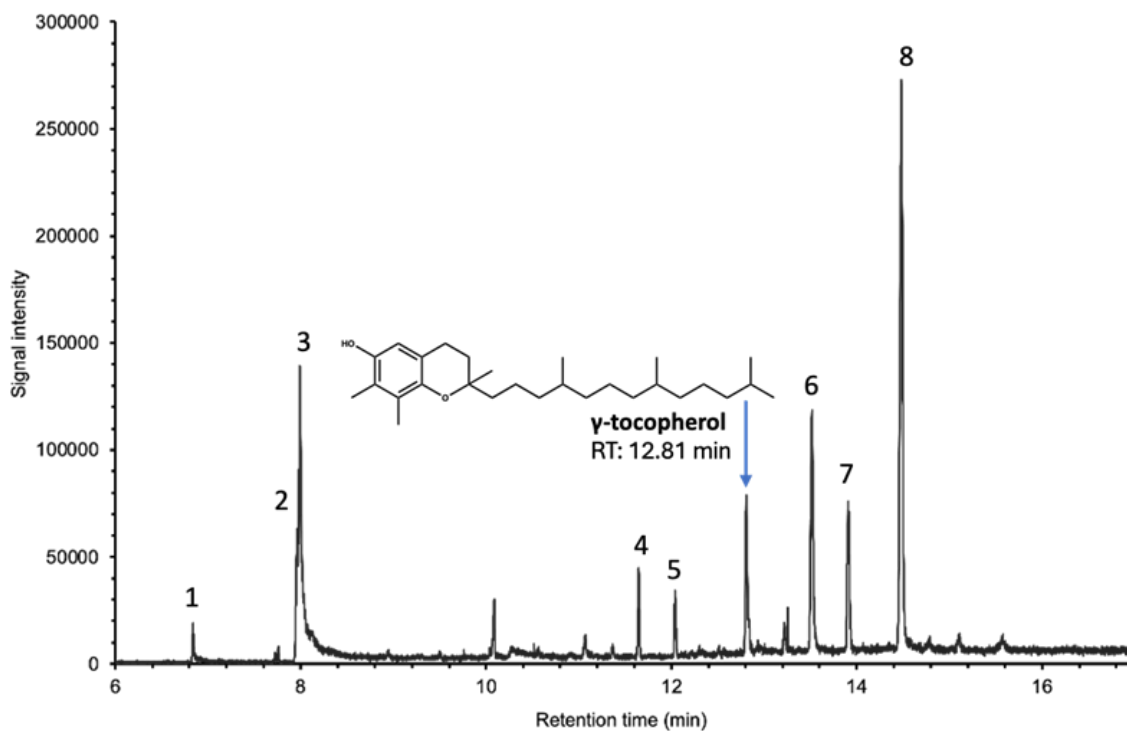
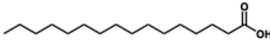

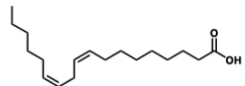
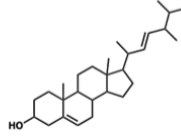
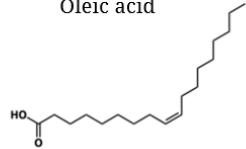
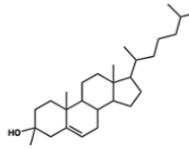
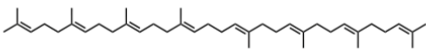
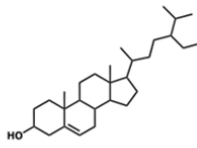


Figure 6. Gas chromatogram of yellow mustard seed oil in ethyl acetate solvent (70 µl/mL).

Table 1. Peak characterization of yellow mustard oil sample

Peak	Characterization	Peak	Characterization
1	Palmitic acid 	5	Tricosane 
2	Linoleic acid 	6	Epibrassicasterol 
3	Oleic acid 	7	23-R-Methylcholesterol 
4	Lycopersene 	8	Clionasterol 

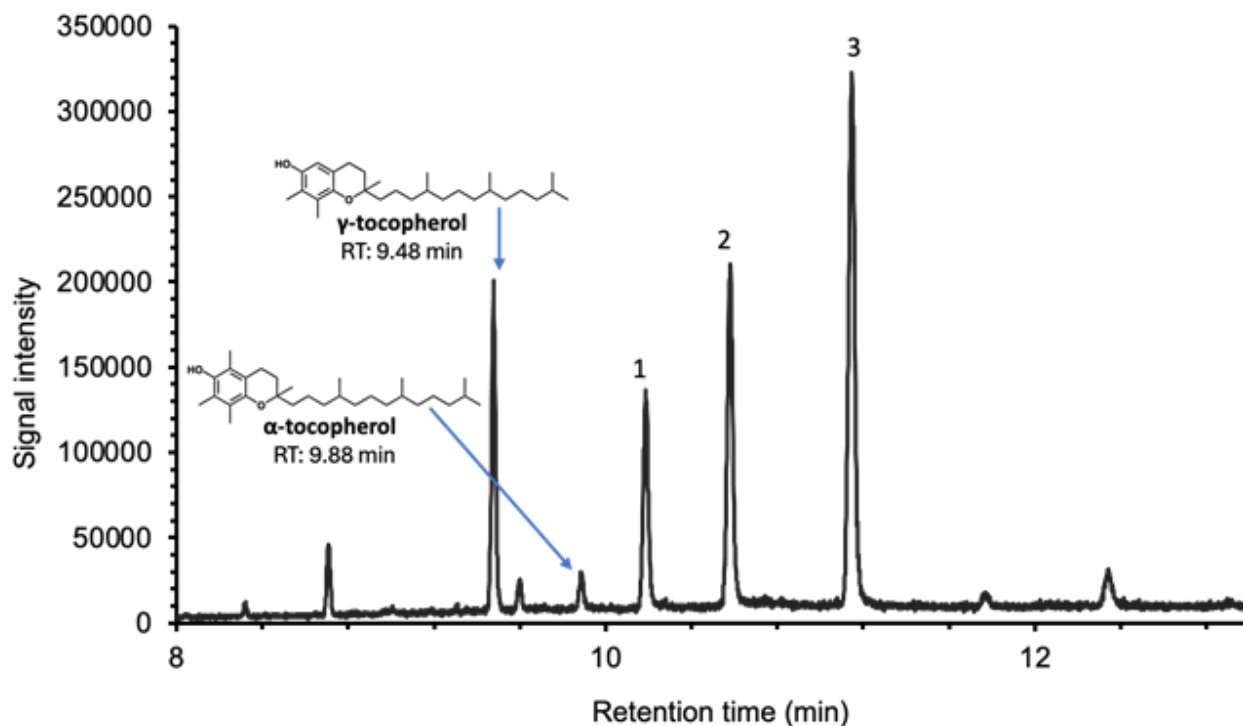
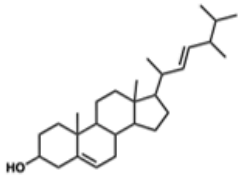
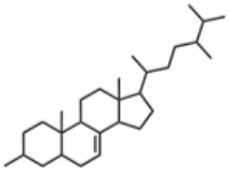
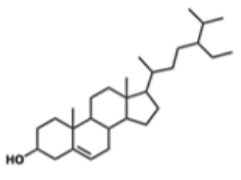


Figure 7. Gas chromatogram of black mustard seed oil in ethyl acetate solvent.

Table 2. Peak characterization of black mustard oil sample

Peak	Characterization
1	Epibrassicasterol 
2	Ergost-7-en-3-ol 
3	Clionasterol 

vitamin E levels indicate it as the better source of antioxidants. The presence of the more biologically active α -tocopherol in black mustard also highlights it as a candidate of interest for future investigations involving topical benefits. This study contributes to a better understanding of mustard oil composition and tocopherol concentrations found in these exact species. As research in this area is limited, further studies are essential for validating reproducibility and optimizing results. Future investments in similar studies could help determine more cost-effective and viable natural sources of beneficial compounds such as vitamin E, unlocking new opportunities for both cosmetic and medicinal applications.

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