

1. Introduction

1.1 Background

In recent years, the valorization of food industry by-products has become a central focus in the pursuit of more sustainable and circular food systems. Valorization refers to the process of transforming industrial by-products and food waste streams into value-added products directly supporting global sustainability objectives, particularly those aimed at minimizing waste and maximizing resource efficiency across the food production chain, in agree with the United Nations 2030 Sustainable Development Goals, especially SDG 12 (Responsible Consumption and Production) and SDG 2 (Zero Hunger) (Abdel-Aal, 2024; Boukid et al., 2018; United Nations, 2015). By repurposing by-products instead of discarding them, industries can both reduce their environmental footprint and generate additional economic value. One promising strategy within this context is the transformation of food by-products into edible materials, which not only preserves valuable resources but also contributes to food security (Abdel-Aal, 2024; Rodríguez et al., 2021). As a result, industries and researchers are increasingly seeking innovative ways to convert underutilized by-products into high-value resources. Among the various by-products of cereal processing, wheat germ (WG) stands out due to its rich nutritional profile and largely untapped potential for human consumption (Boukid et al., 2018; Khosroshahi & Razavi, 2023; Erim Kose, 2022; Mahmoud et al., 2015).

WG constitutes approximately 2.5–3.8% of the wheat kernel, is a by-product of the milling industry, typically separated along with bran (Brandolini & Hidalgo, 2012; Yu et al., 2015). Structurally, WG includes the embryo and the scutellum, the latter functioning both as a storage organ and as the attachment point between the pericarp and the embryo, though most of the scutellum is removed during milling, leaving primarily the embryo (Rizzello et al., 2010). Despite accounting for only 0.2–0.5% of the kernel's industrial output, WG is the most nutrient-rich component of the wheat grain yet remains underutilized in human food systems (Zhu et al., 2006; Khosroshahi & Razavi, 2023; Mahmoud et al., 2015). WG is densely packed with bioactive and health-promoting constituents, including proteins (26–35%), lipids (10–15%), dietary fiber, minerals, and a wide range of phytochemicals such as tocopherols, phytosterols, policosanols, carotenoids, phenolics, and B-complex vitamins (Brandolini & Hidalgo, 2012; Niu et al., 2013; Sun et al., 2015; Zhu et al., 2015). These compositional traits contribute to its antioxidant, hypocholesterolemic, antihyperlipidemic, and anticancer potential (Ghafoor et al., 2017; Mueller & Voigt, 201; Bartolini et al., 2022).

However, its high enzymatic activity and lipid content make WG highly unstable after milling. Lipase and lipoxygenase catalyze the hydrolysis and oxidation of unsaturated fatty acids, resulting in rancidity, off-flavors, and a sharp decline in nutritional value and shelf-life (Arslan et al., 2020; Capitani et al., 2011; Liu et al., 2024). Additionally, the inclusion of WG in flour can impair dough stability and technological quality, prompting milling industries to minimize its presence to meet

bakery standards (Marti et al., 2014; Zhu et al., 2006). The instability of WG poses significant limitations for its application in food systems. Once separated from the endosperm, exposure to air and mechanical stress accelerates oxidation, restricting WG's shelf-life to only a few days (Li et al., 2016; Geng et al., 2015; Sjövall et al., 2000; Xiong et al., 2025). As a result, the majority of WG produced, estimated globally at 25 million tons annually, is used for animal feed or industrial non-food uses (FAOSTAT, 2008; Rizzello et al., 2010). Nevertheless, increasing interest in sustainable processing has led to renewed focus on the valorization of milling by-products like WG. Recent strategies aim to transform WG from an unstable waste to a health-promoting, cost-effective ingredient suitable for human consumption (Boukid et al., 2018).

Stabilization methods, such as thermal treatments, extrusion, microwave processing, oil removal (Matucci et al., 2003; Paradiso et al., 2008), and the application of antioxidants (Paradiso et al., 2008; Arslan et al., 2020; Khosroshahi & Razavi, 2023; Erim Kose, 2022) have been explored to prevent enzymatic degradation. However, these interventions can be costly, reduce nutritional quality, or raise concerns over the safety of synthetic additives (Paradiso et al., 2008). Moreover, consumer preference is shifting towards preservative-free, mildly processed foods with enhanced shelf life and maintained nutritional integrity (Rizzello et al., 2010). Beyond its raw form, WG has also gained relevance through its derivatives. Wheat germ oil is now incorporated into cosmetic, pharmaceutical, and even fertility-enhancing products (Gorusupudi & Baskaran, 2013; Niu et al., 2013; Boukid et al., 2018; Ghafoor et al., 2017). Defatted wheat germ (DWG), a by-product of oil extraction, retains approximately 30% protein (Sun et al., 2015; Mahmoud et al., 2015). Its protein quality is comparable to FAO/WHO standards and rivals that of egg and milk proteins (Ge et al., 2002; Arshad et al., 2007; Rizzello et al., 2010), suggesting that both DWG and its protein isolates (DWGP) may serve as sustainable and functional ingredients in human diets (Boukid et al., 2018; Khosroshahi & Razavi, 2023).

The growing focus on the utilization of WG aligns with broader efforts to reduce agri-food waste and develop nutritionally enriched, sustainable food systems (Abdel-Aal, 2024; Boukid et al., 2018; Socas-Rodríguez et al., 2021). Current research underscores the need for effective, scalable stabilization and separation techniques that optimize recovery, preserve WG's bioactive potential, and simultaneously meet safety and sensory standards for human consumption (Boukid et al., 2018; Khosroshahi & Razavi, 2023; Erim Kose, 2022; Liu et al., 2024; Rizzello et al., 2010; Socas-Rodríguez et al., 2021). Additionally, value-added products such as functional foods and nutraceuticals derived from WG and its oil offer promising avenues for market development and public health promotion (Abdel-Aal, 2024; Ghafoor et al., 2017; Paško et al., 2023; Siraj, 2022; Zargar et al., 2023).

1.2 Milling process

Since WG is removed as a by-product during flour production, understanding the milling process is crucial for exploring its valorization potential. The primary objective of wheat milling is to

extract the starchy endosperm and convert it into fine flour, while removing the bran and germ fractions (Abdel-Aal, 2024; Boukid et al., 2018; Rizzello et al., 2010). The endosperm, which comprises approximately 81–84% of the wheat kernel, is the main component targeted for human consumption, whereas the bran (14–16%) and wheat germ (2–3%) are separated as by-products (Abdel-Aal, 2024; Boukid et al., 2018; Brandolini & Hidalgo, 2012; Liaqat et al., 2021). While the bran is more readily separated due to its peripheral position, the germ poses greater challenges. Wheat germ (WG), located at the base of the kernel, is structurally distinct but partially embedded within the endosperm, making its removal technically complex (Boukid et al., 2018; Rizzello et al., 2010; Zhang et al., 2015). To avoid excessive damage to the cell structure during germ separation, milling machinery must be precisely calibrated across multiple stages. The separation of WG is not achieved in a single step but rather through a gradual, multi-phase process (Boukid et al., 2018). This typically involves multiple grinding and reduction stages, where the action of the machinery must be carefully adjusted (Boukid et al., 2018; Fistes & Rakić, 2015). Over time, milling flow diagrams have undergone significant evolution, adapting to changes in wheat varieties, technological advancements, and market demands for both high-yield flour and functional by-products such as wheat germ (Boukid et al., 2018).

Two general approaches are used for separating WG during the milling process: direct and gradual methods (Boukid et al., 2018). The direct method, known as degermination, involves pre-conditioning the grain by increasing its moisture content (typically 19–25%) to enhance flexibility and reduce breakage (Boukid et al., 2018; Rizzello et al., 2010). The moistened kernels are then gently crushed from the edges to physically release the germ with minimal grinding, leaving the endosperm intact for subsequent processing into flour (Boukid et al., 2018). This method reduces the number of purification steps (Boukid et al., 2018) and allows for the isolation of whole, relatively undamaged germ (Rizzello et al., 2010). Despite its efficiency, degermination is more commonly employed in corn milling, where the germ is economically valuable for oil extraction (Abdel-Aal, 2024; Boukid et al., 2018). In contrast, its application in wheat milling is limited due to the smaller proportion of WG in the grain and the dominance of flour yield optimization in the industry (Boukid et al., 2018; Siraj, 2022; Zou et al., 2018). Nevertheless, as awareness of WG's nutritional and functional value grows, there is increasing interest in optimizing degermination techniques for wheat to allow for more sustainable and value-added utilization of this by-product (Boukid et al., 2018; Khosroshahi & Razavi, 2023; Rizzello et al., 2010).

The gradual separation approach, which remains the standard in wheat milling, involves multiple grinding and sifting stages designed to carefully isolate the germ without contaminating the flour stream or causing excessive mechanical stress. Given the nutrient richness of wheat germ comprising proteins, unsaturated fats, vitamins, and bioactive compounds, its recovery through efficient and non-destructive methods represents both a technological challenge and a nutritional opportunity (Ghafoor et al., 2017; Boukid et al., 2018). Modern milling operations continue to refine these methods, balancing flour purity and yield with the recovery of valuable co-products (Abdel-Aal et al., 2024). Further research and investment into precision milling technologies may

help integrate WG valorization to improve component separation efficiency and value addition, thereby aligning food production with sustainability and nutrition goals (Abdel-Aal et al; 2024).

1.3 Composition and Nutritional Value of Wheat Germ

Understanding the detailed composition of wheat germ helps explain why its valorization is increasingly emphasized in sustainable food systems. Wheat germ is the most nutritious part of the wheat kernel, offering a dense profile of essential macronutrients, micronutrients, and bioactive compounds (Nutrition Data, 2014).

1.4 Macronutrient Composition

Raw wheat germ provides energy around 381 kcal per 100 g (379–381 kcal/100 g), with carbohydrates (51.3 g) as the main component, followed by proteins (28.1 g) and total fats (9.4–9.7 g) (Danish Food Composition Databank; 2009; Boukid et al; 2018; Mahmoud et al; 2015). Dietary fiber accounts for 12.3 g (Khosroshahi & Razavi; 2023; Boukid et al; 2018) and is mostly insoluble (Marti et al; 2014; Srivastava et al; 2006; Sudha et al; 2007). The protein profile is notable for its quality (Rizzello et al; 2010), consisting mainly of albumins, globulins, glutenins, and prolamines (Zhu et al; 2006; Boukid et al; 2018). It contains high levels of essential amino acids, such as lysine, arginine, and glycine (Liu et al; 2017; Zhu et al; 2006), and exhibits good amino acid balance, comparable to egg and milk proteins (Rizzello et al; 2010; FAO; 1995; Zhu et al; 2006). Wheat germ fat content includes healthy unsaturated fatty acids: oleic, linoleic, and α -linolenic acids (Ghafoor et al; 2017; Mahmoud et al; 2015; Paško et al; 2025; Sjövall et al; 2000). These essential fatty acids and high tocopherol content make wheat germ oil (WGO) valuable for dietary and supplemental use (Ghafoor et al; 2017; Siraj; 2022; Giménez et al; 2013).

1.5 Micronutrients and Antioxidants

Wheat germ is exceptionally rich in minerals, with total mineral content (ash) measuring approximately 4.4 g/100 g (Boukid et al., 2018). Specific mineral concentrations per of raw WG, based on the Danish Food Composition Databank, notably include phosphorus (1000 mg), potassium (1046 mg), magnesium (259 mg), zinc (17.8 mg), and iron (5.00 mg) (Khosroshahi & Razavi, 2023; Boukid et al, 2018). Among vitamins, WG is considered an exceptional source of vitamin E (Ghafoor et al, 2017; Bartolini et al, 2022; Siraj, 2022). It is renowned as the richest known source (plant origin) of vitamin E (Boukid et al, 2018; Khosroshahi & Razavi, 2023), with α -tocopherol concentration listed 11.0 mg/100 g and being 16 times higher than in any other wheat fraction (Khosroshahi & Razavi, 2023; Boukid et al., 2018; Wang et al., 2001). WG also provides important amounts of B vitamins, including thiamine, riboflavin, Vitamin B6, and folic acid (Arslan et al., 2020; Rizzello et al., 2010; Eisenmenger et al., 2007)

Furthermore, WG contains several other bioactive compounds that contribute to its antioxidant potential (Khosroshahi & Razavi, 2023; Ghafoor et al., 2017). These include phytosterols (such as β -sitosterol and campesterol), polycosanols (like octacosanol), carotenoids, flavonoids, and glutathione (Boukid et al., 2018; Liu et al., 2024; Zhu et al., 2006). These compounds support cellular protection, and the associated anti-inflammatory properties of these bioactive lipids and phenolics are key to WG's benefits for overall health and cardiovascular protection (Bartolini et al., 2022; Paško et al., 2025).

1.6 Functional and Nutraceutical Use

Due to its nutrient richness and functional properties, wheat germ (WG) is considered a promising ingredient in functional foods and nutraceuticals. WG is particularly valued as a highly attractive, plant-based protein source containing natural anti-cancer and anti-inflammatory properties, reinforcing its role as a key nutraceutical ingredient (Ge et al., 2000; Reddy et al., 2000; Zalatnai et al., 2001).

1.7 Extraction Methods for Wheat Germ Oil (WGO)

Building on the nutritional richness of wheat germ, one of the most effective strategies for its industrial valorization is oil extraction, and the choice of extraction method plays a crucial role in determining the yield, purity, oxidative stability, and overall nutritional quality of the final product (Boukid et al., 2018; Ghafoor et al., 2017). The subsequent choice of extraction method plays a crucial role in determining the yield, purity, oxidative stability, and overall nutritional quality of the final product (Liu et al., 2024; Siraj, 2022; Ghafoor et al., 2017). Different techniques could be employed:

- Cold pressing is a mechanical, solvent-free extraction method performed at temperatures typically below 50 °C. This technique is considered environmentally friendly and is widely adopted for food and cosmetic applications due to its ability to retain natural aroma, color, and sensitive bioactive such as tocopherols and polyunsaturated fatty acids (Karne, 2022; Karne et al., 2025; Liu et al., 2024; Siraj, 2022). Despite lower extraction yields compared to solvent-based techniques, the resulting oil is of high sensory and nutritional quality (Aslan et al., 2021; Boukid et al., 2018; Karne et al., 2025).
- Solvent extraction, predominantly employing non-polar solvents such as *n*-hexane, remains the principal industrial technique for WGO recovery, achieving yields of up to 99% (Ghafoor et al., 2017; Megahad et al., 2002; Plensar et al., 2016). The procedure necessitates subsequent evaporation and refining steps to eliminate residual solvents (Liu et al., 2024; Ghafoor et al., 2017). Despite its efficiency, the use of *n*-hexane raises significant safety and environmental concerns, due to potential solvent residues in the final product and its classification as a hazardous air pollutant (Dunford et al., 2003; Siraj, 2022;

Boukid et al., 2018). Refining processes, including degumming, neutralization, and deodorization, are required to enhance oil quality but may result in the depletion of bioactive compounds, particularly tocopherols, with high-temperature deodorization exacerbating such losses (Ghafoor et al., 2017; Kraljić et al., 2015; Wang et al., 2001).

- Supercritical fluid extraction with carbon dioxide (SC-CO₂) constitutes an environmentally friendly and highly selective approach for WGO recovery. Operating at moderate temperatures (31–80 °C) and elevated pressures (typically >100 bar), SC-CO₂ enables efficient extraction of thermolabile bioactives, including tocopherols, sterols, and carotenoids, without employing toxic solvents (Piras et al., 2009; Parczewska-Plesnar et al., 2016). The use of co-solvents such as ethanol can further enhance the solubility of polar constituents and improve extraction efficiency. Comparative investigations have indicated that SC-CO₂ yields oils exhibiting superior oxidative stability and greater retention of functional compounds relative to cold-pressing methods (Özcan et al., 2013; Bartolini et al., 2022).

Emerging and hybrid techniques are being actively explored to enhance oil recovery while preserving quality (Liu et al., 2024; Ghafoor et al., 2017; Siraj, 2022). These novel technologies include pressurized solvent extraction (PSE), which significantly reduces solvent consumption and extraction time compared to traditional methods (Dunford et al., 2003; Siraj, 2022), ultrasound-assisted extraction (Siraj, 2022; Abdel-Aal et al., 2024), and aqueous enzymatic extraction (AEE) (Siraj, 2022; Xie et al., 2011). These innovative methods, alongside techniques, can be integrated with traditional techniques to enhance selectivity, improve performance efficiency, and reduce overall resource consumption (Abdel-Aal et al., 2024; Liu et al., 2024; Dunford et al., 2003).

The extraction of WGO is a key step in its industrial valorization (Ghafoor et al., 2017; Boukid et al., 2018). Cold pressing and supercritical CO₂ (SC-CO₂) extraction are preferred when preserving oil quality and bioactive compounds is essential, as they operate without toxic solvents and under mild thermal conditions (Karne et al., 2025; Liu et al., 2024; Piras et al., 2009). In contrast, solvent extraction, typically with hexane, remains the dominant industrial method because it provides very high yields, reaching up to 99% (Ghafoor et al., 2017; Megahad et al., 2002). Current researches aim to optimize greener and hybrid extraction strategies to support a more sustainable grain-processing system and enhance the production of high-value functional ingredients (Abdel-Aal et al., 2024; Khosroshahi & Razavi., 2023; Boukid et al., 2018).

1.8 Refining of Wheat Germ Oil (WGO)

Conventional refining methods involve several stages aimed at eliminating undesirable components from crude oil. While these processes are effective in purification, they often lead to the loss of valuable bioactive compounds.

Degumming and Neutralization: these initial steps primarily target the removal of phospholipids and free fatty acids (FFAs). Neutralization, achieved through alkali application, is specifically the

step that eliminates phospholipids and FFAs from the oil (Wang et al., 2001; Ghafoor et al., 2017). FFAs are particularly problematic, as they contribute to undesirable bitter and soapy flavors in food (Ghafoor et al., 2017; Nayak et al., 2016) and also accelerate the oxidation of polyunsaturated fatty acids by catalyzing further oxidation through the solubilization and activation of metal catalysts (Nayak et al., 2016; El-Hadad et al., 2024; Erickson et al., 2022). FFA formation is typically attributed to hydrolysis reactions that break down triacylglycerols. This is caused by the lipase enzyme, which hydrolyzes lipids in the wheat germ, especially under elevated temperatures in the presence of moisture and air (Ghafoor et al., 2017; Megahed., 2011; Liu et al., 2024; Erickson et al., 2022). Consequently, crude WGO may exhibit a high FFA content if the germ is not promptly stabilized (Megahed, 2011; Arslan et al., 2020; Liu et al., 2024; Ghafoor et al., 2017). Alkali neutralization is effective in removing FFAs from WGO (Ghafoor et al., 2017; Wang et al., 2001). However, this process is commonly associated with considerable oil loss and a measurable reduction in tocopherol content (Ghafoor et al., 2017; Boukid et al., 2018; Siraj, 2022). Neutralization also plays a central role in phospholipid removal, as demonstrated by studies showing that phosphorus levels, used as an indicator of phospholipid decline during this step rather than during degumming (Wang et al., 2001; Ghafoor et al., 2017; Kraljić et al., 2015). Given that conventional refining, including neutralization, reduces tocopherols, milder approaches such as supercritical fluid fractionation are increasingly recommended to better preserve the nutritional integrity of the oil (Ghafoor et al., 2017; Siraj, 2022).

Bleaching targets the removal of pigments and other impurities (Ghafoor et al., 2017; Wang et al., 2001). The color of WGO is primarily attributed to carotenoids and other plant pigments, which are potent antioxidants naturally present in plant-based oils (Ghafoor et al., 2017; Paško et al., 2025). Regarding tocopherol stability, the total tocopherol content in WGO did not undergo considerable alteration during the bleaching process (Wang et al., 2001; Ghafoor et al., 2017). This finding contrasts with the subsequent deodorization stage, which can cause an appreciable decrease in tocopherol content, especially when performed at high temperatures or for long periods (Ghafoor et al., 2017; Wang et al., 2001).

Deodorization is regarded as the final refining step, and its primary objective is to eliminate volatile compounds that contribute to undesirable off-flavors and odors (Ghafoor et al., 2017; Liu et al., 2024; Wang et al., 2001). Although the process successfully reduces free FFA content, peroxide values, and the oil's red color (Wang et al., 2001; Ghafoor et al., 2017), it can negatively impact tocopherol levels when conducted at high temperatures and extended durations (Ghafoor et al., 2017; Wang et al., 2001). For instance, research indicates that deodorization performed at for 30 minutes caused a marked reduction in tocopherols, whereas a milder condition of for 9 minutes had a negligible effect (Wang et al., 2001).

However, these conventional refining processes can result in significant losses of nutritional components, particularly tocopherols (Ghafoor et al., 2017; Wang et al., 2001; Farhoosh et al., 2013), with high-temperature deodorization being especially detrimental (Ghafoor et al., 2017; Wang et al., 2001). To mitigate these losses and preserve the oil's quality, alternative refining

techniques have been proposed (Ghafoor et al., 2017; Siraj., 2022). These include supercritical fluid fractionation (SFF) and molecular distillation, which offer a gentler approach that better preserves tocopherols and other bioactive compounds (Ghafoor et al., 2017; Siraj, 2022; Shao et al, 2008). Such refining approaches directly influence the suitability of wheat germ oil (WGO) for various food and nutraceutical applications (Ghafoor et al., 2017; Siraj, 2022).

1.9 Composition, Properties, and Health Benefits of Wheat Germ Oil

Wheat germ oil (WGO) represents one of the most nutrient-dense vegetable oils, owing to its distinctive lipid and bioactive composition. Extracted from the germ fraction of the wheat kernel, it contains an exceptionally high concentration of polyunsaturated fatty acids (PUFAs), natural antioxidants, and phytochemicals that contribute both to its nutritional value and to its functional potential in food and health applications (Boukid et al., 2018; Siraj, 2022).

1.9.1 Fatty Acid Composition

The lipid fraction of WGO is dominated by unsaturated fatty acids (UFAs) (Siraj, 2022; Mahmoud et al., 2015), which typically account for about 80–85% of the total fatty acids (Ghafoor et al., 2017; Mahmoud et al., 2015; Paško et al., 2025; Zou et al., 2018). Linoleic acid (C18:2, ω -6) is the most abundant, constituting around 55–60% (Siraj, 2022; Ghafoor et al., 2017; Zou et al., 2018; Uday Karne et al., 2025), followed by oleic acid (C18:1, ω -9) at approximately 14–25% (Siraj, 2022; Ghafoor et al., 2017; Zou et al., 2018; Sngul Aslan et al., 2021). Saturated fatty acids (SFAs) such as palmitic acid (C16:0) and stearic acid (C18:0) are present in lower proportions (around 15–18%) (Ghafoor et al., 2017; Uday Karne et al., 2025; Sengul Aslan et al., 2021). This high PUFA content also increases the oil's sensitivity to oxidation and degradation (Siraj, 2022; Ghafoor et al., 2017; Aslan et al., 2021; Arslan et al., 2020), severely influencing its storage requirements and stability (Khosroshahi & Razavi, 2023; Boukid et al., 2018).

1.9,2 *Minor Bioactive Components*

Beyond its fatty acid profile, WGO is a rich natural source of tocopherols, phytosterols, carotenoids, phospholipids, and phenolic compounds, all of which contribute significantly to its antioxidant potential and physiological functionality. Wheat germ oil (WGO) contains exceptionally high levels of total tocopherols, frequently ranging up to 300 mg/100 g (Paško et al., 2025; Erim Kose, 2022; Zou et al., 2018; Bartolini et al., 2022). The oil's tocopherol profile is typically dominated by α -tocopherol, which often accounts for approximately 60% of the total content, followed by β -, γ -, and δ -tocopherols (Paško et al., 2025; Zou et al., 2018; Siraj, 2022). These compounds function as potent lipid-soluble antioxidants, interrupting radical chain reactions and thus protecting cell membranes from oxidative stress (Bartolini et al., 2022; Erickson et al., 2022; Paško et al., 2025). Tocopherols are also regarded as valuable quality markers, influencing both oxidative stability and nutritional labeling (Erim Kose, 2022; Siraj, 2022). Moreover, compounds such as β -sitosterol, campesterol, and stigmasterol, have demonstrated the ability to lower circulating cholesterol levels (El-Hadad et al., 2024; Feng et al., 2020; Ghafoor et al., 2017) and reduce low-density lipoprotein (LDL) cholesterol levels in the blood (Kmieciak et al., 2021). These minor components also exhibit valuable technological properties in the food industry, functioning as emulsifiers and emulsion stabilizers (El-Hadad et al., 2024), and contribute to the oil's oxidative stability (OS) (El-Hadad et al., 2024). Eventually, carotenoids and phenolic compounds add to the antioxidant defense system of WGO, providing protection against lipid peroxidation and offering additional health-promoting properties such as anti-inflammatory and anti-aging effects (Paško et al., 2025; Aslan et al., 2021; Ahmed et al., 2016).

1.9,3 *Physicochemical Properties*

The physicochemical characteristics of WGO are directly related to its composition (Boukid et al., 2018; Xiong et al., 2025). Typically, WGO exhibits a low melting point and a golden-yellow color due to the presence of carotenoids and xanthophyll pigments (Aslan et al., 2021; Boukid et al., 2018; Ghafoor et al., 2017). Its specific gravity and refractive index are comparable to other high-polyunsaturated fatty acid oils, whereas its iodine value (typically between 110–130 g I₂/100 g) reflects its high degree of unsaturation (Dunford & Zhang, 2003; Ghafoor et al., 2017; Mahmoud et al., 2015; Özcan et al., 2013). However, this chemical richness also makes WGO particularly prone to oxidative degradation when exposed to heat, light, or air, requiring careful handling and storage conditions (Arslan et al., 2020; Boukid et al., 2018; Ghafoor et al., 2017; Liu et al., 2024; Xiong et al., 2025). Specifically, its high content of PUFAs contributes to its sensitivity to oxidative degradation (Aslan et al., 2021). This degradation is caused by the high activity of lipase (LA) and lipoxygenase (LOX), leading to hydrolytic and oxidative rancidity (Arslan et al., 2020; Boukid et al., 2018; Erim Kose, 2022; Ghafoor et al., 2017).

1.9,4 *Health Benefits*

Due to its bioactive profile, WGO has been widely studied for its nutritional and therapeutic properties. The combination of essential fatty acids, vitamin E, and phytosterols exerts multiple beneficial effects on human health. Tocopherols and phenolics scavenge free radicals, reducing oxidative stress and inflammatory responses. These mechanisms are implicated in the prevention of chronic diseases such as cardiovascular disorders, neurodegeneration, and certain cancers (Aslan et al., 2021; Paško et al., 2025; Bartolini et al., 2022). The high content of linoleic and oleic acids contributes to improved lipid profiles by lowering LDL-cholesterol and increasing HDL-cholesterol levels, thereby reducing the risk of atherosclerosis (El-Hadad et al., 2024; Aslan et al., 2021). WGO supplementation has been associated with enhanced immune response, protection against cellular aging, and improved liver and kidney function due to its antioxidant defense capacity (Boukid et al., 2018; Ghafoor et al., 2017; Bartolini et al., 2022). When applied topically, WGO promotes skin regeneration, hydration, and elasticity. Its emollient nature and vitamin E content make it a common ingredient in anti-aging and restorative cosmetic formulations (Ghafoor et al., 2017; Karne et al., 2025).

1.10 *Mechanisms of Lipid Oxidation*

Lipid oxidation is a complex chain reaction that significantly affects the chemical stability, nutritional quality, and sensory attributes of edible oils (Erickson et al., 2022; Kmiecik et al., 2018). It generally proceeds through three main mechanisms, autooxidation, photo-oxidation, and enzymatic oxidation (lipooxygenase activity), all leading to the formation of primary oxidation products, primarily hydroperoxides, which subsequently decompose into secondary oxidation products, such as aldehydes, ketones, and alcohols, responsible for rancidity and off-flavors (Ahmed et al., 2016; Erickson et al., 2022; Xiong et al., 2025; Wąsowicz et al., 2004). The mechanism of autooxidation, which is the predominant pathway of lipid deterioration during storage, is described as a free radical chain mechanism consisting of three main steps: initiation, propagation, and termination (Erickson et al., 2023; Taghvaei & Jafari, 2015). This process is influenced by factors such as the presence of oxygen, heat (e.g., frying conditions), and catalytic metals (Erickson et al., 2022; Nayak et al., 2016; Taghvaei & Jafari, 2015). Initiation: The oxidation process begins with the initiation step, during which free radicals are generated from the lipid substrates. In unsaturated fats, this involves the radicalization of carbon-hydrogen bonds located next to allylic carbons. Specifically, a hydrogen atom is abstracted from a methylene group through a homolytic reaction, forming an alkyl free radical ($R\cdot$). This process is often catalyzed by external factors such as heat, light, or trace metal ions. (Taghvaei and Jafari 2015; Erickson et al., 2023). Propagation: following initiation, the oxidation reaction enters the propagation phase, a chain process that leads to the rapid formation of hydroperoxides. The alkyl radical generated during initiation reacts with molecular oxygen (O_2) to form a peroxy radical, as represented by the reaction $R\cdot + O_2 \rightarrow ROO\cdot$ (Erickson et al., 2022; Nayak et al., 2015; Taghvaei & Jafari, 2015; Wąsowicz et al., 2004). The peroxy radical then abstracts a hydrogen atom from another lipid molecule (RH), producing a hydroperoxide ($ROOH$) and regenerating a new alkyl radical ($R\cdot$),

thereby perpetuating the chain reaction ($\text{ROO}\cdot + \text{RH} \rightarrow \text{ROOH} + \text{R}\cdot$) (Erickson et al., 2022; Nayak et al., 2015; Taghvaei & Jafari, 2015). Hydroperoxides formed during this stage are the primary products of lipid oxidation; they are non-volatile and odorless but highly reactive, serving as key intermediates that drive further oxidative processes. (Erickson et al., 2022; Xiong et al., 2025; Wąsowicz et al., 2004). Termination: is the final stage of autooxidation where radicals combine to form stable, non-radical products. These termination reactions typically yield peroxide polymers and hydroperoxides (Erickson et al., 2023; Nayak et al., 2015; Wąsowicz et al., 2004). The rate of autooxidation increases under the influence of various external factors such as elevated temperature, the presence of oxygen, light, and catalytic metal ions (Erickson et al., 2022; Nayak et al., 2015). Furthermore, the susceptibility of lipids to oxidation is highly dependent on their structure, so PUFAs oxidize far more rapidly than monounsaturated or saturated ones (Erim Kose, 2022; Erickson et al., 2022; Liu et al., 2019). Specifically, linolenic acid oxidizes approximately 2,500 times faster than saturated stearic acid under similar conditions (Erim Kose, 2022; Liu et al., 2019; Nayak et al., 2015).

On the other hand, photooxidation, is triggered by light exposure (Taghvaei & Jafari, 2015; Wąsowicz et al., 2004). Photosensitizing compounds such as chlorophylls or riboflavin absorb visible or ultraviolet light and transfer energy to molecular oxygen, generating singlet oxygen ($^1\text{O}_2$) (Gordon, 2001; Wąsowicz et al., 2004). This reactive oxygen species directly attacks the double bond of unsaturated fatty acids, producing an allylic hydroperoxide (Wąsowicz et al., 2004). Therefore, protecting WGO from light is crucial, requiring careful handling and storage (Arslan et al., 2020; Aslan et al., 2021; Ghafoor et al., 2017; Liu et al., 2024).

Enzymatic oxidation primarily occurs in raw wheat germ before oil extraction, driven by endogenous enzymes such as lipoxygenase (LOX) and lipase (Arslan et al., 2020; Boukid et al., 2018; Erim Kose, 2022; Ghafoor et al., 2017; Liu et al., 2024). Lipase catalyzes the hydrolysis of lipids, releasing free fatty acids (FFAs) (Erim Kose, 2022; Gili et al., 2017; L. Xiong et al., 2025), which are then oxidized by LOX, leading to hydroperoxide formation (Ahmed et al., 2016; Erim Kose, 2022; Gili et al., 2017). This process not only accelerates oxidative rancidity but also increases the FFA content, consequently significantly reducing oil quality (Ahmed et al., 2016; Boukid et al., 2018; L. Xiong et al., 2025).

1.11 Factors Affecting Oxidative Stability

Several intrinsic and extrinsic factors govern the oxidative stability of WGO:

Fatty acid composition: The inherent composition of WGO, specifically the high content of PUFAs such as linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids (Ghafoor et al., 2017; Özcan et al., 2013; Paško et al., 2025; L. Xiong et al., 2025; Zou et al., 2018), makes the oil highly sensitive to oxidative degradation (Aslan et al., 2021; L. Xiong et al., 2025). The rate of oxidation for these PUFAs is faster than monounsaturated fatty acids (Liu et al., 2019; Nayak et al., 2015).

Natural antioxidant content: native compounds of WGO, such as tocopherols, phytosterols, carotenoids, and phenolic compounds, act as radical scavengers (Erickson et al., 2022; Paško et

al., 2025; Taghvaei & Jafari, 2015), thereby delaying oxidation (Aslan et al., 2021). However, these essential antioxidants degrade during storage due to oxidation (Aslan et al., 2021; Erim Kose, 2022) or are substantially reduced during refining (Ghafoor et al., 2017; K. Kraljić et al., 2015), diminishing their protective capacity.

Storage and environmental conditions: exposure to elevated temperature, light, oxygen, and humidity accelerates the degradation reactions (Arslan et al., 2020; Erickson et al., 2022; L. Xiong et al., 2025; Liu et al., 2024; Taghvaei & Jafari, 2015). To counter this, storage under refrigeration (F. Boukid et al., 2018; G. Rodríguez et al., 2021; Uday Karne et al., 2025) and using an inert gas atmosphere, such as nitrogen, can significantly reduce oxidative damage (Arslan et al., 2020; J. Wang & F. Shahidi, 2017).

Packaging: Protecting WGO from light exposure is crucial (Arslan et al., 2020; Ghafoor et al., 2017), as transparent containers permit photooxidation (Boukid et al., 2018). Using opaque or UV-blocking packaging materials substantially helps to extend the oil's shelf life (Karne et al., 2025).

Processing and refining: high-temperature refining steps, such as alkali application or deodorization, result in the loss or stripping of natural antioxidants like tocopherols and phenolics (Ghafoor et al., 2017; K. Kraljić et al., 2015), further limiting the oil's oxidative resistance (Erim Kose, 2022; Ghafoor et al., 2017).

1.13 Analytical Evaluation of Oxidation

The progression of oxidation in WGO is commonly monitored using chemical indices such as peroxide value for primary oxidation products and *p*-anisidine value for secondary ones (Erickson et al., 2022; Arslan et al., 2020; Aslan et al., 2021; G. Rodríguez et al., 2021). Increasing *p*-AV and PV levels reflect oxidative deterioration (Arslan et al., 2020; Aslan et al., 2021; Choe & Min, 2007), compromising nutritional quality and functional value, and correlate with sensory changes such as rancid odor and flavor (Aslan et al., 2021; Choe & Min, 2007). The degradation rate of tocopherols also serves as an indicator of oxidative progression (Aslan et al., 2021; Choe & Min, 2006; Bartolini et al., 2022), typically following the order: α -tocopherol > (γ + β)-tocopherol > δ -tocopherol (Aslan et al., 2021; Bruscatto et al., 2009).

1.14 Oxidative Stability of Wheat Germ Oil (WGO)

As mentioned before, WGO is known for its nutritional richness but also for its susceptibility to oxidative deterioration (Aslan et al., 2021; Arslan et al., 2020; Erim Kose, 2022). Its stability is influenced by both intrinsic factors, such as fatty acid composition and enzyme activity, and extrinsic factors, including temperature, light, oxygen exposure, and packaging conditions (Erim Kose, 2022; Liu et al., 2024; Arslan et al., 2020). WGO is particularly rich in polyunsaturated fatty acids (PUFAs) (Ghafoor et al., 2017; Siraj, 2022), which make up approximately 80% of its triglyceride content (Arslan et al., 2020; Ghafoor et al., 2017). While nutritionally beneficial, this high PUFA content makes the oil highly prone to oxidative

degradation (Arslan et al., 2021; Erim Kose, 2022; L. Xiong et al., 2025). The multiple double bonds in PUFAs act as reactive sites for lipid peroxidation, leading to the formation of rancid off-flavors (Arslan et al., 2020; L. Xiong et al., 2025).

Moreover, the rapid deterioration of wheat germ during storage is driven by hydrolytic and oxidative enzymes, notably lipase (LA) and lipoxygenase (LOX) (Arslan et al., 2020; Ghafoor et al., 2017; Liu et al., 2024; Erim Kose, 2022; Boukid et al., 2018), which accelerate lipid breakdown (Xiong et al., 2025; Ghafoor et al., 2017). This process initially releases free fatty acids (FFA) by hydrolysis (Erim Kose, 2022; Xiong et al., 2025), accelerating oxidative rancidity (Rizzello et al., 2010), and leading to the formation of volatile compounds such as hexanal (Sjövall et al., 2000; Rizzello et al., 2010). Lipase, in particular, is relatively thermostable, retaining over 20% of its activity even after heating at 60–90 °C for one hour (Arslan et al., 2020; Erim Kose, 2022), although its activity diminishes under acidic conditions (Rao et al., 1991; Ahmad et al., 2010).

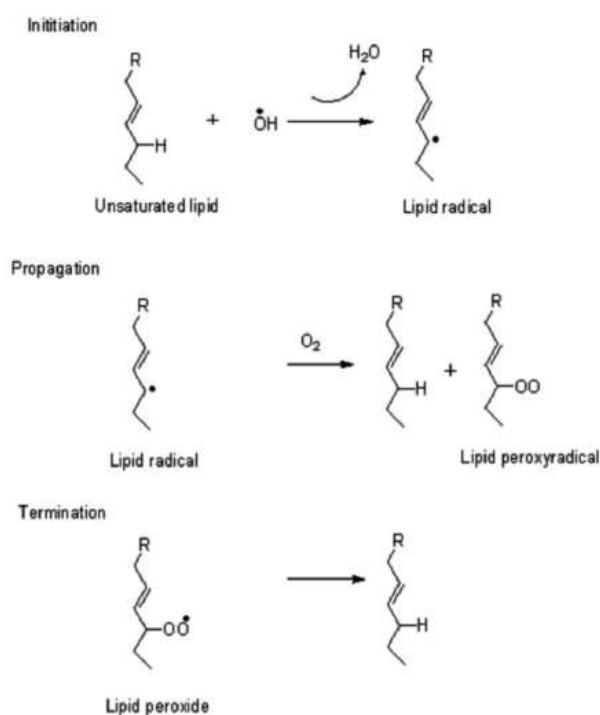


Figure 1: steps of autooxidation (Sachdeva, M., Karan, M., Singh, T., & Dhingra, S, 2014)

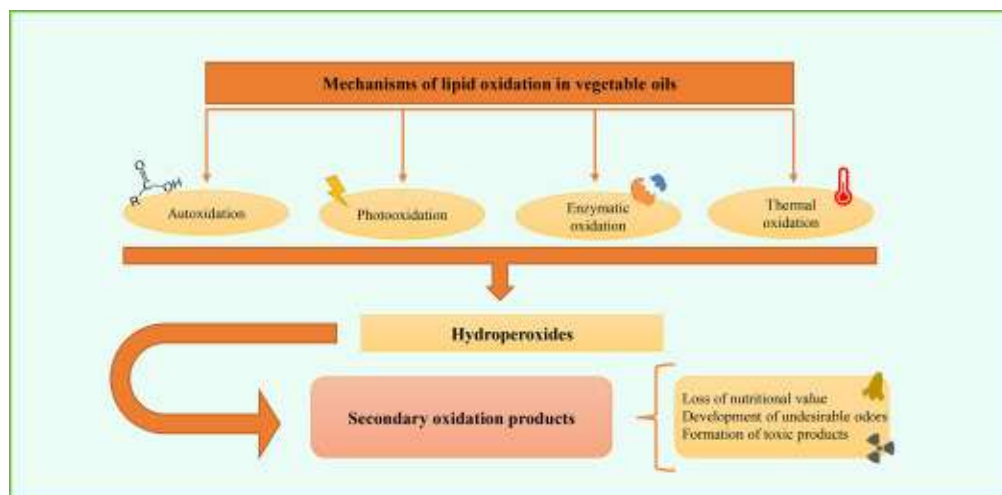


Figure 2: Lipid Oxidation in Vegetable Oils

1.15 Safety Issues and Consequences of Lipid Oxidation in oils

Regardless of the pathway, lipid oxidation produces hydroperoxides as primary products, which subsequently decompose into a variety of secondary products including aldehydes, ketones, alcohols, and acids (Erim Kose, 2022; Ahmed et al., 2016; Erickson et al., 2023; L. Xiong et al., 2025). Lipid oxidation safety issues stem from the formation of potentially harmful compounds such as aldehydes and free radicals, which can damage DNA, increase oxidative stress, and contribute to chronic diseases. These degradation products can be ingested through oxidized foods, leading to health problems such as atherosclerosis, cytotoxicity, and inflammation (Bartolini et al., 2022; Feng et al., 2020; Liaqat et al., 2020; Schaich, 2020). In addition to these health risks, lipid oxidation causes sensory and nutritional deterioration (Aslan et al., 2021; Choe & Min, 2007; L. Xiong et al., 2025). Secondary products are responsible for off-flavors, bitterness, discoloration, and altered viscosity (Aslan et al., 2021; Choe & Min, 2007; Erickson et al., 2023; Nayak et al., 2016; L. Xiong et al., 2025), while the degradation and loss of components such as PUFAs, tocopherols, and other bioactive compounds reduces the nutritional value of oils (Aslan et al., 2021; Choe & Min, 2007; Erickson et al., 2023; L. Xiong et al., 2025). Overall, oxidation diminishes food quality by causing rancidity and negative changes in flavor, texture, and color (Aslan et al., 2021; Ahmed et al., 2016; El-Hadad et al., 2024; Erickson et al., 2022).

1.16 The Frying Process: Physicochemical Reactions and Oil Degradation

Frying is a widely used cooking method that greatly enhances the sensory qualities of food, including its flavor, texture, and appearance (Erickson et al., 2023; Oke et al., 2018; Rodríguez et

al., 2021). The global popularity of fried products is reflected in the growing number of fast-food outlets and the increasing consumption of deep-fried foods (Nayak et al., 2016). Deep-fat frying typically takes place at high temperatures (160–190 °C) (Erickson et al., 2023; Hosseini et al., 2016; Rodríguez et al., 2021), during which complex physicochemical reactions occur between the oil, food, and surrounding environment, exacerbated by prolonged exposure to high heat, oxygen, and moisture (Erickson et al., 2023; Nayak et al., 2016). The major degradation mechanisms during frying include oxidation, hydrolysis, and polymerization (Erickson et al., 2023; Kmiecik et al., 2020; Nayak et al., 2016). Lipid oxidation, the most critical and damaging reaction, is a primary mechanism mediated by free radicals (Erickson et al., 2023; Taghvaei & Jafari, 2015). It is triggered by the exposure of unsaturated fatty acids to oxygen, particularly under high heat, leading to the formation of hydroperoxides (Erickson et al., 2023; Nayak et al., 2016). These primary oxidation products subsequently break down into volatile aldehydes, ketones, and acids (Erickson et al., 2023; Nayak et al., 2016), which are responsible for rancidity and undesirable flavors (Erickson et al., 2023; Razem et al., 2025). Hydrolysis occurs when moisture released from the food reacts with the hot oil, breaking down triglycerides into free fatty acids (FFA), diglycerides, monoglycerides, and glycerol (Erickson et al., 2023; Nayak et al., 2016). This not only reduces oil quality but also accelerates oxidative deterioration, as FFA can catalyze further oxidation (Nayak et al., 2016; Ujong et al., 2023). Finally, polymerization, another key reaction, involves the formation of high molecular weight compounds via free radical pathways (Erickson et al., 2023; Nayak et al., 2016). These reactions increase oil viscosity and result in the formation of foams and sticky residues, ultimately impairing the safety and functionality of the frying medium (Nayak et al., 2016; Razem et al., 2025).

1.17 Oil Stability Requirements

To maintain oil quality during frying, oils must demonstrate resistance to oxidation, hydrolysis, and polymerization under high heat (Erickson et al., 2022; Kmiecik et al., 2018; Nayak et al., 2015; Rodríguez et al., 2021). Achieving stable oil performance generally requires the following characteristics:

- high oxidative stability index (OSI): A high OSI value indicates stability against oxidation, providing a measure of the oil's longevity (Erickson et al., 2023).
- low initial free fatty acid (FFA) content: A low level of FFAs is necessary because FFAs are quickly oxidized and promote further oxidative deterioration by possibly assisting in the catalysis of oxidation of polyunsaturated fats (Nayak et al., 2016; Paradiso et al., 2018).
- The presence of stabilizing compounds: oxidation can be successfully delayed or prevented by endogenous stabilizing compounds like tocopherols (Vitamin E), or by the addition of antioxidants (Erickson et al., 2023; Taghvaei & Jafari, 2015).

Oils naturally rich in PUFAs, such as WGO, present a stability challenge because PUFAs (which can account for up to 80% of WGO's oil content) are prone to oxidative degradation (Arslan et al.,

2020; Erim Kose, 2022; Ghafoor et al., 2017; Siraj, 2022). However, these oils can be suitable if their existing antioxidant profile adequately compensates for their inherent reactivity (Erickson et al., 2023). WGO is notably known for its exceptionally high tocopherol content, which serves as a major natural antioxidant (Erickson et al., 2023; Ghafoor et al., 2017). The addition of WGO to other PUFA-rich oils (like marine oils) has been shown to improve their oxidative stability due to this increased tocopherol content (Wang & Shahidi, 2017).

1.18 Natural Antioxidants, Clean Label Trend, and Applications in Oils

The increasing consumer demand for “clean label” products and growing concerns about the potential long-term health effects of synthetic antioxidants have led to a significant shift towards the use of natural antioxidants in the food industry (Erickson et al., 2023; Taghvaei & Jafari, 2015; Wąsowicz et al., 2004). This shift is necessary because synthetic antioxidants, often investigated for potential links to chronic diseases such as cancer and cardiovascular disease, face public scrutiny and tighter regulations (Erickson et al., 2023; Taghvaei & Jafari, 2015). That trend emphasizes the priority placed on ingredients that are perceived as natural, chemical-free, and sustainably sourced (Bartolini et al., 2022; Razem et al., 2025). Natural antioxidants, derived primarily from plants as phenolic compounds, are generally considered safer alternatives (Razem et al., 2025; Wąsowicz et al., 2004). These compounds provide beneficial functional and nutritional effects beyond their main function of giving hydrogen to free radicals to prevent auto-oxidation in oils (Taghvaei & Jafari, 2015).

1.19 Rosemary Extract as a Natural Antioxidant

Rosemary extract is widely recognized as an effective natural antioxidant, particularly for enhancing the oxidative stability of edible oils under both storage and frying conditions. Its efficacy has been consistently demonstrated across a broad range of lipid systems, making it one of the most studied plant-derived alternatives to synthetic antioxidants (Erickson et al., 2023; Taghvaei & Jafari, 2015; Wąsowicz et al., 2004). The antioxidant activity of rosemary extract is primarily attributed to its phenolic diterpenes, notably carnosic acid and carnosol, along with rosmarinic acid. These compounds act as primary antioxidants by donating hydrogen atoms to lipid-derived free radicals (ROO $\cdot$), thereby interrupting radical chain reactions and effectively inhibiting lipid peroxidation. Through this mechanism, rosemary extract limits both the initiation and propagation phases of oxidative degradation in oils (Erickson et al., 2022; Saoudi et al., 2017; Taghvaei & Jafari, 2015). Under frying conditions, rosemary extract has shown a pronounced ability to delay thermo-oxidative deterioration in a variety of vegetable and animal fats, including sunflower, corn, soybean, palm olein, rapeseed, hazelnut, olive oil, and ghee, at temperatures ranging from 150 to 200 °C. Several studies report significant reductions in primary oxidation markers, such as peroxide value (PV), as well as secondary oxidation products, including p-anisidine value (p-AV), total polar compounds (TPC), and conjugated dienes (CD) and trienes

(Erickson et al., 2023; Razem et al., 2025). In addition to slowing oxidative degradation, rosemary extract contributes to the preservation of endogenous antioxidants, particularly tocopherols and phytosterols, which further supports oil stability during repeated heating cycles.

Importantly, the antioxidant performance of rosemary extract is frequently reported to be comparable to, or even superior to, commonly used synthetic antioxidants such as TBHQ, BHA, and BHT. For example, the addition of 0.2% rosemary extract to sunflower, corn, and soybean oils was found to provide greater protection against oxidation than TBHQ at 0.02 % under accelerated storage conditions, highlighting its potential as an effective natural substitute in lipid-rich food systems (Cordeiro et al., 2013; Erickson et al., 2023; Wąsowicz et al., 2004).

From a sensory perspective, most studies indicate that rosemary extract does not adversely affect the flavor, aroma, or overall acceptability of fried foods, and in some cases may even enhance sensory quality. Nevertheless, excessive concentrations can lead to undesirable sensory changes, such as darker color or herbal off-flavors, as observed at levels around 0.5 % in ghee, underscoring the importance of optimizing dosage for specific applications (Rahila et al., 2018; Erickson et al., 2023).

Overall, the strong thermal stability of rosemary extract allows it to retain its antioxidative activity during prolonged heating and deep-fat frying processes. This stability, combined with its efficacy and generally favorable sensory profile, supports its suitability as a natural antioxidant for improving the oxidative stability of frying oils and fat-based food products (Kmieciak et al., 2020; Taghvaei & Jafari, 2015; Wąsowicz et al., 2004).

1.20 Polyphenols (Including Tannins) as Natural Antioxidants

Polyphenols constitute a large and chemically diverse group of plant-derived bioactive compounds that have attracted considerable attention as natural antioxidants in edible oils. Their relevance in lipid systems arises not only from their ability to protect plants against oxidative stress but also from their demonstrated capacity to enhance oxidative stability and provide potential health benefits when incorporated into food matrices (Khosroshahi & Razavi, 2023; Paško et al., 2025). The antioxidant effectiveness of polyphenols is mainly attributed to their capacity to scavenge free radicals through hydrogen donation, thereby interrupting radical chain reactions responsible for lipid autoxidation. By acting at both the initiation and propagation stages of oxidation, polyphenols can significantly delay the formation of primary and secondary oxidation products in oils. In addition, many polyphenolic compounds possess metal-chelating properties, which reduce the catalytic activity of transition metals that otherwise accelerate oxidative reactions. This dual mode of action—radical scavenging combined with metal chelation—makes polyphenols particularly effective in complex lipid systems (Erickson et al., 2023; Ma et al., 2023; Taghvaei & Jafari, 2015).

A wide range of plant materials has been investigated as sources of polyphenolic antioxidants, including fruits, vegetables, cereals, legumes, and tea. Among cereal-derived sources, wheat bran

extracts have shown notable promise. In particular, Kamut wheat bran extract (KWBE), rich in phenolic acids such as ferulic and p-coumaric acids, flavonoids, quercetin, and alkylresorcinols, has demonstrated strong antioxidant performance in heated oil systems. Experimental evidence indicates that KWBE can outperform the synthetic antioxidant TBHQ as well as individual phenolic standards, effectively reducing peroxide formation, suppressing the development of conjugated dienes and trienes, and limiting the degradation of unsaturated fatty acids during heating. Moreover, oils treated with KWBE have been reported to generate lower levels of aldehydic compounds during frying, indicating improved oxidative stability under high-temperature conditions (Razem et al., 2025). Similarly, polyphenol-rich extracts from green tea and olive plant by-products have been widely reported to enhance oil stability during both storage and thermal processing. Green tea catechins and flavonoids exhibit strong radical-scavenging activity, while olive-derived phenolics from leaves or mill wastewater have been shown to delay oxidative deterioration in various oil matrices. These findings highlight the versatility of polyphenols as natural antioxidants derived from diverse and often underutilized plant sources (Erickson et al., 2022; Taghvaei & Jafari, 2015; Wąsowicz et al., 2004).

Despite their proven antioxidant efficacy, the application of polyphenols in frying oils is not without limitations. Many polyphenolic compounds are sensitive to high temperatures, and their concentration can decrease rapidly during prolonged heating or conventional refining processes, particularly deodorization. As a result, their protective effects may diminish under severe thermal conditions, and their effectiveness can vary substantially depending on the source, chemical structure, and processing history of the extract (Erickson et al., 2022; Farhoosh & Hosseini-Yazdi, 2013; Ghafoor et al., 2017; Kraljić et al., 2015). The growing interest in polyphenols as oil stabilizers is closely linked to the increasing consumer demand for clean-label products and the desire to replace synthetic antioxidants with naturally derived alternatives. Plant-based antioxidants are generally perceived as safer and more sustainable, while also offering potential added health benefits beyond oxidative protection. Nevertheless, achieving a balance between antioxidant efficacy, thermal stability, and sensory acceptability remains a key challenge, particularly for applications involving high-temperature frying (Bartolini et al., 2022; Erickson et al., 2023; Razem et al., 2025).

Within this context, wheat germ oil represents a particularly interesting lipid system due to its naturally high content of antioxidants, including tocopherols, phytosterols, carotenoids, and polyphenols. Although its high polyunsaturated fatty acid content makes it inherently prone to oxidation, the presence of these bioactive compounds contributes to its intrinsic oxidative resistance and reinforces its potential value in health-oriented and clean-label formulations. A deeper understanding of how polyphenols interact with endogenous antioxidants and lipid substrates is therefore essential for developing effective strategies to improve oil stability during storage and frying (Aslan et al., 2021; Ghafoor et al., 2017; Paško et al., 2025).

1.21 *Using Wheat Germ Oil as a Novel Frying Oil*

The growing global population and rising demand for edible fats have intensified the search for novel vegetable oil sources, particularly from underutilized industrial by-products (Abdel-Aal, 2024; Ghafoor et al., 2017; Siraj, 2022). Wheat germ oil (WGO), traditionally exploited as a specialty raw material for pharmaceuticals and cosmetics, remains largely underutilized in culinary applications, with a substantial portion of the 25 million tons produced annually directed toward animal feed (Arslan et al., 2020; Boukid et al., 2018; Erim Kose, 2022; Khosroshahi & Razavi, 2023; Liu et al., 2024). Although rich in unsaturated fatty acids (80–85%), which generally increase susceptibility to thermal degradation, WGO is exceptionally rich in natural antioxidants, primarily tocopherols and phytosterols, which can counteract lipid oxidation and enhance stability under frying conditions (Arslan et al., 2020; Ghafoor et al., 2017; Paško et al., 2025; Siraj, 2022; Zou et al., 2018). This compositional balance suggests that WGO may outperform conventional frying oils, such as sunflower oil, which often have reduced antioxidant levels due to refining (Erickson et al., 2023; Kraljić et al., 2015; Naz et al., 2005; Razem et al., 2025). Therefore, this study aims to evaluate the oxidative stability and nutritional integrity of WGO under deep-frying conditions, comparing its performance to common oils, and assessing its potential as a functional, high-value frying medium while responding to the growing need for sustainable and health-promoting vegetable oils (Boukid et al., 2018; Khosroshahi & Razavi, 2023; Liu et al., 2024; Paško et al., 2025).

1.22 *Synergistic Effects of Tocopherols, Phytosterols, and Squalene*

The oxidative stability of wheat germ oil (WGO) is strongly supported by its rich unsaponifiable fractions of phytosterols, specific sterols, squalene, and carotenoids which plays a central role in counteracting oxidation through complementary and potentially synergistic mechanisms (Ghafoor et al., 2017; Zargar et al., 2023; Paško et al., 2025). Rather than acting independently, these compounds collectively enhance the resistance of triacylglycerols to thermal stress, limiting oxidative pathways that typically lead to polymerization, viscosity increase, and oil deterioration during high-temperature processing (Erickson et al., 2022; Razem et al., 2025). Phytosterols contribute to this protective effect by slowing oxygen diffusion and oxidation kinetics at the oil interface, although their susceptibility to oxidation at elevated temperatures highlights the importance of matrix interactions and protective strategies such as microencapsulation (Aslan et al., 2021; Kmiecik et al., 2021). Squalene further reinforces oxidative stability by acting as an efficient radical scavenger, particularly when tocopherol levels decline during prolonged heating, thereby extending the functional antioxidant capacity of the oil (Erickson et al., 2022; Zhang et al., 2012). In parallel, carotenoids provide additional oxidative defense, and their enhanced stability when structurally protected suggests a meaningful contribution to the overall antioxidant network within WGO (Aslan et al., 2021).

Taken together, these findings indicate that the oxidative behavior of WGO cannot be explained solely by its fatty acid composition. Instead, the collective and interacting action of its natural antioxidants effectively mitigates the inherent vulnerability associated with high PUFA levels, supporting the view that WGO possesses a structurally reinforced antioxidant system capable of maintaining oxidative stability under demanding thermal conditions (Ghafoor et al., 2017; Erim Kose, 2022).

1.23 Research Objectives

Building on these findings, the present study has the objectives of valorize WGO as a beneficial edible frying oil. The oxidative stability of WGO stability and nutritional integrity under frying conditions, including peroxide value, acidity, oxidation products, and antioxidant level was assed. Finally, the WGO performance in producing French fries were compared with those achieved by sunflower oil and mixed seed oil through controlled frying trials and CATA sensory test. By addressing these aims, this thesis seeks to determine whether refined WGO can serve as a viable, functional frying oil, thereby contributing to a sustainable valorization of a major industrial by-product.

2. Materials and methods

2.1 Chemicals and reagents

Three edible oils were selected for the frying experiments: wheat germ oil (WGO, Casillo Next Food, Italy), sunflower oil (SO) and mixed seed oil (MSO) (both purchased from a local Italian retailer, Italy). Frozen pre-fried French fries (Patatine fritte surgelate, brand: Pizzoli, Italy) were employed as the fried food matrix throughout the experimental trials, ensuring product uniformity and high reproducibility of frying conditions. Analytical reagents for chemical analyses were provided as ready-to-use kits for the CDR FoodLab® Junior analyzer (CDR S.r.l., Ginestra Fiorentina, Italy), including: acidity reagent for free fatty acid determination (% oleic acid, ER FOODLAB®); peroxide Reagents 1 and 2 (ER FOODLAB® PEROXIDES); *p*-Anisidine Reagent (ER FOODLAB®). The commercial FAME standards was purchased by Supelco (Bellefonte, PA, USA). 4-methyl-2-pentanol and 5- α -cholestane, used as internal standard in Volatiles organic compounds (VOCs) and fatty acid methyl esters analysis (FAMES) respectively, were obtained from Sigma-Aldrich (Milan, Italy). Methanol, *n*-hexane, propan-2-ol were purchased from Honeywell (Morristown, New jersey, USA). All reagents were of analytical grade and used according to the manufacturer's instructions. Deionized water was employed for reagent preparation and cleaning procedures.

2.2 Frying Protocol and Total Polar Material (TPM) Determination

For the frying experiments, three domestic deep fryers (Aigostar, Agni 300004HEY; 40.5 × 22.7 × 24.7 cm, 3 L capacity) were used. Each fryer was filled with 3 L of oil (SO, MSO, WO) and the experiment was carried out for five consecutive days, without fresh oil refill. The temperature was set to 180 ± 5 °C, actual oil temperature was continuously monitored using an analytical temperature probe to ensure accurate thermal conditions. After the initial heating, Total Polar Materials (TPM) were determined using a Testo T270BT according to the manufacturer's instructions. Before measurement, the device was calibrated, then two independent measurements were taken from different regions of the oil surface, and the mean value was reported. Before the first frying cycle, the oil was held at 180 ± 5 °C for 30 minutes without food to allow temperature equilibration. Frozen pre-fried French fries (100 ± 5 g) were then deep-fried for 4 minutes. A 10-minute interval was allowed between successive frying cycles to restore the operational temperature. Each oil underwent four consecutive frying cycles per day. After the fourth frying cycle, the Testo was measured again two times. The oil was maintained at 180 ± 5 °C for an additional 30 minutes without food to simulate prolonged heating. The fryer was then turned off, and the oil was allowed to cool at room temperature. Finally, approximately 50 mL of each oil sample was collected, placed into labeled tubes and stored in freezer (-18°C) until further analyses. Each experiment was in triplicate ($n=3$).

2.3 Determination of Free Fatty Acids (FFA)

The free fatty acid (FFA) content of the oil samples was determined using the CDR FoodLab Junior analyzer. Initially, 1000 μL of the Acidity Reagent was pipetted into a dedicated cuvette. The cuvette was then placed in incubator for 5 minutes to allow the oil to dissolve completely in the solvent. Following incubation, 5 μL of the oil sample at room temperature was added to the pre-warmed reagent in the cuvette. The mixture was then gently shaken to ensure complete solvation. The prepared cuvette was inserted into the CDR FoodLab JR device. The system automatically measured the absorbance. The acidity value was displayed on the screen as % oleic acid, representing the FFA content of the sample. All measurements were performed in triplicate.

2.4 Determination of Peroxide Value (P)

The peroxide value (PV) of the oil samples was determined using a CDR FoodLab JR analyzer, following the manufacturer's instructions for the peroxide test. Initially, 1.0 mL of Reagent 1 was pipetted into a disposable cuvette using a 1000 μL adjustable micropipette. The cuvette was placed in the instrument's pre-warming block for 5 minutes to reach the appropriate reaction temperature. A dedicated blank cuvette containing only Reagent 1 was also prepared to be used for background correction. Subsequently, 2.5 μL of the oil sample was added to the cuvette containing Reagent 1, followed by the addition of 1 drop (1 μL) of Reagent 2. The mixture was gently shaken to ensure complete solvation. The same amount of Reagent 2 was also added to the blank cuvette. Both cuvettes (sample and blank) were then inserted into the analyzer. The instrument automatically measured absorbance after 3 minutes of reaction time, and PV results were expressed in meq O_2/kg oil. All measurements were performed in triplicate.

2.5 Determination of p-Anisidine Value (p-AV)

The *p*-anisidine value (*p*-AV) of the oil samples was determined using a CDR FoodLab JR analyzer, following the manufacturer's instructions for the *p*-anisidine test. Initially, 1000 μL of the *p*-anisidine reagent was pipetted into a disposable cuvette. The cuvette was placed in the instrument's pre-warming block and incubated for 5 minutes to allow the reagent to reach the required reaction temperature. The cuvette was then gently shaken to ensure homogeneity of the reagent solution. Subsequently, 20 μL of the oil sample was added to the cuvette and mixed gently by shaking. The prepared cuvette was inserted into the analyzer and the reaction was allowed to proceed for 1 minute. The instrument automatically measured the absorbance and displayed the *p*-anisidine value, expressed as *p*-AV. All measurements were performed in triplicate.

2.6 Determination of Fatty Acid Composition (FAME, GC-FID)

The fatty acid composition of oils was determined after trans-esterification to obtain fatty acid methyl esters (FAMEs), which was necessary to increase their volatility and thermal stability, allowing proper separation and quantification by GC-FID. Oxidized oil samples (20 mg) were dissolved in 1 mL of *n*-hexane:propan-2-ol (3:2, v/v) containing 1.05 mg of 5- α -cholestane as internal standard (IS). An aliquot of 1 μ L was injected (split ratio 1:50) into a GC-2010 equipped with an AOC-20i auto-injector and a flame ionization detector (GC-FID) (Shimadzu, Kyoto, Japan). Separation was carried out using a silica capillary column Stabilwax (20 m $\times$ 0.18 mm $\times$ 0.18 μ m; Restek, Bellefonte, PA, USA). The oven temperature was programmed as follows: 100 $^{\circ}$ C to 180 $^{\circ}$ C at 10 $^{\circ}$ C/min (held 2 min), then increased to 240 $^{\circ}$ C at 10 $^{\circ}$ C/min (held 5 min). The injector and FID temperatures were set at 248 $^{\circ}$ C and 250 $^{\circ}$ C, respectively. Helium was used as the carrier gas at a linear velocity of 42 cm/s. Identification of fatty acids was performed by comparing retention times with commercial FAME standards (Supelco, Bellefonte, PA, USA) injected under the same chromatographic conditions. Quantification was based on the internal standard method, with response factors determined using appropriate commercial standards. Results were expressed as grams per 100 g of oil (%).

2.7 Determination of Volatile Organic Compounds (VOCs, GC-MS)

Volatile compounds were analyzed by headspace solid-phase microextraction (HS-SPME) coupled with GC-MS (QP-2010 Plus, Shimadzu, Kyoto, Japan). Accurately weighed oil samples (3.0 g) were placed in 20 mL headspace vials sealed with PTFE-silicone septa (Agilent Technologies, Santa Clara, CA, USA). After equilibration at 40 $^{\circ}$ C for 10 min, a DVB/CAR/PDMS-coated fused silica fiber was exposed to the headspace for 20 min. Extraction was carried out using a Combi Pal autosampler system (CTC Analytics AG, Zwingen, Switzerland). After extraction, the fiber was thermally desorbed in the GC-MS injector at 260 $^{\circ}$ C for 5 min with a split ratio of 1:10. VOC separation was achieved using a Zebron ZB-WAXplus capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m; Phenomenex, Torrance, CA, USA). The oven temperature program was: 40 $^{\circ}$ C initial (held), ramped at 10 $^{\circ}$ C/min to 200 $^{\circ}$ C, then at 4 $^{\circ}$ C/min to 250 $^{\circ}$ C, and held for 5 min. Helium was used as carrier gas at a constant linear velocity of 36.2 cm/s. The ion source temperature was 200 $^{\circ}$ C, and the interface temperature was 260 $^{\circ}$ C. Mass spectra were acquired in scan mode over the range of 33–350 *m/z*. VOCs were identified by comparison with the NIST08s mass spectral library (National Institute of Standards and Technology, Gaithersburg, USA). Results were expressed as the relative percentage of each peak area with respect to the total VOC peak area.

2.7 Check-All-That-Apply (CATA) and Hedonic Evaluation

To complement the chemical analyses, a sensory evaluation was conducted combining a hedonic test and a Check-All-That-Apply (CATA) test to assess consumers' perception of French fries fried

in WO, SO and MO. A total of 88 consumers participated in the blind test. The samples (warm French fries) were presented in a randomized order and coded with three-digit numbers to avoid bias. Participants completed online questionnaires corresponding to each sample. In the hedonic test, consumers evaluated five quality attributes — color, aroma, taste, crispness, and overall acceptance — using a nine-point hedonic scale ranging from 1 (*extremely unpleasant*) to 9 (*extremely pleasant*). They were also asked a purchase intent question (“If this product were available on the market, would you buy it?”), rated on a 9-point scale. In the CATA test, participants were instructed to select all descriptors that applied to the sample from a predefined list, organized into three sensory dimensions:

- Aroma: pleasant, mild, unpleasant, fish-like
- Taste: pleasant, mild, oily, green, fish-like, rancid, burnt, spicy
- Texture: crispiness, lack of crispiness, chewy

The frequency of citation for each descriptor was calculated as the percentage of consumers who selected that term for each oil. Descriptive analysis was then used to identify the most frequently perceived attributes and to compare sensory profiles among the oils.

2.9 Statistical analysis

Statistical analysis was performed using Microsoft Excel 2019 (Microsoft Corporation, USA). All measurements were carried out in triplicate, and the results are expressed as mean $\pm$ standard deviation (SD) of three independent replicates ($n=3$). For most parameters, a one-way analysis of variance (ANOVA) was applied to evaluate significant differences among samples, followed by Tukey’s post hoc test when appropriate. In contrast, differences in fatty acid methyl ester (FAME) composition were analyzed using Student’s *t*-test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Chemical analysis

3.1.1 Free Fatty acid

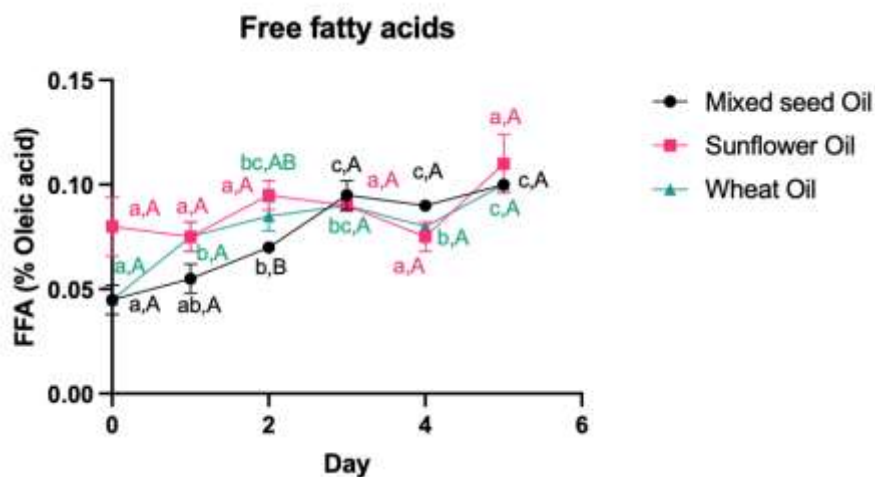


Figure 1: Changes in free fatty acid (FFA) content of Wheat Germ Oil (WGO), Sunflower Oil (SO), and Mixed Seed Oil (MSO) during five days of frying. Different lowercase letters indicate statistically significant differences ($p < 0.05$) within the same oil over time, while uppercase letters denote significant differences ($p < 0.05$) among oils at the same sampling day.

The free acidity provides an essential indicator of hydrolytic degradation during frying, reflecting the extent of triacylglycerol cleavage under high heat and moisture. Among the three oils, sunflower oil showed the highest FFA levels throughout the whole frying period. The FFA content increased slightly from 0.08% at the beginning (Day 0) to 0.11% at the end (Day 5), but the change was not statistically significant, indicating that its hydrolytic degradation remained stable. The consistently uniform statistical grouping suggests that sunflower oil reached an early equilibrium between the formation and breakdown of free fatty acids, showing a stable performance under thermal stress despite its high baseline value. In contrast, mixed seed oil, exhibited a statistically significant increase in FFA content over time, indicating an ongoing hydrolytic process during repeated frying. Starting from a low initial level, 0.045%, its FFA values rose steadily, confirming measurable degradation of triacylglycerols. Wheat germ oil followed a similar increasing pattern, with FFA levels rising significantly from 0.045% at the beginning to 0.10% at the end of the frying period. Although minor fluctuations occurred midway, these changes were not statistically significant, and the overall trend indicated a steady accumulation of free fatty acids over time. Overall, the results indicate that all three oils followed a similar hydrolytic pattern during repeated frying. Although sunflower oil showed slightly higher FFA values throughout the experiment,

these differences were not statistically significant. The increase in FFA over time was comparable among the oils, suggesting that hydrolytic degradation progressed at a similar rate for all. This trend confirms that, despite small numerical variations, the three oils exhibited equivalent hydrolytic stability under the applied frying conditions.

3.1.2 Peroxide Value

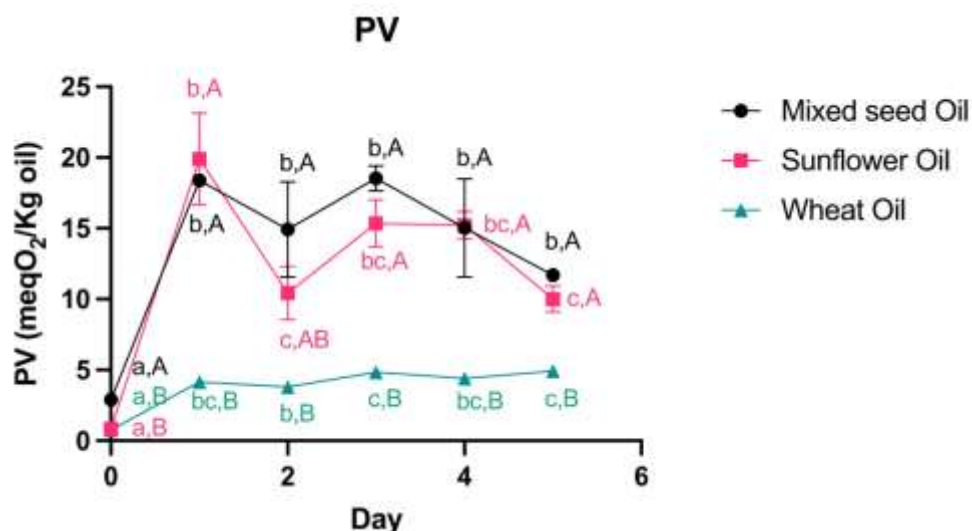


Figure 2: Peroxide Value (PV) of SO, MSO, and WGO during 5 days of frying cycles. Uppercase letters indicate statistically significant differences among oils on the same day, while lowercase letters indicate intra-oil differences over time

The Peroxide Value (PV) monitors the formation of hydroperoxides, the primary oxidation products of lipid oxidation. The results showed clear differences in the oxidation response of the three oils, particularly during the first frying cycles. SO exhibited a sharp rise in PV, increasing from 0.85 meq O₂/kg (Day 0) to 19.91 meq O₂/kg (Day 1), followed by a marked decrease up to 10.42 meq O₂/kg (Day 2) due to hydroperoxide decomposition. Afterwards, the PV fluctuated moderately, highlighting its instability under repeated frying. MSO followed a similar but more sustained trend, increasing from 2.92 meq O₂/kg at the beginning to a peak of 18.54 meq O₂/kg on Day 3, then gradually declining to 11.69 meq O₂/kg by Day 5, indicating persistent hydroperoxide formation and slower degradation compared to SO. WGO showed a distinctly milder oxidation response, starting from 0.76 meq O₂/kg and rising gradually to a maximum of 4.85 meq O₂/kg on Day 3, after which values stabilized. This pattern reflects slower hydroperoxide accumulation and higher oxidative stability. Overall, these findings demonstrate that SO and MSO underwent rapid

and pronounced primary oxidation, with SO showing the strongest fluctuations, while WGO maintained low and steady PVs, confirming its superior oxidative stability under repeated frying.

3.1.3 Total Oxidation Value

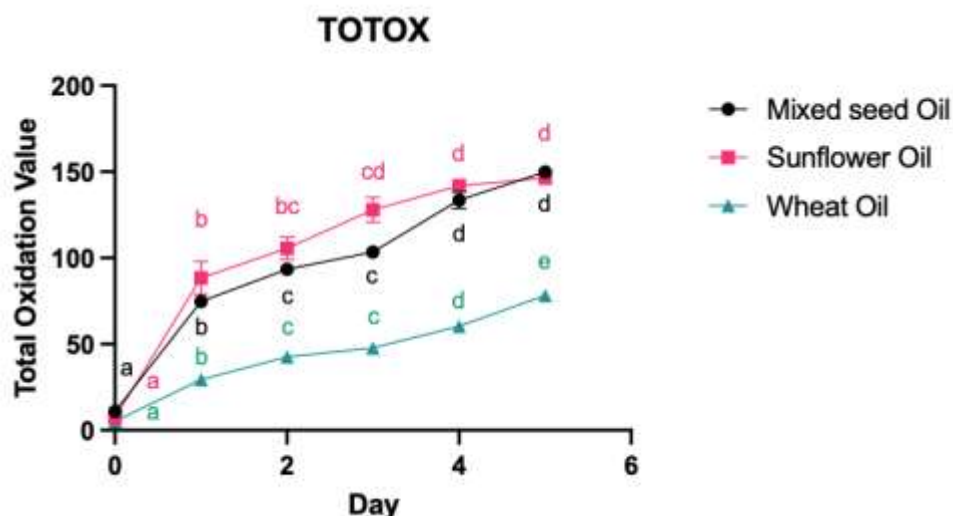


Figure 3: Changes in Total Oxidation Value (TOTOX) of SO, MSO, and WGO during 5 days of repeated frying cycles. Uppercase letters indicate significant differences ($p < 0.05$) among different oils on the same day, while lowercase letters indicate significant differences within each oil over the frying period.

The TOTOX index, which combines both primary (peroxide) and secondary (*p*-anisidine) oxidation products, provides an overall indication of the oxidative status of the oils during frying. SO demonstrated the fastest and highest increase in TOTOX, rising sharply from about 80 at Day 0 to nearly 130 at Day 3 and stabilizing close to 150 by Day 5. The values consistently remained the highest among the three oils, confirming pronounced oxidative instability under repeated frying conditions. MSO exhibited an intermediate oxidation rate. It showed a sharp increase from approximately 80 at Day 0 to 120 by Day 3. From Day 3 to Day 5, the TOTOX values rose further, eventually converging with SO at approximately 150 by the end of the experiment. This indicates that MSO underwent progressive oxidative degradation, with a rate similar to SO in the later stages. WGO showed the greatest oxidative stability throughout the 5 days period. Its TOTOX values remained low and increased gradually, from around 60 at Day 0 to approximately 80 by Day 5. Two moderate increases were observed on Day 1 and Day 4, but overall trend remained steadily upward and significantly lower than those of SO and MSO, demonstrating strong resistance to both primary and secondary oxidation under repeated frying. Overall, these results suggest distinct oxidative behaviors among the tested oils. SO was the least stable oil, showing rapid and high accumulation of oxidation products, MSO had moderate resistance initially but

eventually reached high oxidation levels, while WGO maintained superior stability, resisting substantial oxidative degradation throughout the frying period.

3.1.4 Total polar compound

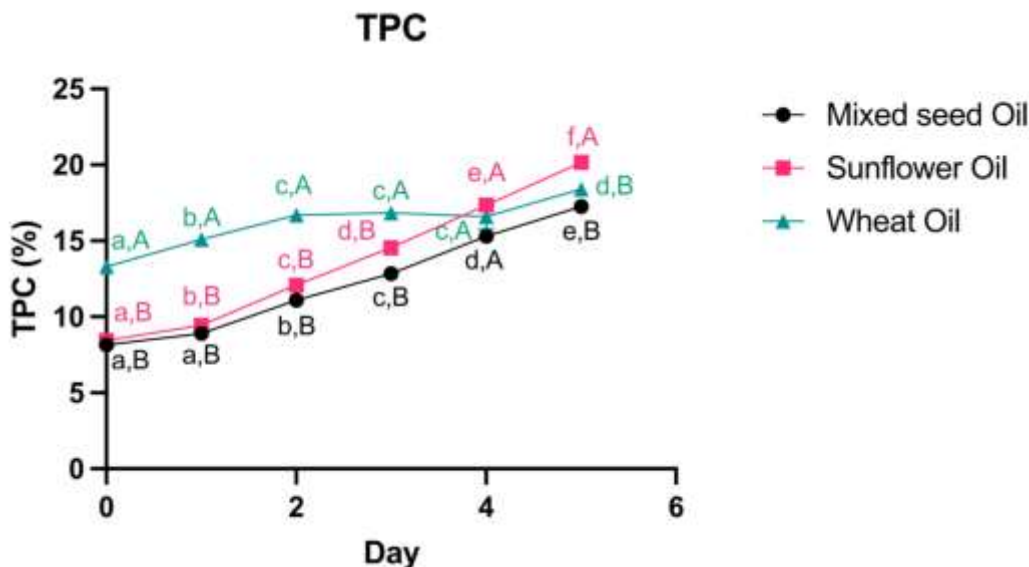


Figure 4: Total Polar Compounds (TPC) in SO, MSO, and WO during a 5-day frying period. Different lowercase letters indicate statistically significant differences among sampling days within the same oil, while uppercase letters indicate significant differences among oils at the same time point

TPCs are chemical degradation products formed during the thermal oxidation of oils, including oxidized triglycerides, free fatty acids, and polymerized compounds. TPC levels are widely used as an indicator of oil quality and oxidative stability, with higher values reflecting greater degradation and reduced frying performance. SO demonstrated the fastest and highest accumulation of TPC during the 5-day frying period. Starting at 8.45% on Day 1, TPC increased continuously and significantly each day, reaching 20.15% by Day 5. This pattern indicates that SO was the least stable oil under repeated frying, with a consistently rapid rate of TPC formation. MSO increased progressively from 8.15% on Day 1 to 17.25% on Day 5, following a clear and steady upward trend. The increase was moderate but continuous throughout the experiment. Statistical analysis confirmed that most daily increments were significant, except between Day 0 and Day 1. Throughout the frying period, MSO consistently exhibited lower TPC values than both WGO and SO, indicating relatively higher oxidative stability. WGO started at 13.30% on Day 1 and showed a gradual increase over the 5-day frying period. Statistically significant changes were observed on Days 2, 3, and 5, while the other days showed minor, non-significant variations. Despite its slower rate of increase compared to SO, WGO maintained intermediate TPC values, higher than MSO initially but lower than SO in the later stages, reflecting moderate oxidative

stability. Overall, the TPC trends highlight differences in oxidative stability among the oils: SO underwent the most rapid degradation, MSO showed a steady but moderate oxidation rate, and WGO exhibited the slowest increase, confirming its relatively better resistance to TPC accumulation under repeated frying.

3.2 Fatty Acid Composition (FAME analysis)

The fatty acid profile of MSO remained relatively stable throughout the 5-day frying period. MUFA content was consistently above 30%, starting at 32.48% on Day 0 and slightly decreasing to 31.24% on Day 5, showing a non-significant variation over time (Figure 5). PUFA was the dominant fraction, decreasing from 50.28% to 42.94% over the frying period. This moderate but statistically significant reduction indicates some degradation of polyunsaturated fatty acids during thermal exposure, while SFA remained nearly constant around 10–11%. Overall, MSO exhibited a balanced composition with moderate MUFA and PUFA levels, maintaining good stability throughout the frying cycle. SO showed PUFA as the predominant fatty acid, starting at 58.66% on Day 0 and declining to 50.93% by Day 5, indicating some degradation of unsaturated fatty acids during frying. MUFA content remained below 30% (from 27.01% to 26.79%), and SFA stayed stable below 10% (around 9.85% to 9.71%). The trend suggests that sunflower oil maintains its general fatty acid composition under repeated frying but experiences a notable reduction in PUFA content. WGO displayed the lowest MUFA fraction, starting at 20.17% and slightly increasing to 21.35% by Day 5. PUFA remained the dominant class, slightly decreasing from 51.18% to 50.04%, while SFA stayed stable at approximately 14–15%. Overall, WGO maintained its fatty acid profile more steadily than MSO and SO, with minor changes observed in MUFA and PUFA fractions. Across the three oils, PUFA was the major fatty acid class, particularly in sunflower and wheat germ oils. MSO showed a more balanced profile, with relatively high MUFA and PUFA content. Over the 5-day frying period, PUFA decreased notably in MSO and SO, reflecting their higher susceptibility to thermal degradation, whereas WGO exhibited the most stable fatty acid composition.

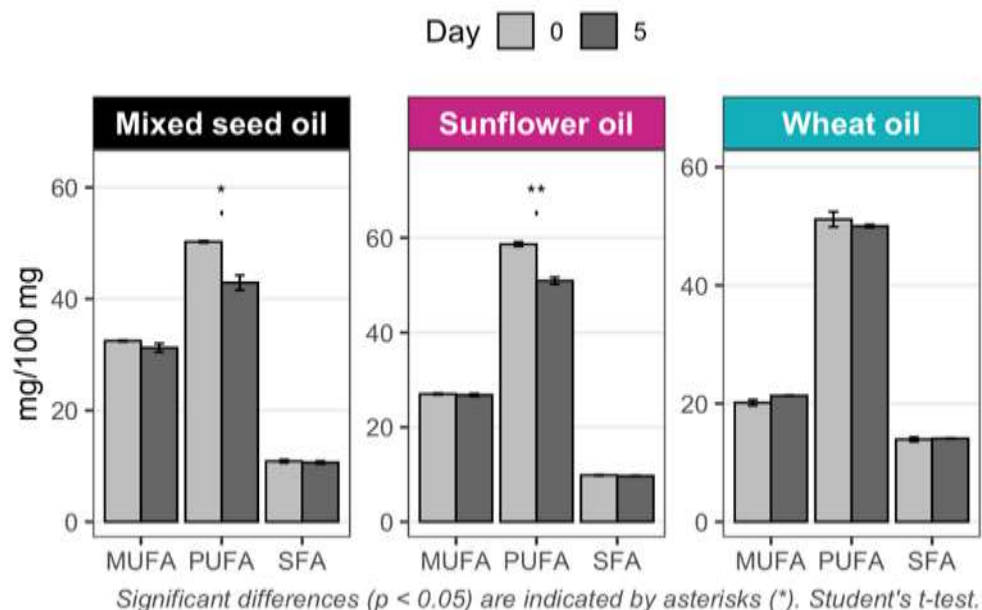


Figure 5: Comparison of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid contents in MSO, SO, and WGO at day 0 and day 5 of frying. Results are expressed as mg per 100 mg of oil.

3.3 Volatile Compounds

The heat map illustrates the evolution of seven major volatile compounds (Acetone, Pentanal, Hexanal, 2,4-Heptadienal, 1-Octen-3-ol, Nonanal, and 2-Octenal) throughout the five-day frying period in Wheat germ oil (WO), Sunflower oil (SO), and Mixed seed oil (MSO). Color intensity represents the relative concentration of each compound, ranging from deep blue (lowest intensity) to deep red (highest intensity), allowing a visual interpretation of oxidative progression rather than quantitative comparison (Figure 6). The heat map clearly differentiates between fresh and oxidized samples. On the left side of the map, Day 0 samples of all three oils are grouped together and appear predominantly in shades of blue, reflecting low volatile compound concentrations and higher freshness. In contrast, Day 5 samples are clustered on the right, characterized by red and orange tones indicative of advanced oxidation. This visual clustering confirms that the frying process caused a consistent increase in oxidation-derived volatiles. The transition from cool to warm colors across the frying period depicts the degradation trajectory common to all oils, although the rate and extent of oxidation varied. Hexanal showed intense positive correlation in Day 5 samples of SO, indicating a strong increase in its relative concentration during frying. Pentanal and 2,4-heptadienal reached their highest correlation in WO by Day 5, showing a clear rise in these volatiles toward the end of the frying period. Nonanal exhibited strong correlation in MO, particularly at Day 5, suggesting a notable accumulation over time. Acetone and 1-octen-3-ol presented moderate to high color intensities in SO and WGO, with

evident increases compared to their initial levels. SO displayed the most drastic color shift from blue to deep red by Day 5, clearly distinguishing it from the other two oils. This pattern reflects a stronger accumulation of volatile compounds and a faster overall oxidation rate. WGO presented a distinctive distribution, with intense positive correlation with acetone and 2,4-heptadienal. Although its overall color intensity was slightly lower than SO, the localized increase in these volatiles indicates that oxidation was concentrated in specific compounds rather than occurring uniformly across all markers. MSO showed a more balanced color pattern, with moderate increases in positive correlation across all compounds by Day 5. Compared to SO and WGO, this gradual change suggests a slower and more stable oxidative progression during repeated frying. The overall color gradient observed in the heat map visually reinforces the progressive degradation pattern evidenced by other oxidation indices. The left-to-right color transition mirrors the increase in TPC and TOTOX over time, confirming that the accumulation of volatile compounds aligns with the overall oxidative deterioration of the oils.

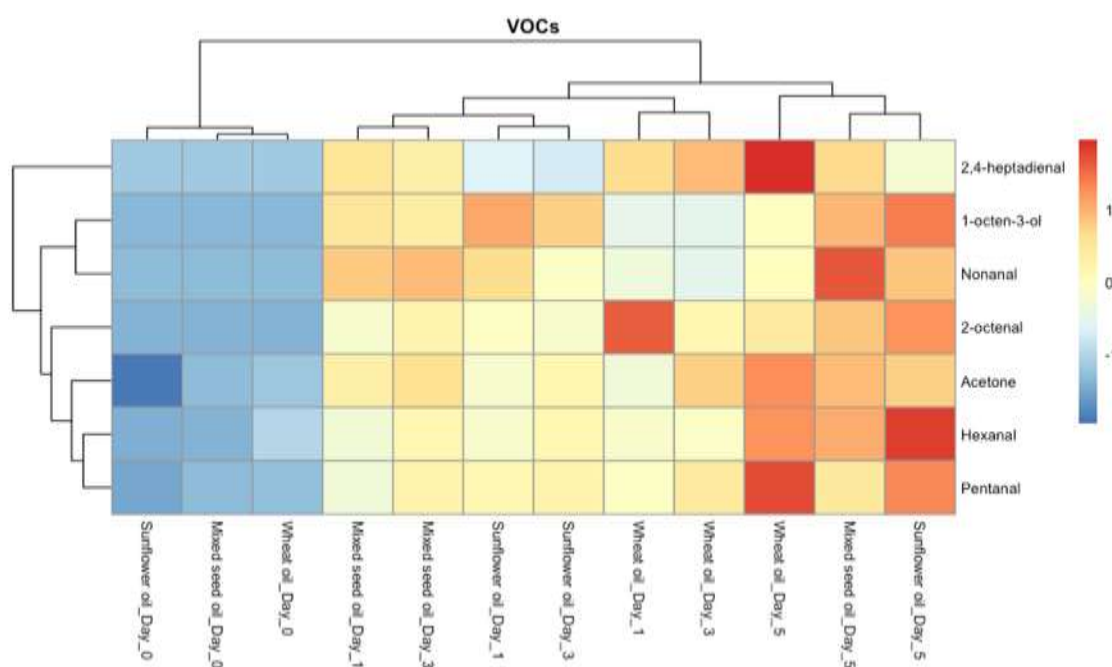


Figure 6. Heat map representation of seven volatile compounds (acetone, pentanal, hexanal, 2,4-heptadienal, 1-octen-3-ol, nonanal, and 2-octenal) detected in SO, MSO, and wheat germ oil WO samples during five days of frying. Color intensity ranges from deep blue (low concentration) to deep red (high concentration).

3.4 Sensory analysis

3.4.1 Color

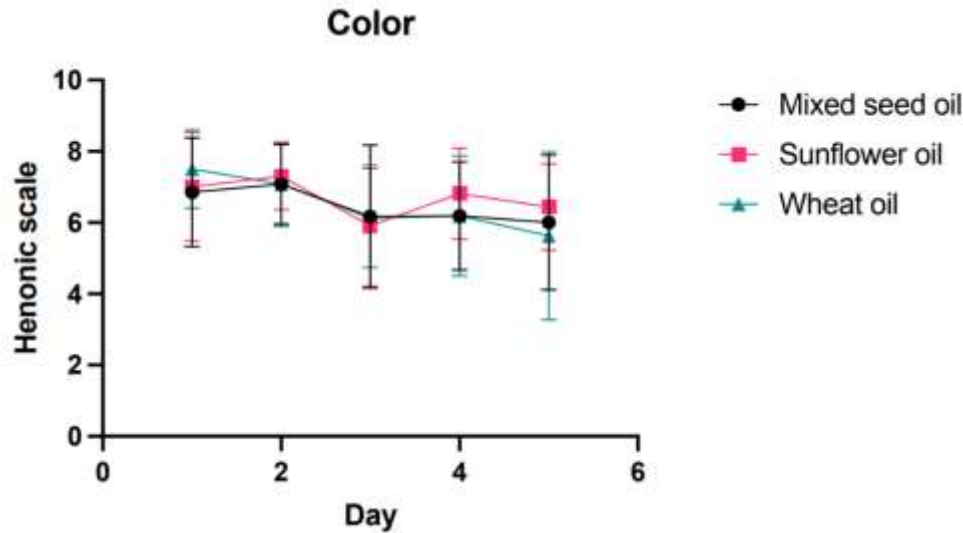


Figure 7. Changes in color scores of French fries fried in WGO, SO and MSO over five consecutive frying days. Scores were assigned by consumers using a 9-point hedonic scale (1 = extremely unpleasant, 9 = extremely pleasant). Different lowercase letters indicate significant differences ($p < 0.05$) among days within the same oil, while uppercase letters denote differences among oils on the same day.,

The color evaluation (Figure 7) of French fries obtained with different oils revealed distinct patterns of change during the 5-day frying process. In SO, the color index showed minor variations in the first two days, followed by a marked decline on Day 3. Although partial recovery was observed afterwards, the overall trend indicated progressive darkening of the fries over time, a phenomenon generally associated with reduced consumer acceptability in fried products (Pedreschi, 2012; Kita, 2014). This behavior suggests that oil oxidation and the accumulation of degradation compounds accelerated browning reactions during frying, resulting in darker color and lower visual acceptability in the later stages. MSO showed a numerically stable color trend throughout the frying process, with only minor fluctuations across the five days. The variations were small and not statistically significant, indicating that, overall, all oils experienced a similar extent of color change during repeated frying. WGO exhibited the strongest and most consistent decline in color values. Starting from the highest initial brightness, its scores progressively decreased throughout the frying period, with no recovery phase. The continuous loss indicates higher pigment oxidation or polymerization reactions, consistent with its tendency toward faster darkening. Overall, all oils showed a similar reduction in color quality as frying progressed, with no statistically significant differences in the extent of decline among them.

3.4.2 Aroma Score

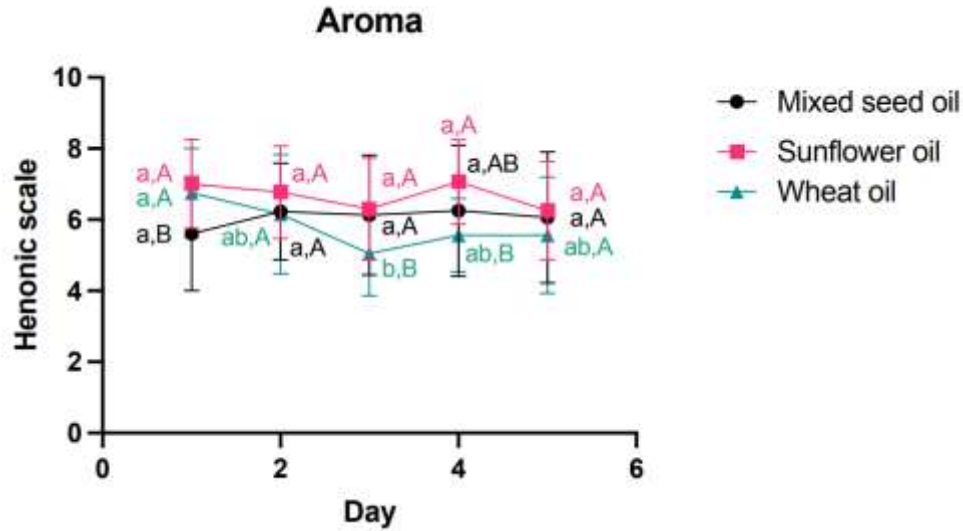


Figure 8. Changes in aroma scores of French fries fried in SO, MSO, and WGO during five consecutive frying days. Aroma was evaluated using a 9-point hedonic scale (1 = extremely unpleasant, 9 = extremely pleasant). Values represent mean sensory scores. Different lowercase letters indicate statistically significant differences ($p < 0.05$) within each oil over time.

The aroma scores (Figure 8) showed distinct but generally moderate variations during the frying period. In SO, the scores fluctuated slightly, but no statistically significant changes were detected across the five days, indicating stable aroma perception despite minor day-to-day variations. In MSO, the aroma remained almost unchanged throughout the frying cycles, with small, non-significant variations. However, SO showed the most consistent aroma quality overall, maintaining greater stability under thermal stress. In contrast, WGO exhibited a significant decrease in aroma between Day 1 and Day 3, after which the scores stabilized. This decline reflects early oxidative changes that affected sensory quality, followed by a steady phase toward the end of the experiment. Overall, the results indicate that mixed seed oil preserved its aroma most effectively, sunflower oil remained stable without significant variation, and wheat germ oil experienced a notable early decline before reaching equilibrium.

3.4.3 Taste

The evolution of taste scores (Figure 9) revealed distinct stability behaviors among the oils. SO maintained the most stable taste profile across the frying period. The values remained nearly constant during the first three days, followed by a gradual but not significant decline toward the end. This stability suggests that SO was less affected by oxidative by-products and retained its sensory acceptability better than the other oils.

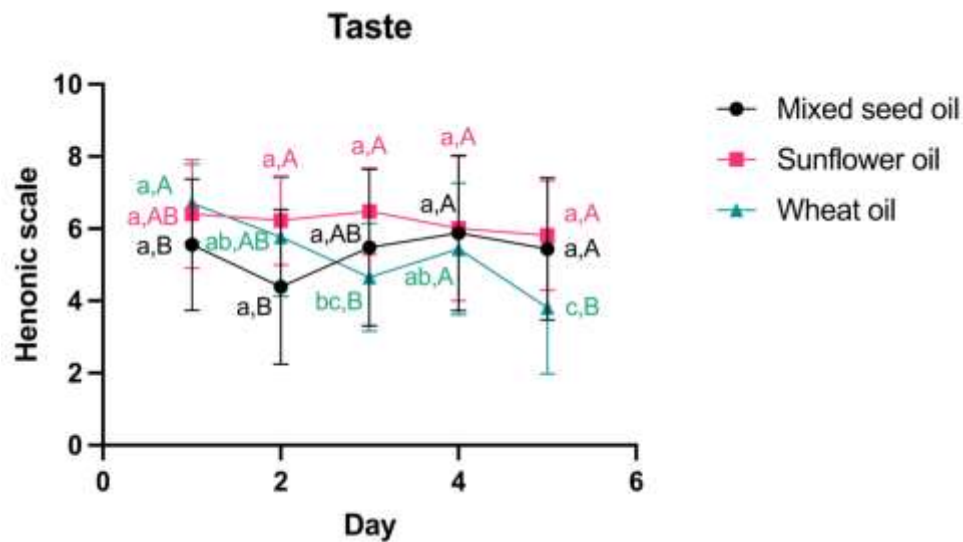


Figure 9. Changes in taste scores of French fries fried in WGO, SO, and MSO during five consecutive frying days. Taste was evaluated using a 9-point hedonic scale (1 = extremely unpleasant, 9 = extremely pleasant). Different lowercase letters indicate significant differences ($p < 0.05$) among days within the same oil, while uppercase letters denote differences among oils on the same day

WGO, in contrast, showed a progressive and statistically significant deterioration. The most notable decrease occurred between Days 2 and 3, marking the sharpest sensory decline among the oils. the final taste score on Day 5 remained the lowest overall, confirming WO's high susceptibility to degradation under repeated heating. MSO displayed greater variability without a defined trend. After an initial decrease, the taste improved mid-experiment before dropping again on the final day. Overall, SO demonstrated the greatest taste stability, MSO exhibited moderate fluctuations, and WGO underwent the most pronounced sensory decline.

3.4.4 Crispiness

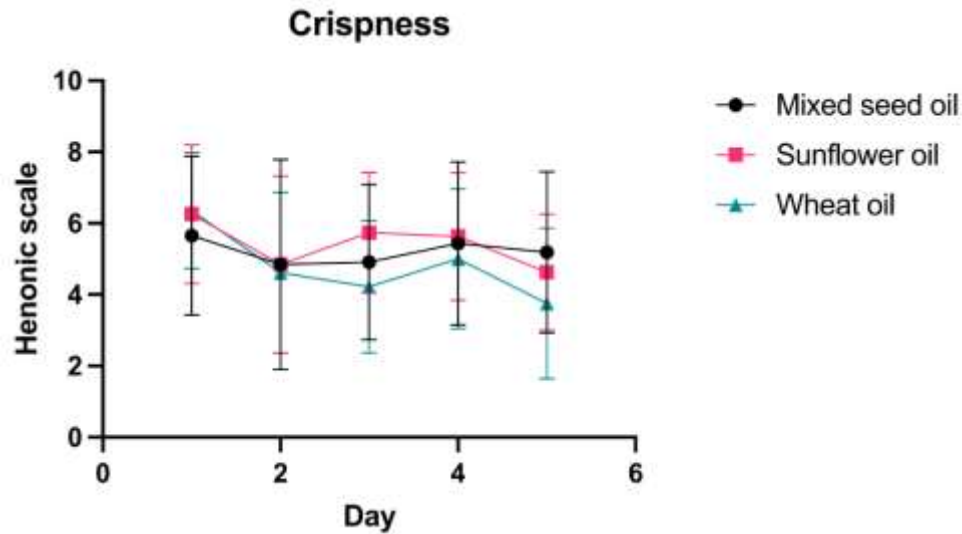


Figure 10. Changes in crispiness scores of French fries fried in WGO, SO, and MSO over five consecutive frying days. Crispiness was evaluated using a 9-point hedonic scale (1 = extremely unpleasant, 9 = extremely pleasant). Different lowercase letters indicate significant differences ($p < 0.05$) among days within the same oil, while uppercase letters denote differences among oils on the same day.

The evolution of crispiness revealed distinct degradation patterns among the oils. SO exhibited pronounced fluctuations rather than a steady trend. After an initial drop from high crispiness to a lower value on Day 2, the texture recovered noticeably on Day 3, suggesting transient moisture loss and structural tightening. However, the score declined again toward the end of frying, indicating that prolonged heating ultimately weakened texture integrity despite temporary recovery. WGO showed the most consistent and significant loss of crispiness throughout the experiment. The sharp decline during the first two days reflected early surface softening, likely due to oxidative polymerization and oil absorption. Although a modest recovery occurred on Day 4, the final value was the lowest among all oils, confirming severe texture degradation with continued frying. MSO maintained the highest crispiness stability overall. While minor decreases occurred early in the process, the texture partially recovered and stabilized toward the end, with the final score nearly matching the initial one. This steady behavior suggests a more balanced fatty acid composition, allowing for better heat performance and reduced textural breakdown. Overall, all oils showed minor variations in crispiness during the frying period, but none of the changes were statistically significant either among the different oils or across the days within the same oil. The general trend suggests stable crispiness perception throughout the frying cycles.

3.4.5 Overall acceptance

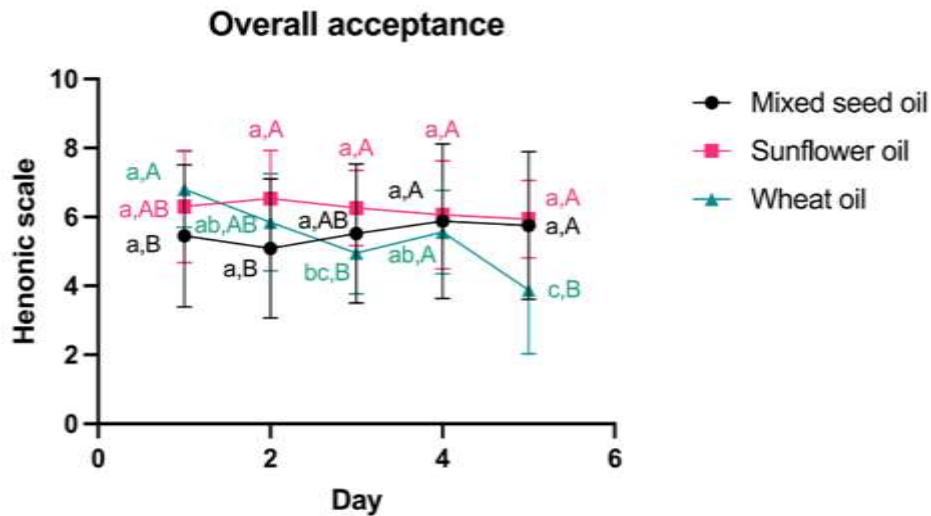


Figure 11. Changes in overall acceptance scores of French fries fried in WGO, SO, and MSO during five consecutive frying days. Overall acceptance was evaluated using a 9-point hedonic scale (1 = extremely unpleasant, 9 = extremely pleasant). Different lowercase letters (a–f) indicate significant differences ($p < 0.05$) within the same oil over time, while uppercase letters (A–C) denote differences ($p < 0.05$) among oils at the same time point.

Throughout the frying period, the overall acceptance of fries showed clear differences among the tested French fries in the different oils. French fries with SO maintained a consistently stable profile, with only minor fluctuations over time, confirming its suitability and reliability for repeated frying. Its acceptability remained high from the beginning to the end of the experiment, with no statistically significant variations across days. In contrast, WGO exhibited a progressive and statistically significant decline in overall acceptance. While its initial score was comparable to that of sunflower and mixed seed oils on the first day, the sensory quality deteriorated sharply over subsequent frying cycles. By the end of the experiment, WGO recorded the lowest acceptance values, indicating that thermal degradation and volatile accumulation negatively impacted consumer perception. MSO showed moderate variability but remained relatively stable, with final values close to those of SO. Its fluctuations were minor and did not represent a significant decline in perceived quality. Overall, SO demonstrated the highest and most stable overall acceptance, while WGO, although initially comparable to the other oils, showed a significant decline in overall acceptance, particularly between Day 3 and Day 5. These findings confirm that while WGO can perform acceptably at the early stages of frying, its suitability diminishes with repeated cycles compared to SO and MSO.

3.4.6 Willingness to buy

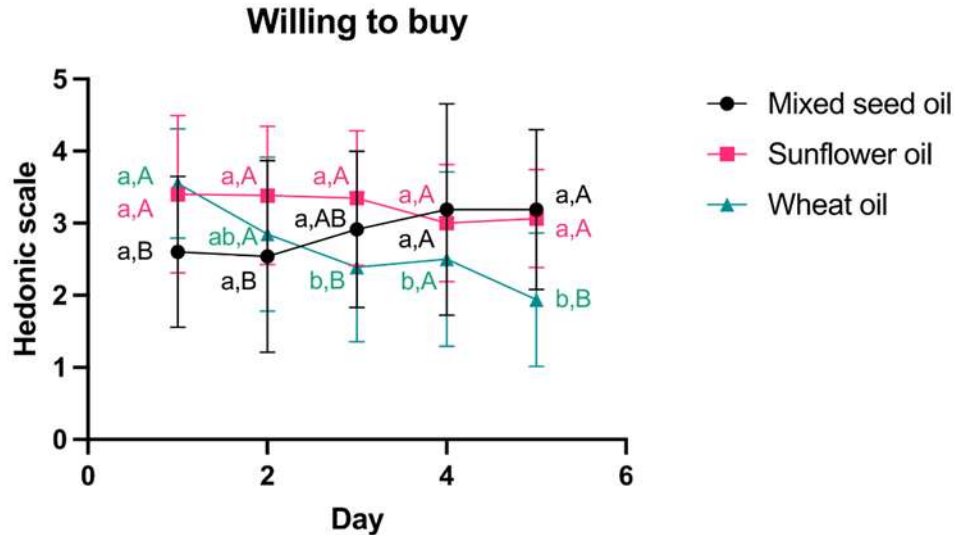


Figure 12. Changes in willingness-to-buy scores of French fries fried in WGO, SO, and MSO during five consecutive frying days. Willingness-to-buy was evaluated using a 9-point scale (1 = definitely would not buy, 9 = definitely would buy). Different lowercase letters (a–b) indicate statistically significant differences ($p < 0.05$) within the same oil over time, while uppercase letters (A–B) denote significant differences ($p < 0.05$) among oils at the same time point.

SO maintained a stable willingness to buy throughout the frying period. The scores fluctuated only slightly and showed no statistically significant changes over time. This steady trend reflects SO's consistent sensory performance and sustained consumer acceptability, even after multiple frying cycles. WGO, on the other hand, displayed a progressive and significant decline. The score dropped markedly after the second day and continued to decrease toward the end of the experiment. The final willingness to buy was the lowest among the three oils, confirming that prolonged frying substantially reduced consumer intention to purchase products fried in WGO. MSO exhibited a gradual improvement over time. After a minor initial decrease, the willingness-to-buy scores increased steadily from mid-frying onward, reaching their highest level by the end of the experiment. Despite these improvements, the differences were not statistically significant, indicating that MSO maintained a moderately stable trend rather than a true upward shift in consumer preference. SO demonstrated the highest stability in consumer willingness to buy, while WGO showed a significant loss of acceptance with extended frying. MSO presented moderate and fluctuating behavior, improving slightly toward the end. These findings suggest that while WGO can perform comparably at the beginning, its market acceptability declines under prolonged use, whereas SO maintains consumer appeal across all frying stages.

3.5 CATA Results

Descriptor	WGO	SO	MSO
Oily aspect	23	24	23
Golden color	68	70	76
Dark color	1	1	0
Pleasant aroma	24	36	60
Mild aroma	54	48	37
Unpleasant aroma	14	7	21
Fish-like aroma	21	3	17
Pleasant taste	16	40	22
Burnt taste	1	0	2
Mild taste	37	34	18
Oily taste	21	26	31
Rancid taste	7	2	11
Spicy taste	0	0	1
Fish-like taste	16	3	18
Green taste	17	10	19
Crispiness	29	36	37
Lack of crispiness	49	44	42
Chewy	19	17	24
Participant number	88	88	88

Table 1: Frequency of sensory descriptors selected by consumers in the CATA evaluation of fries fried in Wheat Germ Oil (WGO), Sunflower Oil (SO), and Mixed Seed Oil (MSO).

The sensory evaluation using the Check-All-That-Apply (CATA) method provided insights into consumers' perception of fries fried in the three tested oils. The results are presented separately for wheat germ oil (WGO), sunflower oil (SO), and mixed seed oil (MSO), considering four sensory dimensions: appearance, aroma, taste, and texture. The frequency of each descriptor selection was expressed as a percentage of participants who identified that attribute. Consumers characterized fries based on appearance, aroma, taste, and texture descriptors.

French fries fried in WGO were predominantly described as golden (77.3%), indicating that the oil preserved an appealing visual quality during frying. The lower frequency of oily aspect (26.1%) suggests that surface oiliness was noticeable but not dominant, while the very low selection of dark color (1.1%) confirms that visual degradation or excessive browning did not occur. The aroma profile was mostly mild (61.4%) and occasionally pleasant (27.3%), showing an overall neutral aromatic character. However, the appearance of unpleasant (15.9%) and fish-like (18.2%) notes indicates that slight off-odors were perceived by part of the panel, especially during later frying cycles. The predominance of mild (42.0%) and pleasant (23.9%) taste descriptors reflects a

generally acceptable flavor perception. Still, the moderate presence of oily (23.9%), green (19.3%), and fish-like (18.2%) tastes suggests early development of atypical or oxidation-related notes. Rancid (8.0%) and burnt (1.1%) tastes were rare, confirming that intense off-flavors remained limited. Lack of crispiness was the most recurrent attribute (55.7%), indicating that fries fried in WGO were perceived as relatively soft. The lower selection of crispiness (33.0%) and chewy texture (21.6%) supports this interpretation. Overall, WGO produced fries with a pleasant appearance but a softer texture and some early negative flavor notes.

Golden color (79.5%) dominated the visual perception of SO-fried fries, indicating a consistently attractive surface appearance. The moderate selection of oily aspect (27.3%) suggests normal oil absorption, while the rarity of dark color (1.1%) confirms stable color quality throughout frying. The high frequency of mild (54.5%) and pleasant aroma (40.9%) shows that SO maintained a stable and generally agreeable aromatic profile. The very low presence of unpleasant (8.0%) and fish-like (3.4%) descriptors indicates minimal off-odor development, even after repeated frying cycles. Pleasant (45.5%) and mild (38.6%) tastes were predominant, reflecting high flavor acceptability. Although oily taste was mentioned moderately (29.5%), negative descriptors such as green (11.4%), fish-like (3.4%), and rancid (2.3%) remained infrequent, confirming that SO produced the most stable flavor profile among the oils. Lack of crispiness (50.0%) slightly exceeded crispiness (40.9%), indicating moderate texture loss during frying. However, compared with WGO, crispiness was better retained. Chewy texture (19.3%) was relatively uncommon, supporting a generally favorable texture perception.

The very high frequency of golden color (86.4%) shows that MSO produced the visually most appealing fries. Oily aspect (26.1%) was moderate, while dark color (0.0%) was never selected, confirming excellent visual quality across all frying cycles. Pleasant aroma (68.2%) was dominant, suggesting strong aromatic appeal. Mild aroma (42.0%) was also common, but the noticeable presence of unpleasant (23.9%) and fish-like (19.3%) descriptors indicates some aromatic instability during prolonged frying. The high frequency of oily taste (35.2%) suggests that MSO imparted a richer or heavier flavor. Pleasant (25.0%) and mild (20.5%) tastes remained important contributors to acceptability, although green (21.6%) and fish-like (20.5%) tastes indicate more variable sensory behavior. Rancid (12.5%) and burnt (2.3%) were infrequent but higher than in SO, suggesting a slightly less stable flavor profile. The similar frequencies of lack of crispiness (47.7%) and crispiness (42.0%) reflect intermediate texture stability. Chewy texture (27.3%) was more common than in the other oils, indicating slightly greater textural softening over time.

Overall, golden color was highly perceived across all oils, with the highest frequency in MSO (86.4%), indicating superior visual appeal. In terms of aroma, MSO exhibited the strongest pleasant aroma (68.2%), while SO maintained the most balanced and stable aromatic profile with minimal unpleasant or fish-like notes. Regarding taste, pleasant taste was most frequent in SO (45.5%), followed by MSO (25.0%) and WGO (23.9%). Negative taste descriptors such as fish-like and rancid were more associated with WGO and MSO, suggesting slight oxidative or flavor degradation. Texturally, lack of crispiness was common across samples, though MSO and SO

maintained better crispiness perception compared to WGO. In summary, fries fried in SO received the most favorable sensory balance overall, characterized by a stable aroma, pleasant taste, and good appearance, while mixed seed oil offered excellent color but slightly more aroma and taste variability. WGO acceptable sensory qualities but tended toward a softer texture and minor off-flavor notes.

4. Discussion

The sustained oxidative stability of frying oils is a critical determinant of both their nutritional value and practical usability during high-temperature cooking. Lipid oxidation follows a free radical chain mechanism, comprising initiation, propagation, and termination stages that accelerates markedly under the elevated temperatures and oxygen exposure characteristic of deep frying (Erickson et al., 2023; Taghvaei & Jafari, 2015). To systematically evaluate the oxidative degradation kinetics of the tested oils such as wheat germ oil (WGO), sunflower oil (SO), and mixed seed oil (MSO), five classical indices were monitored: Peroxide Value (PV), *p*-Anisidine Value (*p*-AV), Total Oxidation Value (TOTOX), Free Fatty Acid (FFA) content, and Total Polar compounds (TPC) (Choe & Min, 2007).

The observed differences in PV among the oils highlight the protective effect of endogenous antioxidants in WGO against primary oxidation. While SO exhibited rapid hydroperoxide formation and subsequent decomposition, WGO maintained low PV levels throughout frying, indicating its superior oxidative stability. This behavior underscores the role of tocopherols and phytosterols in stabilizing highly unsaturated fatty acids under thermal stress, consistent with previous reports demonstrating the protective effect of natural antioxidants in vegetable oils (Erickson et al., 2023; Ghafoor et al., 2017). MSO showed intermediate stability, suggesting that blending oils can partially enhance oxidative resistance but may not match the inherent stability conferred by WGO's antioxidant-rich profile. These findings support the potential of WGO as a functional frying medium capable of preserving oil quality during repeated heating, aligning with literature emphasizing the importance of antioxidant content in mitigating lipid peroxidation during deep-frying (Paško et al., 2025; Aslan et al., 2021).

The *p*-Anisidine Value (*p*-AV) (Figure 2), reflects the formation of secondary oxidation products, mainly aldehydes and ketones, resulting from hydroperoxide decomposition. During frying, SO showed a sharp and rapid increase in *p*-AV, especially after the second day, indicating accelerated aldehyde generation and advanced oxidative degradation. This rise corresponded with the decline in its PV, confirming the thermal breakdown of primary oxidation products. In contrast, WGO exhibited the lowest and slowest *p*-AV increase, signifying stronger resistance to secondary oxidation. This stability is attributed to its rich content of α -tocopherol, phytosterols, and phenolic compounds, which effectively neutralize free radicals and stabilize hydroperoxide intermediates. These results align with the reports of Aslan et al. (2021), Ma et al. (2023), and Erickson et al. (2023), who all emphasized the protective role of endogenous antioxidants in delaying secondary

oxidation under thermal stress. The MSO displayed intermediate behavior; the slower *p*-AV increase observed in WGO and MSO compared with SO demonstrates the pivotal influence of antioxidant composition in limiting carbonyl compound accumulation and improving oxidative stability.

To provide a holistic measure of oxidative deterioration, the TOTOX index was evaluated. This combined indicator integrates both primary and secondary oxidation stages and is widely used to assess the overall oxidative status of frying oils (Farhoosh & Hoseini-Yazdi, 2013). As expected, SO displayed the highest and fastest TOTOX increase, reaching approximately 150 by Day 5, reflecting substantial oxidation and thermal decomposition of its polyunsaturated fatty acids. In contrast, WGO maintained the lowest TOTOX levels throughout frying, confirming its robust oxidative stability and efficient antioxidant defense system. MSO exhibited a moderate TOTOX trajectory between SO and WGO, demonstrating the partial protective effect of oil blending and antioxidant synergy. The overall pattern reinforces the hierarchy of oxidative resistance (WGO > MSO > SO), in agreement with recent reports by Erickson et al. (2023) and Razem et al. (2025), which highlight that incorporating antioxidant-rich or wheat-derived oils can effectively lower TOTOX development during repeated frying.

The Free Fatty Acid (FFA) content, which indicates the extent of hydrolytic cleavage of triacylglycerols, increased gradually in all oil samples throughout the frying period. This rise results from the hydrolysis of ester bonds under high temperature and the presence of water vapor released from the French fries, which accelerates the breakdown of glycerides into free fatty acids (Nayak et al., 2016). SO exhibited slightly higher initial FFA values, likely due to a greater proportion of unsaturated triacylglycerols that are more susceptible to hydrolysis, while the overall rate of increase remained comparable among WGO, MSO, and SO. These findings are consistent with the general pattern reported in the literature, where repeated deep-frying cycles lead to progressive FFA accumulation due to combined thermal and moisture-induced hydrolysis (Hosseini et al., 2016; Ujong et al., 2023). Importantly, although the extent of hydrolysis was similar across the three oils, the oxidative behavior diverged significantly, implying that compositional factors such as antioxidant content and fatty acid saturation exerted greater influence on oxidative rather than hydrolytic degradation. This observation aligned with the view that FFA accumulation can indirectly accelerate oxidation by increasing the oil's polarity and promoting metal-catalyzed reactions, thereby linking hydrolytic degradation with subsequent oxidative instability (Choe & Min, 2007).

The Total Polar Compounds (TPC) content represents the accumulation of oxidized, dimerized, and polymerized triglycerides, key indicators of oil deterioration and frying life. TPC is widely recognized as the most reliable criterion for determining frying oil discard limits, with values exceeding 24–27% generally considered unacceptable for consumption (Choe & Min, 2007; Bansal et al., 2010). In the present study, TPC increased steadily in all samples during the five days of frying period, but the rate of accumulation differed significantly among the oils. SO showed the most rapid rise, surpassing 20% by Day 5, a level approaching commonly cited discard limits (24-

27%) for frying oils, as recommended in several quality guidelines (Choe & Min, 2007; Bansal et al., 2010), indicating extensive thermal and oxidative degradation. In contrast, WGO exhibited the slowest TPC formation, while MSO showed intermediate behavior, consistent with its blended fatty acid and antioxidant profile. This hierarchy confirms that the non-saponifiable constituents of WGO, particularly tocopherols, phytosterols, and other minor bioactive that enhance triacylglycerol stability by suppressing polymerization and limiting the formation of high-molecular-weight oxidation products (Erickson et al., 2023; Chen et al., 2021). The observed TPC trends closely parallel those of PV, *p*-AV, and TOTOX, reinforcing the established oxidative stability ranking of WGO > MSO > SO.

The Fatty Acid Methyl Ester (FAME) analysis provides a critical understanding of the compositional and structural transformations that occur in oils during deep frying. Fatty acid distribution governs an oil's thermal and oxidative stability since the degree of unsaturation determines susceptibility to oxidation, polymerization, and isomerization under high-temperature conditions (Erickson et al., 2023; Ma et al., 2023).

The degradation kinetics observed through FAME analysis indicated that polyunsaturated fatty acids (PUFAs), particularly linoleic and linolenic acids, undergo the most rapid transformation during frying. Their multiple double bonds act as primary initiation sites for oxidation, resulting in hydroperoxide formation and subsequent fragmentation into smaller volatile aldehydes (Zou et al., 2018; Erickson et al., 2023).

In SO, which initially contained the highest PUFA proportion, the results showed a marked reduction in linoleic acid content after repeated frying cycles. This significant PUFA depletion paralleled the steep increase in PV and TOTOX observed earlier, illustrating a direct link between compositional and oxidative degradation, consistent with Ujong et al. (2023), that has reported similar behavior in PUFA-rich oils. The relative increase in SFA (Figure 5) was interpreted as a concentration effect, caused by the preferential oxidation of unsaturated bonds rather than actual fatty acid synthesis (Farhoosh & Hoseini-Yazdi, 2013; Liu et al., 2024). That instability is primarily attributed to the high sensitivity of linoleic acid (C18:2), the dominant PUFA in SO, to oxidation during prolonged thermal exposure. The total PUFA content in SO significantly ($p < 0.05$) decreased from 58.66% on Day 0 to 50.93% on Day 5. This 7.73% absolute decline reflects substantial degradation of polyunsaturated fatty acids during frying and is consistent with literature reports indicating marked linoleic acid loss under thermal stress (Nagy et al., 2024). Together, these changes position SO as the least stable frying medium among the oils tested, with pronounced compositional and oxidative deterioration under repeated heating. In contrast, WGO demonstrated exceptional resistance to compositional alteration. Over the 5 day frying period, PUFA content declined only marginally from 51.18% to 50.04%, MUFA slightly increased from 20.17% to 21.35%, and SFA remained stable at approximately 14–15% (Figure 5). This minimal change in fatty acid distribution suggests a robust oxidative stability profile. These findings are consistent with previous literature reporting high PUFA levels in WGO, typically ranging from 54% to 58%, with MUFA between 14–20% and SFA values around 16–18% (Sakhawat Ali et al., 2013; Kara,

2016). Although the PUFA levels in our sample are slightly lower, the observed stability under thermal stress aligns with studies showing that WGO maintains its fatty acid composition even under heating conditions (Özcan et al., 2015). WGO's compositional resilience is attributed to its abundance of α -tocopherol, along with β - and γ -tocopherols, phytosterols, and carotenoids, all of which act synergistically to neutralize peroxy radicals and stabilize hydroperoxide intermediates (Aslan et al., 2021; Bartolini et al., 2022; Ghafoor et al., 2017). α -Tocopherol acts as a chain-breaking antioxidant by donating hydrogen atoms to peroxy radicals ($\text{ROO}\cdot$), thereby terminating the propagation phase of lipid oxidation (Erickson et al., 2023). Moreover, its higher saturated fatty acid fraction (13.95%) (Figure 5) provides additional thermal resilience (Ma et al., 2023). The interplay between high antioxidant concentration and moderate saturation explains the preservation of WGO's unsaturated structure despite prolonged exposure to frying conditions. This observation aligns with Zou et al. (2018), who reported that controlled thermal treatment of WGO enhances stability by releasing bound phenolics and forming Maillard-derived stabilizers. Similar findings have been noted in Sacha Inchi oil, where high tocopherol levels mitigated degradation despite high unsaturation (Rodríguez et al., 2021). Thus, the FAME data reinforce that oxidative resistance is not determined solely by PUFA content, but rather by the dynamic balance between unsaturation and antioxidant protection, a defining characteristic of WGO's stability.

MSO exhibited an intermediate degradation pattern, characterized by moderate PUFA reduction and partial preservation of oleic acid (MUFA) (Figure 5). This relative oxidative resistance is due to the lower susceptibility of oleic acid to peroxidation compared to linoleic or linolenic acids, owing to its single double bond and reduced tendency to form conjugated intermediates (Erickson et al., 2023; Ma et al., 2023). The intermediate behavior of MSO in oxidative and sensory parameters suggests the presence of both unsaturated fatty acids and some level of antioxidant protection. This was reflected in its moderate TOTOX and TPC levels throughout the frying cycles. The relative stability may be attributed to potential blending effects, consistent with previous findings that oil blends with balanced MUFA:PUFA ratios can slow oxidative progression compared to high-PUFA single oils (Ujong et al., 2023; Ma et al., 2023). While the FAME data alone do not confirm exact formulation strategies, the observed trends support the broader concept that combining oils of differing oxidative profiles can improve thermal resilience under repeated frying conditions (Erickson et al., 2023; Taghvaei & Jafari, 2015; Razem et al., 2025). The depletion of polyunsaturated fatty acids (PUFAs), particularly in SO and MSO, represents a loss of essential fatty acids (linoleic and linolenic acids), thereby reducing the nutritional value of both the oil and the fried product (Erickson et al., 2023).

Volatiles organic compounds (VOCs), derived from the oxidative cleavage of unsaturated fatty acids, are primarily responsible for rancid odors and off-flavors. GC-MS analysis identified hexanal, 2,4-heptadienal, and nonanal as key contributors, served as principal oxidation markers, reflecting both the chemical and sensory evolution of the oils. Volatile aldehydes arise from the decomposition of lipid hydroperoxides formed during the propagation stage of oxidation. Hexanal, the most abundant volatile across all samples, is primarily generated through the thermo-oxidation

of linoleic acid (C18:2), forming via cleavage of the 13-hydroperoxide intermediate (Choe & Min, 2007; Wang & Shahidi, 2017). 2,4-Heptadienal originates from the oxidation of linolenic acid (C18:3) through conjugated diene intermediates, indicating a higher degree of unsaturation and oxidative stress (Zou et al., 2018). Nonanal results mainly from the oxidation of oleic acid (C18:1) and signifies degradation of monounsaturated hydroperoxides (Hosseini et al., 2016). SO showed the highest and fastest accumulation of hexanal and nonanal, particularly in the early frying stages (Figure 6). The early surge in hexanal corresponded with the initial PV spike and subsequent rise in TOTOX index, confirming the rapid oxidative deterioration typically seen in PUFA-rich oils (Ujong et al., 2023; Wang & Shahidi, 2017). This pattern agrees with literature, confirming hexanal as a primary volatile marker of linoleic acid peroxidation, and its sharp increase strongly reflects the onset of lipid oxidation (Zhang et al., 2022; Nogueira et al., 2019). In thermally degraded SO, the TOTOX elevation and hexanal formation has been widely validated as a hallmark of early oxidative stress (Oakley et al., 2018).

In contrast, WGO maintained significantly lower total volatile levels. Despite its high PUFA content, 51.18% at Day 0, (Figure 5), its rich endogenous antioxidant profile, chiefly α -tocopherol, phytosterols, and phenolic compounds, effectively inhibited hydroperoxide decomposition and delayed aldehyde formation (Aslan et al., 2021; Bartolini et al., 2022). The GC–MS chromatograms revealed a notable late-stage accumulation of 2,4-heptadienal in WGO, with the highest intensity observed on Day 5, while hexanal and nonanal remained relatively stable throughout the frying period (Figure 6). This pattern underscores WGO's selective oxidative stability, where its robust radical-scavenging and hydroperoxide-stabilizing capacity effectively delayed general aldehyde formation. However, despite only a marginal reduction in linolenic acid (C18:3) content of 0.19% over five days, thermally sensitive volatiles such as 2,4-heptadienal still emerged in the final stages, indicating that even minimal compositional changes in highly unsaturated fractions can yield potent odor-active compounds under extended thermal exposure (Erickson et al., 2023; Ma et al., 2023).

MSO exhibited an intermediate volatile formation pattern, showing moderate accumulation of hexanal and a distinctly higher concentration of nonanal by Day 5, while 2,4-heptadienal intensity remained higher than in SO. The elevated nonanal levels correspond to MSO's relatively high oleic acid (C18:1) fraction (≈ 31), suggesting that oxidation in this oil is influenced by the oleic acid pathway rather than the polyunsaturated degradation routes typical of SO and WGO. The balanced distribution of PUFAs and MUFAs, together with the moderate presence of endogenous antioxidants, contributed to a more gradual oxidative progression, consistent with its intermediate TOTOX and TPC profiles. These trends align with Zhang et al. (2022), who reported strong correlations ($R = 0.70\text{--}0.94$) between aldehyde concentrations particularly hexanal, nonanal, and (E,E)-2,4-heptadienal—and the *p*-AV of thermally oxidized oils, highlighting the compositional control of volatile generation. Likewise, Razem et al. (2025), demonstrated that supplementing frying oils with wheat-bran phenolics significantly reduced these aldehydes, illustrating the antioxidant efficiency of wheat-derived bioactives analogous to those naturally present in WGO.

Although WGO exhibited the lowest TOTOX and overall chemical stability, the late-stage rise in 2,4-heptadienal was associated with a distinct decline in sensory scores, characterized by fishy or oily off-flavors (Zou et al., 2018; Zhang et al., 2022).

The sensory evaluation represents a crucial measure of frying oil performance, directly linking chemical degradation processes to consumer perception. In the present study, hedonic scores and Check-All-That-Apply (CATA) data were used to evaluate the sensory quality and the results revealed clear relationships between the evolution of chemical oxidation indices such as PV, *p*-AV, TOTOX, and VOCs, and sensory attributes such as color, odor, taste, and texture. A consistent pattern emerged in which the generation of secondary oxidation products, measured by *p*-AV and summarized by TOTOX (Figure 3), directly corresponded to declining sensory scores (Farhoosh & Hoseini-Yazdi, 2013; Ma et al., 2023). These findings illustrate how chemical oxidation dynamics translate directly into sensory rejection, a relationship widely documented in frying oil studies (Hosseini et al., 2016; Zhang et al., 2022). SO maintained the most stable taste profile and the highest overall acceptability throughout the five-day frying period, while WGO initially exhibited comparable acceptability levels up to Day 3. However, a progressive sensory decline was observed thereafter, with statistically significant deterioration in taste by Day 5 (Figure 11). This pattern aligns with previous findings where the sensory acceptability of oils declined as a function of accumulated oxidation-derived volatiles, particularly aldehydes and ketones (Zou et al., 2018; Zhang et al., 2022). Similar observations were reported by Razem et al. (2025), who noted that oils rich in polyunsaturated fatty acids exhibit early flavor degradation due to the rapid formation of 2,4-alkadienals, despite showing temporary chemical stability. The contrasting trends between chemical and sensory parameters confirm a crucial finding. Although WGO exhibited the lowest TOTOX values and the highest overall chemical stability, a marked increase in 2,4-heptadienal was detected during the final frying stages. This rise was associated with a distinct decline in sensory scores, characterized by noticeable fishy and oily off-flavors (Zou et al., 2018; Zhang et al., 2022). Initially, WGO displayed sensory acceptability levels comparable to the other oils up to Day 3. However, from that point onward, a progressive and statistically significant deterioration in taste was observed, leading to the lowest overall acceptance by Day 5 (Figure 11). This pattern is consistent with previous findings, which reported that the sensory acceptability of frying oils decreases as oxidation-derived volatiles—particularly aldehydes and ketones—accumulate over time (Zou et al., 2018; Zhang et al., 2022).

The contrasting trends between chemical and sensory parameters highlight a critical insight: chemical stability does not necessarily ensure sensory persistence. Although WGO maintained low *p*-AV and stable TOTOX values, indicating effective chemical protection, its sensory quality declined sharply between Days 2 and 3. This discrepancy suggests that the oxidation pathway of WGO produced specific potent odor-active molecules, capable of triggering off-flavors even under relatively mild oxidation. The evolution of volatile organic compounds (VOCs), especially aldehydes, closely paralleled the increases in TPC and TOTOX, confirming their decisive role in driving sensory deterioration (Zhang et al., 2022; Razem et al., 2025). Hexanal, produced via

oxidation of linoleic acid (C18:2), accumulated rapidly in SO by Day 5. Associated with grassy, fatty, or green notes, hexanal's presence typically signals advanced oxidation. However, despite its high TOTOX and volatile accumulation, SO recorded the lowest frequency of negative sensory descriptors such as unpleasant aroma (8.0%) and rancid taste (2.3%), implying that its oxidation products, though abundant, were less sensory-active (Choe & Min, 2007; Hosseini et al., 2016; Wang & Shahidi, 2017). WGO's sensory decline was primarily linked to elevated concentrations of 2,4-heptadienal, formed from linolenic acid (C18:3). This compound, with its extremely low odor threshold, produced pronounced fishy and oily notes detected by 18.2% of the CATA panel. Despite WGO's chemical stability (low TOTOX) (Figure 3), the generation of this specific aldehyde was sufficient to cause rapid sensory rejection, aligning with findings by Zou et al. (2018) and Razem et al. (2025), who emphasized the high sensory potency of (E,E)-2,4-alkadienals. Nonanal, formed through the oxidation of oleic acid (C18:1), was most prominent in MSO by Day 5 (Figure 6). Since MSO contains the highest oleic acid content among the oils, it correspondingly generated the most amount of nonanal. Its fatty and slightly soapy sensory profile likely contributed to the moderate decline in acceptability (unpleasant aroma 23.9%, rancid taste 12.5%), aligning with previous reports linking nonanal accumulation to sensory degradation in oxidized oils (Shahidi & Zhong, 2010).

From a sensory standpoint, PUFA degradation directly promotes volatile aldehyde formation, particularly hexanal and 2,4-heptadienal. These aldehydes not only impart undesirable aromas but also act as oxidation toxicity markers due to their low sensory thresholds and cytotoxic potential (Wang et al., 2023; Erickson et al., 2023). By the fifth frying day, WGO samples displayed a unique volatile profile dominated by acetone and 2,4-heptadienal (Figure 6). Hence, the FAME analysis highlights a central paradox in frying oil performance: chemical stability does not inherently ensure sensory persistence. Effective stabilization strategies must therefore target both lipid molecular integrity and the suppression of odor-active aldehydes, for example, through antioxidant fortification or optimized oil blending (Taghvaei & Jafari, 2015). SO exhibited the highest chemical instability, as indicated by its elevated TOTOX (Figure 3) and PV values (Figure 2), yet maintained comparatively stable sensory performance throughout the frying cycles. This paradox is explained by the nature of its major volatile oxidation product, hexanal, which, although abundant, is less odor-intense and more tolerable to consumers than the potent aldehydes (e.g., 2,4-heptadienal) that dominated WGO's volatile profile (Zou et al., 2018; Zhang et al., 2022). Thus, despite accelerated oxidation, SO's oxidative by-products exerted milder sensory effects. MSO displayed intermediate chemical and sensory stability, marked by moderate TOTOX increases and balanced sensory attributes. Initially, MSO achieved the highest panel scores for *golden color* (86.4%) and *pleasant aroma* (68.2%), reflecting its favorable fatty acid balance and moderate antioxidant content (Table 1). However, as oxidation progressed, negative sensory descriptors such as unpleasant aroma (23.9%) and rancid taste (12.5%) increased proportionally, consistent with the rise in TPC, p-AV, and TOTOX values, indicating cumulative oxidative degradation. This behavior confirms that MSO's blended composition delayed, but did not prevent, oxidation-driven sensory decline (Ma et al., 2023; Ujong et al., 2023; Erickson et al., 2023).

All oils retained a generally golden color during the initial frying cycles, with gradual darkening observed toward the end of the experiment (Figure 7). This darkening correlated with increased TPC and the formation of polymerized carbonyl structures typical of prolonged thermal oxidation (Choe & Min, 2007; Nayak et al., 2016). Texturally, panelists noted a progressive loss of crispiness across all samples (Figure 10), consistent with oil absorption and surface polymerization that impair the structural integrity of fried products. These changes are well-documented outcomes of thermal stress and repeated oil use (Zhang et al., 2022).

5. Conclusion

This study aimed to assess whether wheat germ oil (WGO), an unconventional oil extracted from cereal by-products, could serve as a viable frying medium in comparison with conventional options such as sunflower oil (SO) and mixed-seed oil (MSO). The hypothesis was that WGO's rich antioxidant composition would confer superior thermo-oxidative stability, potentially enabling its valorization as a functional frying oil. The results confirm that WGO possesses outstanding chemical stability under frying conditions, outperforming SO and MSO across all oxidation indices. Its TOTOX values rose moderately from ~60 to ~80 over five days, compared to ~150 for SO and MSO. Similarly, WGO exhibited the slowest accumulation of total polar compounds, and its peroxide value remained under 5 meqO₂/kg, reflecting robust resistance to both primary and secondary oxidation. From a compositional standpoint, WGO maintained a remarkably stable fatty acid profile, with only marginal PUFA depletion and a consistent SFA content. That stability is attributable to its high concentration of natural antioxidants (especially α -tocopherol) and its relatively high SFA content, which collectively suppress free radical propagation and polymerization. These findings substantiate WGO's potential as a nutritionally rich and oxidation-resistant oil, suitable for food systems requiring chemical resilience. However, a critical limitation emerged from the sensory analysis. Despite its chemical integrity, WGO displayed a significant decline in sensory acceptance after Day 3, culminating in the lowest acceptability scores by day 5. Volatile analysis revealed late-stage accumulation of 2,4-heptadienal, a highly odor-active aldehyde derived from linolenic acid oxidation, perceived by 18.2% of panelists as producing "fish-like" off-flavors. This occurred even as Totox and TPC values remained low, revealing a decoupling between chemical and sensory stability. By contrast, SO and MSO, despite exhibiting higher oxidation and PUFA degradation maintained more stable sensory profiles. In SO, the high linoleic acid (C18:2) content led to the highest hexanal formation among the oils; however, because hexanal has a relatively high sensory threshold compared to other volatiles, its accumulation did not translate into stronger negative sensory perception. Thus, WGO's disadvantage lies not in its chemistry, but in its volatile profile, highlighting the paradox that superior oxidative stability does not inherently ensure sensory persistence.

Given these findings, WGO is not recommended as a standalone oil for industrial or prolonged frying applications. However, it may be highly suitable for short-term, single-use frying in domestic contexts, where its nutritional benefits can be fully realized without incurring significant

sensory deterioration. Alternatively, WGO may serve as a functional blending component, used to fortify oxidation-prone oils like SO with natural antioxidants, enhancing their thermal stability without compromising flavor.

In conclusion, this research supports the valorization of wheat germ as a functional food ingredient, not by promoting WGO as a commercial frying oil in isolation, but by leveraging its antioxidant and compositional advantages in multi-oil systems. Bridging the gap between chemical functionality and sensory acceptability, potentially through deodorization, esterification, or blending, will be essential for aligning WGO's technical merits with consumer preferences in real-world frying applications.

6. References

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