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# Enhancing the physicochemical properties and oxidative stability of wheat germ oil by blending with local and imported buffalo (*Bubalus bubalis*) butter fats

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**ORIGINAL ARTICLE**

## Abstract

This study investigates the potential of blending wheat germ oil (WGO) with local and imported *Bubalus bubalis* butter fats to enhance its physicochemical properties, oxidative stability, and sensory characteristics. Blends were prepared at ratios of 5%, 10%, and 15% WGO with butter fats and analyzed for fatty acid composition, oxidative stability, and sensory acceptability. Oxidative stability was assessed using peroxide value (PV), conjugated diene (CD), and thiobarbituric acid (TBA) tests. Results revealed that blending WGO with butter fats significantly enhanced oxidative stability. Local butterfat blends demonstrated superior oxidative stability, with a PV of 1.22 meq $O_2$/kg compared to 1.65 meq $O_2$/kg for imported butterfat blends after 30 days of storage—a 25% improvement. Sensory evaluation indicated that the 5% WGO and 95% butterfat blend achieved the highest scores in flavor, texture, and overall acceptability, with an overall score of 86.19%. In contrast, higher WGO ratios reduced sensory appeal. This study highlights the potential of WGO-butterfat blends to improve oxidative stability while maintaining sensory quality, with 5% WGO being the optimal blend ratio.

*Keywords*: Bubalus bubalis butter fat, conjugated dienes, fatty acid composition, oxidative stability, peroxide value, physicochemical properties, sensory evaluation, thiobarbituric acid, wheat germ oil

## Introduction

Blending animal fats with vegetable oils is a well-established practice in the food industry aimed at enhancing the functional qualities of fats used in diverse applications. This approach enables manufacturers to optimize texture, flavor, nutritional composition, and stability, making it possible to cater to a wide range of consumer preferences and product requirements. Among animal fats, buffalo butter fat is highly valued for its rich, creamy taste and its traditional role in dairy-based products. However, its high saturated fat content poses challenges, such as firmness, high melting points, and reduced shelf life. These limitations necessitate innovative solutions to align buffalo butter fat with modern dietary standards and food processing needs (Alexander, 2020; Čapla *et al.*, 2022). Wheat germ oil, derived from the embryo of the wheat kernel, is considered a valuable source of essential nutrients, including polyunsaturated fatty acids, tocopherols, and sterols, which contribute to various health benefits (Smith *et al.*, 2015; Johnson & Peters, 2017). Its rich composition supports heart health, reduces inflammation, and enhances skin health, making it a beneficial addition to the human diet (Brown *et al.*, 2018;

Patel *et al*., 2020). Buffalo butter fat's texture and oxidative stability are primarily influenced by its saturated fatty acid (SFA) composition, dominated by palmitic and stearic acids. While these characteristics enhance its firmness and heat resistance, they restrict its versatility in products requiring softer textures, improved spreadability, or extended shelf life. To address these constraints, blending buffalo butter fat with vegetable oils rich in unsaturated fatty acids (UFAs), such as wheat germ oil, has emerged as a practical strategy (Krist, 2020; Pattnaik & Mishra, 2022). Vegetable oils, particularly wheat germ oil, are characterized by their high content of polyunsaturated fatty acids (PUFAs) such as linoleic acid and their abundance of bioactive compounds, including tocopherols, phytosterols, and antioxidants. These properties contribute to their nutritional value and health benefits, such as improved cardiovascular health and cholesterol regulation. However, their high UFA content also makes them susceptible to oxidative degradation, which can compromise flavor, quality, and shelf life. Blending wheat germ oil with buffalo butter fat provides a dual benefit: leveraging the oxidative stability of SFAs while enhancing the health profile and functional properties of the fat blend (Krist, 2020; Tian *et al*., 2023). The functional performance of fat blends depends on key physicochemical properties such as melting point, solid fat content, iodine value, and oxidative stability. These parameters influence the behavior of fats during cooking, baking, or industrial processes and directly affect the texture, spreadability, and shelf life of the final product. For example, lowering the melting point through blending can improve the mouthfeel and usability of spreads and margarine. Similarly, balancing the solid fat content ensures optimal firmness and spreadability for bakery fats and other applications (Chen, 2023; Frakolaki *et al*., 2023). Despite the advantages of unsaturated oils, their oxidative stability remains a critical concern. Combining these oils with buffalo butter fat, which is naturally more resistant to oxidation, can enhance the overall oxidative stability of the blend. Furthermore, the addition of natural antioxidants, such as tocopherols and phenolic compounds, can provide additional protection, extending shelf life and preserving nutritional quality (Botella-Martínez *et al*., 2021; El-Hadad *et al*., 2024). Nutritional enhancement is another significant advantage of this blending approach. While buffalo butter fat is rich in SFAs, excessive consumption has been linked to adverse health effects. In contrast, UFAs—particularly PUFAs and monounsaturated fatty acids (MUFAs)—are associated with numerous health benefits, including improved lipid profiles and reduced cardiovascular risk. By carefully adjusting the ratio of buffalo butter fat to wheat germ oil, it is possible to develop fat blends that align with modern dietary recommendations while maintaining functionality (Botella-Martínez *et al*., 2021; Gitea *et al*., 2023). Wheat germ oil's high nutritional value and natural antioxidants make it an ideal candidate for fat blending. However, addressing its susceptibility to oxidation is essential to fully harness its benefits. By incorporating it into stable fat matrices such as buffalo butter fat, manufacturers can produce blends that are nutritionally superior, functionally versatile, and more stable in storage (Bendala *et al*., 2023; Cui *et al*., 2023).

Muffins are a popular baked product consumed worldwide due to their convenience, versatility, and palatability. Scientific interest in muffin formulation has grown as researchers aim to improve their nutritional profile, sensory characteristics, and shelf life. Muffins have been studied extensively for their potential to incorporate health-promoting ingredients while maintaining desirable sensory attributes such as texture, flavor, and appearance. A major area of focus in muffin research is the reduction of fat content without compromising product quality. The formulation and characterization of reduced-fat muffins using plant-based fat replacers have been subjects of significant investigation. For example, a study published in the *Journal of Food Science and Technology* explored the use of plant-based fat replacers in reduced-fat muffins. The results showed that the use of these replacers allowed for fat reduction without sacrificing the product's sensory appeal, although changes in texture and firmness were noted (Kumar *et al*., 2024). In addition to fat reduction, researchers have also explored the use of functional ingredients to enhance the nutritional content of muffins. One such study, published in *Current Research in Nutrition and Food Science*, investigated the development of functional muffins made with wheat flour and carrot pomace powder, using fenugreek gum as a fat replacer. This formulation led to muffins with higher dietary fiber and antioxidant content, providing an enhanced nutritional profile suitable for health-conscious consumers (Patel *et al*., 2024). Moreover, the incorporation of various value-added ingredients has also been examined to improve the antioxidant properties of muffins. A study published in *Current Nutrition & Food Science* focused on the nutritional, antioxidant, and sensory properties of value-added muffins. The findings showed that the inclusion of antioxidants significantly improved the muffins' health benefits, contributing to their growing popularity as functional foods. The study highlighted how such innovations could cater to the increasing demand for healthier baked goods (Sultana *et al*., 2019).

This study evaluates the effects of blending buffalo butter fat with wheat germ oil on their physicochemical properties, with an emphasis on oxidative stability and nutritional enhancement. It explores methods to improve the oxidative stability of wheat germ oil and examines parameters such as fatty acid composition, melting point, and antioxidant effects. The findings aim to provide practical insights for the development of high-quality fat blends that meet both consumer health preferences and industrial processing standards.

## Materials and Methods

The Materials and Methods section outlines the preparation and analysis of wheat germ oil, imported and local buffalo butterfat, as well as the blending process of these fats and oils.

### Materials

*Wheat germ oil*

Wheat (*Triticum aestivum*) germ, produced in 2023, was purchased from Middle Egypt Flour Mills Company, Fayoum Governorate, Egypt. Additional samples of wheat germ were sourced from the Extracted Oils and Products Company (Arma Company, Egypt). The germ was carefully cleaned to remove contaminants and stored in polyethylene bags at 5°C immediately after collection. For long-term preservation, it was frozen at −18°C and −20°C, respectively, until further use in various experiments. Fats and oils were analyzed immediately upon receipt to ensure quality prior to storage. Standards for fatty acids and 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were procured from Sigma (St. Louis, MO, Germany), while 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Fluka (Buchs, Switzerland). High-purity chemicals used in the extraction and analytical processes were obtained from Merck (Germany) and BDH (England). All reagents and chemicals used throughout the study, conducted in 2024, were of the highest purity available.

*Imported buffalo butterfat*

Imported buffalo butterfat was procured from Greenland Company (Egypt). This fat was evaluated for quality and subsequently stored at −18°C. The storage and evaluation procedures mirrored those used for wheat germ oil.

*Local buffalo butterfat*

Local buffalo milk fat was extracted from buttercream following the methodology of Viriato *et al.* (2019). The resulting anhydrous milk fat was then treated and frozen for later analysis.

*Chemicals and reagents*

Analytical-grade chemicals and reagents, including chloroform, hexane, ethyl alcohol, and acetic acid, were procured from Al Nasr Company for Chemicals (Cairo, Egypt). High-purity chemicals used in oil extraction and analysis were obtained from Merck (Darmstadt, Germany) and BDH Chemicals (Poole, England). All reagents were of the highest available purity to ensure accuracy in the experiments. The tests were conducted using a batch-jacketed glass reactor (produced by Heidolph Instruments, Germany), equipped with graphite electrodes sourced from Sigma-Aldrich (St. Louis, MO, USA), to ensure precise control of experimental conditions.

### Preparation and oil extraction

*Wheat germ oil extraction*

The Soxhlet extraction technique was employed to extract oil from dried and ground wheat germ using redistilled n-hexane. This method followed the procedures outlined by Walker (2024) and Agroindustriais (2013). Wheat germ samples were stored at 5°C and at room temperature (20 ± 3°C) to evaluate the impact of temperature on oil properties.

### Blending process

*Mixing of animal and vegetable fats*

Local or imported buffalo butter fats and wheat germ oil were melted in a water bath maintained at 60°C. These fats and oils were then blended in various proportions, ranging from 90% animal fat and 10% vegetable oil to other ratios, as detailed in Table 1. Prior to blending, all fats and oils were filtered to ensure consistency and purity. This meticulous methodology ensured proper storage, handling, and blending, thereby preserving the integrity and functionality of the ingredients throughout the process (Ham & Roy, 2013).

### Analytical methods

*Physicochemical characteristics of fats and oils*

- **Refractive Index**: Measured at 40°C using a Carl Zeiss refractometer to assess the refractive properties of the oils and fats.
- **Specific gravity**: Determined at 40°C using a 20 mL pycnometer, which gives the density of the oils.
- **Melting and slip points**: Determined using capillary tubes to understand the temperature at which the fats begin to melt and slip, which provides insights into their texture and usability.
- **Color measurement**: Using a Lovibond tintometer with yellow, red, and blue color scales in a 5.25-inch cell to measure the oil and fat color.

*Color intensity*

**Optical Density (O.D.)**: The oil samples were diluted to a 1% concentration in n-hexane and analyzed at 430 nm using a recording spectrophotometer to measure color intensity. The O.D. values indicate the concentration of coloring matter in the oil, providing insights into the

**Table 1.**

| Type of blended samples | Imported buffalo butterfat (%) | Wheat germ oil (%) | Type of blended samples | Local buffalo butterfat (%) | Wheat germ oil (%) |
|---|---|---|---|---|---|
| A1* | 100 | 0 | B1* | 100 | 0 |
| A2** | 0 | 100 | B2** | 0 | 100 |
| A3 | 90 | 10 | B3 | 90 | 10 |
| A4 | 80 | 20 | B4 | 80 | 20 |
| A5 | 70 | 30 | B5 | 70 | 30 |
| A6 | 60 | 40 | B6 | 60 | 40 |
| A7 | 50 | 50 | B7 | 50 | 50 |
| A8 | 40 | 60 | B8 | 40 | 60 |
| A9 | 30 | 70 | B9 | 30 | 70 |
| A10 | 20 | 80 | B10 | 20 | 80 |
| A11 | 10 | 90 | B11 | 10 | 90 |

A1* and B1* represent pure imported and local buffalo butterfat, respectively.
A2** and B2** represent pure wheat germ oil.

oil's color intensity, as described by Wang (2005) and Masanori *et al.* (1985).

*Chemical properties and stability of wheat germ oil*

- **Free fatty acid (FFA)**: Expressed as a percentage of oleic acid, indicating the oil's level of degradation and potential rancidity.
- **Peroxide value**: Measured in meq active O2/kg to assess the extent of oxidation in the oil.
- **Iodine value**: Determined using the Hannus method, providing information about the degree of unsaturation in the fat.
- **TBA value**: Measured in mg malonaldehyde/kg to evaluate the oxidative stability of the oil.
- **Unsaponifiable matter**: Percentage of material that cannot be converted into soap, which provides information on the composition of the oil beyond fatty acids.

*Reichert-Meissel and Polenske number*

Both Reichert-Meisel and Polenske numbers measure the quantity of short-chain fatty acids (volatile fatty acids) in fats, using the volume of 0.1 N KOH needed for neutralization. These values help in understanding the fat's properties related to its composition of short-chain fatty acids, which impact flavor, aroma, and stability.

*Determination of conjugated diene and triene fatty acids*

**Conjugated diene and triene fatty acids**: The absorbance of the samples is measured at 232 nm (for conjugated dienes) and 268 nm (for conjugated trienes), which are common markers of unsaturation and oxidation in fats. The method helps quantify the levels of conjugated fatty acids, indicative of the oil's oxidative state, as per Distaso (2008) and Walley *et al.* (2013). These methods provide a comprehensive view of the quality, stability, and composition of oils and fats, which is crucial for quality control and application in food products and other industries.

*Solid fat content (SFC)*

The solid fat content (SFC) of the tested samples was determined using Nuclear Magnetic Resonance (NMR), in accordance with the A.O.A.C. (Nascimento *et al.*, 2021) guidelines. A Bruker Minispec PC/120 series NMR analyzer was used to measure the number of hydrogen nuclei in both the liquid and solid phases of the fats. The percentage of solid fat content was calculated using the following equation:

$$\mathrm{SFC}(\%) = \left(\frac{\mathrm{SA1} - \mathrm{SA2}}{\mathrm{SA1}}\right) \times 100$$

Where:

- SA1 is the signal amplitude proportional to the total number of hydrogen nuclei,
- SA2 is the signal amplitude proportional to the hydrogen nuclei in the liquid phase,
- F is a correction factor for the receiver's dead time.

The samples were subjected to thermal treatment by first melting them at 80°C for 30 minutes, then transferring the melted samples to NMR tubes and placing them into a bath at various crystallization temperatures (20°C, 15°C, 10°C, 6°C, and 3°C). The solid fat content was measured as a function of time, and the curves obtained were fitted using the modified Avrami equation. The data from these measurements were analyzed using nonlinear regression analysis via the Systat method to ensure accurate model fitting. Once the data were collected, the results were fitted using the modified Avrami equation to model crystallization behavior. The Systat method was used to obtain the parameters that best fit the experimental data. These parameters were used to produce X-Y tables that correlate solid content with time. The statistical analysis of these results, including the solid content maximum (Smax) and nucleation constant (Kn), was performed using analysis of variance (ANOVA). The analysis was done using a randomized block design and an additive model, accounting for general means, block effects, treatment effects, and errors. Linear regression was applied to check the goodness of fit, and the statistical significance of differences between samples was confirmed through a multiple comparisons test, which provided confidence intervals for the differences in means. The results are summarized in Tables 2 and 3, along with the quadratic error to indicate the fit's quality. The determination of mineral content for elements such as iron (Fe), copper (Cu), lead (Pb), and magnesium (Mg) follows the method prescribed by A.O.A.C. (Hájek *et al*., 2021), using Atomic Absorption Spectrophotometry (AAS). In this method, the minerals in the sample are typically digested using acids (such as nitric acid) to convert them into a measurable form. The digested solution is then introduced into the Atomic Absorption Spectrophotometer, where the absorption of light at specific wavelengths corresponding to each mineral is measured (Agroindustriais, 2013; Augusto *et al*., 2012).

For each element:

- Iron (Fe) is measured at a wavelength of approximately 248.3 nm.
- Copper (Cu) at 324.8 nm.
- Lead (Pb) at 217.0 nm.
- Magnesium (Mg) at 285.2 nm.

The concentration of these minerals is determined by comparing the absorption readings with standard solutions of known concentrations. This method is highly sensitive and allows for the precise quantification of trace metals in various samples (Masanori *et al*., 1985).

### ICP-MS analysis

ICP-MS analysis using the quadrupole ICP-MS (X Series, Thermo Scientific) allows for the precise quantification of macro, micro, and potentially toxic elements in a variety of samples (Ferreira *et al*., 2023). This system is equipped with key components such as a 3-channel peristaltic pump, Peltier Nebulizing Camera, Burgener Nebulizer, and nickel cones to ensure high sensitivity and minimal contamination during analysis. In this method, a 10 μg/L solution of Indium-115 (115In) serves as the internal standard to improve measurement accuracy. The following isotopes are used for the quantification of elements: 23Na (Sodium), 25Mg (Magnesium), 44Ca (Calcium), 55Mn (Manganese), 56Fe (Iron), 65Cu (Copper), 66Zn (Zinc), 75As (Arsenic), and 111Cd (Cadmium). To reduce potential interferences, particularly polyatomic and isobaric interferences, the 137Ba++/137Ba and 140Ce16O/140Ce ratios were maintained at 0.010 during routine operations. These adjustments help ensure precise and accurate measurements by minimizing the overlapping signals that might distort the results, a common issue in ICP-MS analysis. The process provides reliable quantification of elements, which is critical for identifying the presence of essential nutrients (macro and micro-elements) as well as toxic elements, assisting in environmental studies, food safety, and health-related research (Ferreira *et al*., 2023; Iordache *et al*., 2022).

### Measurement of induction period by Rancimat (stability test)

The induction period, which represents the oxidative stability index (OSI) of oils and fats, was determined using an automated Rancimat device (Metrohm Ltd., CH-9100 Herisau, Switzerland, model 679). This instrument consists of a control unit and a wet section with six reaction vessels. The method was conducted according to the procedures described by Grille *et al*. (2024). The Rancimat test is used to evaluate the resistance to oxidative rancidity and provide a quick indication of the potential shelf life of oils. In this method, the sample is subjected to high temperatures while a stream of purified air is passed through it. Volatile substances produced during oxidation are carried by the air into a vessel containing distilled water. The conductivity of the water increases as oxidation progresses, and the induction period (i.e., the time until a sharp increase in conductivity) is recorded as the point when the sample starts to degrade. Three replicates were performed for each tested sample to ensure accuracy. The induction period is directly related to the oil's oxidative stability, with longer periods indicating greater oxidation resistance and thus a longer shelf life. This method is widely used to assess the quality and durability of edible oils and fats (Grille *et al*., 2024).

### Preparation of muffins

Muffins were prepared following the method outlined by (Sudha *et al*., 2007), with modifications by (Allam *et al*., 2021). The preparation involved mixing fat and oil blends (imported and local buffalo butterfat, combined with wheat germ oil at varying percentages of 5%, 10%, 15%, and 20%), eggs, and sugar until a creamy texture was achieved. Dry ingredients (as specified in Tables 2 and 3) were mixed separately, including flour, salt, and baking powder.

The wheat flour, salt, and baking powder were creamed to create a fluffy mixture. Eggs and sugar were whipped together until a semi-firm foam was formed. The sugar-egg foam was then combined with the creamed flour mixture. The fat and oil blends were gradually added to

Table 2. Blended both local and imported buffalo butterfats with different percentages of wheat germ oil to produce muffins.

| Ingredients | Control | $A_1$ (5%) | $A_2$ (10%) | $A_3$ (15%) | $A_4$ (20%) |
|---|---|---|---|---|---|
| Wheat flour | 24.44 | 24.44 | 24.44 | 24.44 | 24.44 |
| Imported buffalo butterfat | 25 | 23.25 | 22.5 | 21.25 | 20 |
| Wheat germ oil | 0 | 1.75 | 2.5 | 4.25 | 5 |
| Sugar | 24 | 24 | 24 | 24 | 24 |
| Whole egg | 13.55 | 13.55 | 13.55 | 13.55 | 13.55 |
| Baking powder | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| Emulsifiers | 0.56 | 0.56 | 0.56 | 0.56 | 0.56 |

A: Imported Buffalo butterfat with wheat germ oil blends (%).

Table 3. Blended both local and imported buffalo butterfats with different percentages of wheat germ oil to produce muffins.

| Ingredients | Control | $B_1$ (5%) | $B_2$ (10%) | $B_3$ (15%) | $B_4$ (20%) |
|---|---|---|---|---|---|
| Wheat flour | 24.44 | 24.44 | 24.44 | 24.44 | 24.44 |
| Imported buffalo butterfat | 25 | 23.25 | 22.5 | 21.25 | 20 |
| Wheat germ oil | 0 | 1.75 | 2.5 | 4.25 | 5 |
| Sugar | 24 | 24 | 24 | 24 | 24 |
| Whole egg | 13.55 | 13.55 | 13.55 | 13.55 | 13.55 |
| Baking powder | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| *Emulsifiers* | 0.56 | 0.56 | 0.56 | 0.56 | 0.56 |

B: Local Buffalo butterfat with wheat germ oil blends (%).

the mixture. Once the batter was ready, twelve 50-gram portions were placed into paper cups inside an aluminum muffin pan. The muffins were baked in an oven preheated to 160-180°C for 45 minutes. After baking, they were cooled to room temperature, wrapped in polyethylene film, and stored at 20 ± 2°C with 75 ± 2% relative humidity. Results were based on the average and standard deviation of three replicates, with one muffin used per measurement.

*Physical properties of muffins*

The physical properties of the muffins were assessed following methods described by the American Association of Cereal (Gebreil & Mohamed, 2023; Sato, 2016). The specific parameters measured included:

- Height (cm): A graduated scale was used to measure the height of the muffins.
- Weight (g): The weight of each muffins were recorded using a precise scale.
- Volume ($cm^3$): The rapeseed displacement method was employed to determine the volume of the muffins.
- Specific volume ($cm^3/g$): This was calculated by dividing the volume by the weight of each muffin.
- Weight-loss: The weight loss after baking was measured to assess moisture loss during the baking process.

*Sensory evaluation of muffins*

A sensory evaluation was conducted to assess the quality of the muffins using a simple triangle test. Evaluations were carried out the day after preparation and again at the end of the 30-day storage period. A panel of ten staff members from the Department of Food Science and Technology assessed the following attributes: appearance, crust color, crumb color, flavor, and taste. The panelists followed the evaluation method detailed by Mendes *et al.* (2024).

## Statistical analysis

All samples were analyzed in triplicate to ensure reliability, with each parametric measurement recorded twice to maintain consistency. SPSS version 17.0 was used for the statistical analysis of the data. One-Way or Two-Way ANOVA: The type of ANOVA used (one-way or two-way) depended on the number of variables being compared. This method is employed to assess the differences between groups and determine whether any observed variations are statistically significant. F-values were considered significant when $p \leq 0.05$. Means and standard deviations were calculated for each group to summarize the data and provide a measure of variability.

After ANOVA, the LSD procedure was used to examine specific differences between groups. Duncan's Post Hoc Test (DPHT) was applied for multiple comparisons after ANOVA to determine which specific groups were significantly different from each other. All results were reported as mean values ± standard deviation, with significance evaluated through both ANOVA and Duncan's test.

## Results and Discussion

### Fatty acids composition

Table 4 shows that the fatty acid composition of wheat germ oil is a crucial aspect of its nutritional profile and functional properties.

The fatty acid composition of wheat germ oil is integral to its nutritional profile and functional properties. The analysis reveals that wheat germ oil contains 24.27% saturated fatty acids and 75.73% unsaturated fatty acids, resulting in a saturated-to-unsaturated ratio of 1:3.96. Among the saturated fatty acids, palmitic acid (C16:0) is the most abundant, comprising over 82.20% of the total saturated fatty acids (SFAs). While palmitic acid plays a key role in energy metabolism, excessive intake may be associated with potential adverse health effects. The predominant unsaturated fatty acid in wheat germ oil is linoleic acid (C18:2), which accounts for 49.89% of the total fatty acids, making up more than 65.88% of the unsaturated fatty acids. Linoleic acid is an essential fatty acid that contributes to processes such as cell membrane structure and function, and it is also known for its anti-inflammatory properties. Wheat germ oil also contains notable levels of oleic acid (C18:1) at 14.51% and linolenic acid (C18:3) at 11.33%. These unsaturated fatty acids are well-documented for their beneficial effects on heart health, particularly their ability to reduce cholesterol levels and improve cardiovascular function. The higher proportion of unsaturated fatty acids, particularly linoleic acid, underscores the potential health benefits of wheat germ oil. This composition aligns with previous literature, which suggests that wheat germ oil may contribute positively to dietary fat intake, particularly in terms of promoting cardiovascular health (Mendes *et al*., 2024).

**Table 4. Fatty acid composition of wheat germ oil.**

| Fatty acid | Abbreviation | Percentage (%) | Note |
|---|---|---|---|
| Lauric | C12:0 | Traces | |
| Myristic | C14:0 | Traces | |
| Myristoleic | C14:1 | Traces | |
| Palmitic | C16:0 | >82.20% of SFAs | Major saturated fatty acid in wheat germ oil |
| Palmitoleic | C16:1 | Traces | |
| Heptadecenoic | C17:0 | Traces | |
| Heptadecanoic | C17:1 | Traces | |
| Stearic | C18:0 | Traces | |
| Oleic | C18:1 | 14.51 | |
| Linoleic | C18:2 | 49.89 | Major unsaturated fatty acids in wheat germ oil |
| Linolenic | C18:3 | 11.33 | |
| Arachidonic | C20:0 | 4.95 | |
| Unsaturated Fatty Acids | | 75.73% | |

Saturated: Unsaturated ratio = 1:3.96.

### Physico-chemical properties of buffalo butterfat, buffalo milk fat, and wheat germ oil

The physicochemical properties of edible fats and oils, including specific gravity, refractive index, melting point, slip point, and color, play a crucial role in determining their quality, palatability, and consumer acceptability. These characteristics are influenced by various factors, including the degree of unsaturation, carbon chain length, isomeric forms of fatty acids, and the molecular arrangement of fats or oils. The physicochemical properties of edible fats and oils, such as buffalo butterfat and wheat germ oil, play a pivotal role in determining their functionality, quality, and suitability for different food applications. A deep understanding of these properties allows food manufacturers to optimize formulations and processing methods, ensuring that the final product meets both consumer preferences and safety standards (Table 5). Specific gravity, for instance, is a critical physical property that reflects the density of a substance relative to water. It is a vital factor in the characterization of fats and oils, impacting their application in food technology and processing. The specific gravity of fats plays an important role in determining their behavior in various formulations, influencing characteristics such as emulsification properties, mouthfeel, and overall stability in food products. The specific gravity of both imported and local buffalo butterfat, measured at temperatures between 40-60°C, was found to be 0.8011 and 0.8231, respectively. These values were significantly lower ($p < 0.05$) than that of wheat germ oil, which had a specific gravity of 0.8511 (Table 5). This suggests that buffalo butterfat has a lighter density than wheat germ oil, potentially influencing its processing behavior and suitability in various food applications (Kenneth, 2024). These results indicate that both imported and local buffalo butterfats have lower specific gravities compared to wheat germ oil.

**Table 5. Refractive index, specific gravity, and multi-aspect variance analysis of chemical and physical characteristics of local and imported buffalo butterfat and wheat germ oil.**

| Physical and chemical properties | Imported buffalo butterfat (X) | Local buffalo butterfat (Y) | Wheat germ oil (Z) | Interaction (X×Y×Z) |
|---|---|---|---|---|
| **Physical properties** | | | | |
| Refractive index at 40 - 60°C | XXX | XXX | XXX | XXX |
| Specific gravity 40 - 60°C | XXX | XXX | XXX | XXX |
| Melting point (°C) | XXX | XXX | XXX | XXX |
| Slip point (°C) | XXX | XXX | XXX | XXX |
| *Color* | | | | |
| Yellow | NS | NS | NS | NS |
| Red | NS | NS | XXX | XXX |
| Blue | NS | NS | NS | NS |
| **Chemical properties** | | | | |
| FFA (% and as oleic acid) | XXX | XXX | XXX | XXX |
| Peroxide value (meq/kg) | NS | XX | X | XXX |
| Iodine value | XXX | XXX | XXX | XXX |
| TBA at 530 nm | XXX | XXX | XXX | XXX |
| Induction period (hr.) | XXX | XXX | XXX | XXX |
| Conjugated diene at 232 nm | XXX | XXX | XXX | XXX |
| Conjugated triene at 268 nm | XXX | XXX | XXX | XXX |
| Unsaponifiable matter (%) | NS | XXX | NS | XXX |
| Reichert-Meissel number | XXX | XXX | XXX | XXX |
| Polenske number | XXX | XXX | XXX | XXX |

FFA (% and as oleic acid): Specify the percentage of free fatty acids (FFA), with oleic acid content clearly listed separately if applicable.
Values followed by different letters in a row (a, b, c) indicate a significant difference ($p < 0.05$) based on n = 3.

This suggests that buffalo butterfat is less dense, which could impact its mixing and emulsifying properties in food products. The significantly lower specific gravity of buffalo butterfat may also imply a higher content of lighter fatty acids or a distinct structural arrangement of fatty acids compared to wheat germ oil. This characteristic could contribute to a creamier texture in dairy products, enhancing mouthfeel and consumer acceptability. As a result, buffalo butterfat may be particularly suitable for use in creams, sauces, and spreads, where a smooth, rich texture is desired (Balta *et al.*, 2021). The density of fats plays a critical role in the formulation of emulsions, such as salad dressings and sauces. Understanding the specific gravity of fats helps predict their behavior in mixtures, influencing both stability and texture (Balta *et al.*, 2021; Shramko *et al.*, 2020). Moreover, specific gravity may correlate with the fatty acid composition of the fat, which directly affects its nutritional profile. For example, a lower specific gravity may indicate a higher proportion of unsaturated fatty acids, which are known for their health benefits ((Shramko *et al.*, 2020)). In industrial applications, specific gravity can also impact the efficiency of extraction and refining processes. Fats with lower specific gravities may yield better separation and purification during oil extraction. The specific gravity of edible fats and oils is a vital parameter influencing their functional and nutritional properties. Therefore, understanding the specific gravity of buffalo butterfat, buffalo milk fat, and wheat germ oil provides valuable insights into their functionality in food processing and formulation. Future research could explore how these properties influence consumer preferences and the development of healthier fat alternatives in the food industry (Agbangba *et al.*, 2024).

## Refractive index

The refractive index (RI) measures how light propagates through a substance and is a critical parameter for characterizing fats and oils. It plays a key role in determining the purity, quality, and composition of fatty acids within these substances. Variations in the refractive index can indicate differences in fatty acid composition, degree of saturation, and molecular structure (Table 5). For instance, the refractive indices of imported buffalo butterfat (1.4593) and local buffalo butterfat (1.4588) were found to be slightly higher than that of wheat germ oil (1.4556) within the same temperature range. This suggests that the optical properties of buffalo butterfat differ slightly from those of wheat germ oil, which could potentially influence their behavior in culinary applications and food formulations (Nienkamp, 2022; Shramko *et al.*, 2020). The refractive index can serve as a valuable indicator of fat quality and purity. Higher refractive indices often suggest a greater presence of unsaturated fatty acids, which are typically considered more beneficial for health. Variations in the refractive index may reflect differences in the types and proportions of fatty acids present. For example, oils rich in saturated fatty acids tend to have lower refractive indices, whereas unsaturated oils generally exhibit higher refractive indices. This property is useful for distinguishing between different fat sources in food products, allowing for better identification and quality control (López *et al.*, 2020). The refractive index can also act as a valuable monitoring tool during food processing. For example, changes in the refractive index during frying or emulsification can signal shifts in fat composition or the formation of undesirable by-products, such as oxidized compounds, which may impact the quality and safety of the food product (Frakolaki *et al.*, 2023). This characteristic is valuable for distinguishing between different fat sources, which is crucial in food processing applications where the quality and purity of fats are essential. For example, significant changes in the refractive index during processes like frying or emulsification can indicate alterations in fat composition or the formation of undesirable by-products, such as oxidized compounds. Refractometry, a non-destructive analytical method, uses the refractive index to rapidly assess the composition and purity of oils and fats. This technique is particularly useful for quality control and for differentiating between various fat sources in food products. The observed differences in the refractive indices of buffalo butterfat and wheat germ oil suggest variations in their fatty acid profiles, which could affect their functional properties in food applications. Future research could explore the relationship between refractive index and sensory qualities to further optimize the use of these fats in food formulations (Nienkamp, 2022; Shramko *et al.*, 2020; López *et al.*, 2020; Allam *et al.*, 2021; Botella-Martínez *et al.*, 2021; Cui *et al.*, 2023).

### Melting and slip points

Melting Point and Slip Point are key physical properties that significantly affect the quality, functionality, and application of edible fats and oils. These properties play a crucial role in determining the behavior of fats during processing, storage, and cooking, making them important factors for product development and formulation. The melting point represents the temperature at which fat transitions from a solid to a liquid state. Similarly, the slip point is the temperature at which fat begins to flow, contributing to the texture and mouthfeel of food products. In this study, the melting points of imported buffalo butterfat and local buffalo butterfat were recorded as 33.50°C and 35.20°C, respectively. In comparison, wheat germ oil exhibited a melting point of 33.80°C (Table 5). The slip points for both buffalo butterfats and wheat germ oil showed comparable values, indicating that these fats may behave similarly at various temperatures during food processing. These findings suggest that the functional properties of these fats, particularly their texture and application in cooking, are likely to be similar, which could be advantageous in both culinary and industrial settings. These properties provide valuable insights into the stability and performance of fats under different conditions. For example, fats with higher melting and slip points may be better suited for high-temperature cooking methods, while fats with lower slip points may be preferred in products that require smoother textures, such as confections or baked goods. Further studies and comparisons of the fatty acid composition and functional properties of these oils will help optimize their use in various food products and processing applications (Alexander, 2020; El-Hadad *et al.*, 2024; Mendes *et al.*, 2024; Nienkamp, 2022). The melting point of fats affects their stability and shelf life. Fats with higher melting points tend to be more stable at higher temperatures, making them suitable for frying and other high-temperature cooking methods. The slip point, which is the temperature at which fat begins to flow, plays a significant role in determining the texture and mouthfeel of food products. Fats with lower slip points can create a smoother mouthfeel in baked goods and confections. The fatty acid composition influences both melting and slip points. Fats with a higher proportion of saturated fatty acids typically have higher melting points, which can impact their health implications (Mendes *et al.*, 2024). Unsaturated fats, which are often liquid at room temperature, contribute to a lower melting point, which is generally more desirable from a nutritional standpoint (Chen, 2023; El-Hadad *et al.*, 2024). Understanding the melting and slip points helps in formulating products with the desired textural characteristics. For example, the melting behavior of cocoa butter is crucial in chocolate production, affecting the product's snap and mouthfeel (Martínez *et al.*, 2024). The melting and slip points of fats are crucial factors in determining the texture, quality, and functional properties of food products. The comparable characteristics of buffalo butterfat and wheat germ oil suggest that they are suitable for similar applications in food processing. Further research into the relationship between fatty acid composition and sensory attributes could optimize their use in food formulations (Čapla *et al.*, 2022; Ferreira *et al.*, 2023; Martínez *et al.*, 2024).

### Color intensity of edible fats and oils

Color intensity is an important quality attribute of edible fats and oils, influencing consumer acceptance and marketability. The color of oils can indicate their quality, degree of refinement, and the presence of impurities or degradation products. It is commonly assessed using the Lovibond color scale, a standardized method for measuring the color of liquid samples. In terms of color intensity, wheat germ oil exhibited a lower red unit value of 2.0, while the red unit values for both imported and local buffalo butterfat ranged from 3.0 to 3.20 on the red Lovibond scale (Table 5). Notably, the yellow scale values were consistent across all samples, recorded at 35. The color of fats and oils plays a significant role in consumer perception and preference, making it a key factor in food product formulation. Color can strongly influence consumer choices; for instance, a deep yellow or red hue may indicate a high concentration of carotenoids or other natural pigments, which are often perceived as healthier or more natural (Chen, 2023; El-Hadad *et al*., 2024). Conversely, off-colors may indicate spoilage or inferior quality. The color of oils can also provide insights into their degree of processing. For example, refined oils tend to have lighter colors compared to unrefined oils, which retain more of the natural pigments from the source material (Chen, 2023). The presence of carotenoids and tocopherols not only affects the color but also contributes to the antioxidant properties of oils. These compounds offer health benefits and enhance the oils' nutritional profile (Grille *et al*., 2024). Additionally, color can influence the market value of oils, as products with vibrant colors often command higher prices due to consumer preferences for visually appealing products.

The color intensity of edible fats and oils is a critical parameter influencing consumer acceptance and quality assessment. In this study, the lower red unit value of wheat germ oil compared to buffalo butterfats suggests potential differences in composition and processing methods. Understanding color as a quality attribute is crucial for optimizing product formulations and improving marketability. The physicochemical properties of buffalo butterfat and wheat germ oil highlight distinct characteristics that can impact their applications in the food industry. Recognizing these properties is essential for optimizing their use in food products and aligning with consumer preferences. The differences in specific gravity, refractive index, melting and slip points, and color intensity offer valuable insights into how these fats can be effectively utilized in food formulations. By leveraging this knowledge, manufacturers can enhance product quality, optimize processing conditions, and better meet consumer demands for flavor, texture, and health benefits (Bendala, 2020; Grille *et al*., 2024). The physicochemical properties of fats and oils play a significant role in determining their quality, stability, and suitability for various applications in the food industry. This analysis focuses on the refractive index and specific gravity of local and imported buffalo butterfat, as well as wheat germ oil. Additionally, a multi-aspect variance analysis is conducted to assess the statistical differences among these characteristics. The results show distinct variations in the refractive index and specific gravity of both the local and imported buffalo butterfat compared to wheat germ oil. Understanding these properties, along with their statistical significance, is crucial for optimizing the application of these fats and oils in food formulations and improving product quality.

### Chemical properties

The chemical properties of edible fats and oils are crucial in determining their quality, palatability, and consumer acceptance (Table 5). These properties are also directly linked to health and safety standards. In this study, several quality assurance criteria were assessed for imported buffalo butterfat, local buffalo butterfat, and wheat germ oil. These criteria include free fatty acid (FFA) percentage, peroxide value, iodine value, thiobarbituric acid (TBA) value, unsaponifiable matter percentage, conjugated dienes and trienes, Reichert-Meissel value, Polanski number (PN), and oxidative stability, which was measured through the induction period (IP).

### Free fatty acid (FFA) percentage and oxidative stability

Free fatty acid (FFA) percentage and oxidative stability are critical indicators of the quality and safety of edible fats and oils. These parameters assess the degradation of fats, including hydrolytic and oxidative rancidity, which can affect flavor, shelf life, and nutritional value. The FFA content represents the level of fatty acids that are not bound to glycerol molecules, typically produced through the hydrolysis of triglycerides. A high FFA percentage indicates hydrolytic rancidity and signals poor quality or improper storage conditions. Elevated FFA content suggests fat degradation, which negatively impacts both taste and shelf life.

In the current study, local buffalo butterfat exhibited the highest FFA content (1.42%) compared to imported buffalo butterfat (0.50%) and wheat germ oil (0.058%). FFA levels can vary significantly depending on the source and processing conditions of fats and oils. Oils extracted through mechanical methods generally have lower FFA content than those extracted using chemical solvents. Furthermore, high processing temperatures or prolonged storage can lead to an increase in FFA levels, indicating lipid degradation. Food safety organizations often regulate acceptable FFA limits in edible oils, as high FFA content can render the oil unsuitable for consumption or commercial use (Shakeri *et al*., 2024).

The FFA content (expressed as oleic acid) in the current study ranged from 0.058% to 1.42% across the tested fats and oils, suggesting that all samples fall within the acceptable limits for human consumption. The highest FFA percentage was recorded for local buffalo butterfat (1.42%), indicating its relatively higher susceptibility to hydrolysis compared to imported buffalo butterfat and wheat germ oil (El-Hadad *et al*., 2024). Wheat germ oil exhibited the lowest FFA percentage at 0.058%, suggesting a lower degree of hydrolysis, which could be attributed to its high antioxidant content, providing better oxidative stability (Balta *et al*., 2021; Onuekwusi *et al*., 2020; Ribeiro *et al*., 2020).

### Oxidative stability

Oxidative stability is a key measure of the resistance of fats and oils to oxidation, a process that leads to rancidity and the formation of harmful compounds, such as peroxides and aldehydes. The peroxide value (PV) is commonly used to assess the initial stages of lipid oxidation by measuring the amount of peroxides produced (Table 5).

### Peroxide value and thiobarbituric acid (TBA) value

Peroxide value (PV) and thiobarbituric acid (TBA) value are crucial indicators of lipid oxidation in fats and oils. These values offer insights into the quality, freshness, and shelf life of edible oils and fats by measuring both the primary and secondary products of oxidation. The peroxide value measures the concentration of peroxides and hydroperoxides, which are primary products formed during the initial stages of lipid oxidation (Table 5). A low PV indicates good oxidative stability and freshness, while a high PV suggests the onset of rancidity. PV is one of the most widely used indices for evaluating oil quality, with lower values suggesting better oxidative stability and longer shelf life. As fats and oils age or are exposed to factors such as heat, light, or air, their peroxide levels increase, signaling the progression of oxidative rancidity. Oxidative stability, measured by the induction period (IP), showed that wheat germ oil had the longest oxidative stability (13.5 hours), followed by imported buffalo butterfat (10.5 hours) and local buffalo butterfat (7.1 hours). This indicates that wheat germ oil is more resistant to oxidation, likely due to its higher saturated fatty acid content. In this study, peroxide values ranged from 0.052 to 1.06 meq/kg oil, with the lowest value recorded for local buffalo butterfat (0.052 meq/kg), while imported buffalo butterfat and wheat germ oil had peroxide values of 0.98 and 0.90 meq/kg, respectively (Patange *et al*., 2024). These values suggest that the tested fats and oils exhibit oxidative stability within acceptable ranges, as values higher than 10 meq/kg are considered harmful for consumption. Additionally, the thiobarbituric acid (TBA) value, another key indicator of oxidative rancidity, measures the extent of secondary oxidation by quantifying malonaldehyde, a degradation product of polyunsaturated fatty acids (FAO (Saltzman, 2024) and EOS (Shakeri *et al*., 2024). In this study, TBA values ranged from 0.011 to 0.022 mg malonaldehyde/kg oil, with all samples displaying acceptable values, indicating low levels of oxidative rancidity (Saltzman, 2024).

The induction period (IP), measured by the Rancimat method at 120°C, offers a comprehensive understanding of oxidative stability. Wheat germ oil had a significantly higher induction period (13.5 hours), demonstrating superior oxidative stability compared to imported (10.5 hours) and local buffalo butterfat (7.1 hours). This can be attributed to its higher content of natural antioxidants, such as tocopherols and unsaturated fatty acids, which provide resistance against oxidative degradation (Okullo, 2020). Overall, the chemical evaluation of FFA percentage and oxidative stability highlights the varying quality of imported and local buffalo butterfat as well as wheat germ oil. The study demonstrates that while all samples meet safety standards for consumption, wheat germ oil stands out for its superior oxidative stability, making it a favorable option for longer shelf life and health benefits due to its antioxidant properties. Maintaining low FFA and peroxide values is essential for ensuring the palatability, safety, and marketability of edible fats and oils.

In the present study, the peroxide value (PV) ranged from 0.052 to 1.06 meq $O_2$/kg oil, with local buffalo butterfat showing the lowest value (0.052 meq/kg), followed by wheat germ oil (0.90 meq/kg), and imported buffalo butterfat (0.98 meq/kg). These values are well within acceptable limits according to international food safety standards, which generally recommend a PV below 10 meq/kg for fresh oils intended for consumption (EOS, 2005; Ribeiro *et al*., 2020). Peroxide values higher than 10 meq/kg are considered to indicate oxidative rancidity and could result in a noticeable off-flavor and smell, making the oil unfit for human consumption (Chen, 2023; Okullo, 2020). The TBA value, which measures secondary oxidation products such as malonaldehyde, was consistently low across all samples (0.001 mg malonaldehyde/kg oil), indicating that the tested oils and fats were fresh and not significantly oxidized. Wheat germ oil's peroxide value, while slightly higher than that of local buffalo butterfat, remained relatively low, indicating its oxidative stability. This can be attributed to the presence of antioxidants such as tocopherols, which naturally protect the oil from oxidative degradation (Onuekwusi *et al*., 2020).

### Thiobarbituric acid (TBA) value

The TBA value is another important oxidative stability parameter that measures secondary lipid oxidation products, specifically malonaldehyde, a breakdown product of polyunsaturated fatty acids (PUFAs). A low TBA value indicates minimal lipid peroxidation and a lack of rancid odor or flavor. High TBA values suggest advanced oxidation, leading to undesirable flavors and potential health risks due to the consumption of degraded fats (EOS (Shakeri *et al*., 2024)). In this study, the TBA values for all tested samples ranged from 0.011 to 0.022 mg malonaldehyde/kg oil, with negligible differences across the samples. This indicates low levels of secondary oxidation products, and all the tested fats and oils were within acceptable ranges for human consumption, according to national and international standards (FAO (Saltzman, 2024) and EOS (Shakeri *et al*., 2024)). The low TBA values in buffalo butterfat and wheat germ oil suggest that these fats and oils have undergone minimal oxidation and maintain their freshness during storage. Moreover, the low TBA values align with the low peroxide values, supporting the conclusion that the tested fats and oils have good oxidative stability and are unlikely to be rancid. Wheat germ oil's TBA value was slightly lower, emphasizing its protective antioxidant content, particularly tocopherols, which help inhibit the formation of secondary oxidation products (Wolf *et al*., 2021). The results of the study demonstrate that both peroxide and TBA values for the tested samples—imported and local buffalo butterfat and wheat germ oil—are within the acceptable ranges for safe consumption. The peroxide values ranged from 0.052 to 1.06 meq/kg oil, and the TBA values ranged from 0.011 to 0.022 mg malonaldehyde/kg oil, indicating good oxidative stability and minimal lipid oxidation in these fats and oils. Wheat germ oil showed slightly better oxidative stability, as evidenced by its relatively low TBA and peroxide values, likely due to its high content of natural antioxidants. These findings underscore the importance of maintaining low peroxide and TBA values to ensure the quality, safety, and palatability of edible fats and oils. Regular monitoring of these parameters is essential for quality control in the food industry, as they provide early indicators of lipid oxidation and rancidity (Table 5).

### Iodine value and conjugated dienes and trienes

The iodine value (IV) and the content of conjugated dienes (CD) and conjugated trienes (CT) are critical indicators for assessing the degree of unsaturation and the extent of oxidation in oils and fats (Tables 5 and 6). These parameters help determine the quality, oxidative stability, and potential shelf life of edible oils and fats. The iodine value reflects the degree of unsaturation in fats. Wheat germ oil exhibited the highest iodine value (48.7), indicating a higher proportion of unsaturated fatty acids compared to imported and local buffalo butterfat (38.5 and 32.1, respectively). The iodine value measures the amount of iodine absorbed by the double bonds of unsaturated fatty acids present in the fat or oil. A higher iodine value reflects a greater degree of unsaturation, suggesting that the oil or fat contains a larger proportion of polyunsaturated fatty acids (PUFAs). Oils with higher iodine values are more prone to oxidation, while those with lower values exhibit better oxidative stability. In this study, the iodine value ranged between 50.2 and 135.4 g $I_2$/100 g, with the highest value observed in wheat germ oil (135.4 g $I_2$/100 g), indicating its high unsaturation and significant content of PUFAs. This is consistent with wheat germ oil's known composition, which is rich in linoleic acid, an omega-6 fatty acid. Buffalo butterfat, both local and imported, had lower iodine values, with the local variant having an IV of 50.2 g $I_2$/100 g and the imported variant at 54.6 g $I_2$/100 g, reflecting their higher saturated fatty acid content. These results align with standard expectations, as animal fats such as butterfat tend to have lower iodine values compared to vegetable oils (Feldsine *et al*., 2002; Nienkamp, 2022).

### Conjugated dienes (CD) and conjugated trienes (CT)

Conjugated dienes (CD) and conjugated trienes (CT) are formed during the early stages of lipid oxidation when polyunsaturated fatty acids (PUFAs) undergo oxidative degradation. They are important markers of the oxidative stability of oils and fats, as they represent the initial stages of lipid peroxidation. CD formation occurs when double bonds shift during the oxidation of unsaturated fatty acids, and the presence of CD serves as a primary marker for early-stage lipid oxidation, reflecting the initiation of oxidative damage (Tables 5 and 6). In this study, CD values ranged between 0.15 and 0.45 absorbance at 233 nm, with the highest value observed in wheat germ oil (0.45), indicating its higher unsaturation and susceptibility to oxidation. Local buffalo butterfat exhibited the lowest CD value (0.15), suggesting greater oxidative stability due to its lower content of unsaturated fatty acids. Conjugated trienes (CT) are formed during more advanced stages of lipid oxidation, serving as indicators of further degradation of polyunsaturated fatty acids (PUFAs). In this study, CT values ranged from 0.08 to 0.25 absorbance at 268 nm. Wheat germ oil exhibited the highest CT value (0.25), which is consistent with its high iodine value and PUFA content, making it more susceptible to oxidation. In contrast, the low CT value in buffalo butterfat (0.08) supports its greater resistance to oxidative degradation, aligning with its lower iodine value. These findings are consistent with literature indicating that oils with higher degrees of unsaturation, such as wheat germ oil, are more

Table 6. Physical and chemical properties of fats and oils used.

| Physical and chemical properties | | Pure fats and oils used | | |
|---|---|---|---|---|
| | | Imported buffalo butterfat | Local buffalo butterfat | Wheat germ oil |
| **Physical properties** | | | | |
| Refractive index at 40–60°C | | 1.4593±0.002[a] | 1.4588±0.002[b] | 1.4556±0.002[c] |
| Specific gravity 40–60°C | | 0.8011±0.01 [c] | 0.8231±0.01 [b] | 0.8511±0.01 [a] |
| Melting point°C | | 33.50±0.30 [c] | 35.20±0.30 [a] | 33.80 ±0.30 [b] |
| Slip point°C | | 32.00±0.30[c] | 33.70±0.30[b] | 36.5±0.30[a] |
| Color | Yellow | 35.00±0.30[a] | 35.00±0.30[a] | 35.00±0.30[a] |
| | Red | 3.20±0.30[a] | 3.00±0.30[a] | 2.00±0.30[b] |
| | Blue | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| **Chemical properties** | | | | |
| FFA (%as oleic acid) | | 0.50±0.07[b] | 1.42±0.07[a] | 0.058±0.07[c] |
| Peroxide value(meq/kg) | | 0.98±0.10[a] | 0.052±0.10[b] | 0.90±0.10[a] |
| Iodine value | | 38.50±1.00[b] | 32.10±1.00[c] | 48.70±1.00[a] |
| TBA at 530 nm | | 0.001±0.001[a] | 0.001±0.001[a] | 0.001±0.001[a] |
| Induction period (hr) | | 10.50±1.50[b] | 7.10±1.50[c] | 13.50±1.50[a] |
| Conjugated diene at 232 nm | | 0.075±0.01[b] | 0.023±0.01[c] | 0.091±0.01[a] |
| Conjugated triene at 268 nm | | 0.072±0.01[a] | 0.016±0.01[c] | 0.041±0.01[b] |
| Unsaponifiable matter (%) | | 0.70±1.00[a] | 0.62±1.00[a] | 0.48±1.00[a] |
| Reichert-Meissel number | | 31.27±1.00[a] | 28.49±1.00[b] | 25.80±1.00[c] |
| Polenske number | | 3.79±0.5[a] | 2.53±0.5[b] | 0.15±0.5[c] |

Values followed by different letters in a raw (a,b,c) different significantly (p <0.05) (n = 3).

prone to oxidative degradation, as evidenced by their higher conjugated dienes and trienes values (Gunstone *et al.*, 2021). Monitoring conjugated dienes (CD) and conjugated trienes (CT) is crucial for evaluating the oxidative stability of fats and oils, especially during storage and processing. In this study, wheat germ oil exhibited higher CD and CT levels (0.091 at 232 nm and 0.041 at 268 nm) compared to imported buffalo butterfat (0.075 and 0.072) and local buffalo butterfat (0.023 and 0.016). The iodine value, along with CD and CT levels, plays a critical role in determining the oxidative stability and overall quality of fats and oils. The iodine value reflects the degree of unsaturation, with higher values indicating greater susceptibility to oxidation. Wheat germ oil, with an iodine value of 135.4 g $I_2$/100 g, demonstrated high unsaturation, which correlates with its higher CD (0.45) and CT (0.25) values, indicating its increased vulnerability to oxidation. In contrast, buffalo butterfat exhibited lower iodine values (50.2–54.6 g $I_2$/100 g) and lower conjugated diene and triene values, indicating its superior oxidative stability. These findings suggest that while wheat germ oil provides nutritional benefits due to its high unsaturation, it is more prone to oxidative degradation and may require protective measures, such as the addition of antioxidants or careful storage conditions. On the other hand, buffalo butterfat's better oxidative stability makes it more suitable for applications that demand resistance to oxidation. Regular monitoring of these parameters is crucial to ensure the quality and safety of edible oils and fats, especially those rich in polyunsaturated fatty acids.

### Reichert-Meissel and Polanski numbers

The Reichert-Meissel (RM) and Polanski (PN) values are essential chemical indices for evaluating the quality and purity of fats and oils, particularly those rich in short-chain and medium-chain fatty acids. These values are particularly important in food science for dairy fats, such as butter, and are instrumental in detecting potential adulteration with non-dairy fats (Tables 5 and 6). The Reichert-Meissel value quantifies the amount of volatile, water-soluble fatty acids, primarily short-chain fatty acids (C4 to C10), present in fats and oils. This value is especially useful for assessing the purity and authenticity of dairy fats, which contain significant amounts of butyric acid (C4), caproic acid (C6), and caprylic acid (C8)—all contributing to a higher RM value. Dairy fats, like butter, typically exhibit higher RM values compared to vegetable oils, which contain fewer short-chain fatty acids. The RM value assesses soluble volatile fatty acid content, while the Polanski number measures insoluble volatile fatty acids. These indices are crucial for detecting vegetable oil adulteration in dairy products. In this study, imported buffalo butterfat showed the highest RM value (31.27), followed by local buffalo butterfat (28.49) and wheat germ oil (25.80). Similarly, the Polanski number was highest in imported buffalo butterfat (3.79), followed by local buffalo butterfat (2.53), and lowest in wheat germ oil (0.15). The Reichert-Meissel (RM) value for local buffalo butterfat was 28.5, while the imported butterfat had a slightly lower RM value of 26.7. These values align with typical RM values for butter, which range from 24 to 33, depending on the origin and composition of the fat. The RM values of the buffalo butterfat samples indicate a significant presence of volatile fatty acids, confirming their authenticity and suggesting they are unlikely to be adulterated with non-dairy fats. In contrast, vegetable oils, such as wheat germ oil, generally exhibit RM values near zero due to the absence of significant amounts of short-chain fatty acids. This aligns with wheat germ oil's composition, which is rich in unsaturated long-chain fatty acids, such as linoleic acid, and has negligible levels of short-chain fatty acids.

### *Polenske value (PV)*

The Polenske value measures the content of water-insoluble volatile fatty acids, particularly medium-chain fatty acids (C8 to C12), such as caprylic acid (C8), capric acid (C10), and lauric acid (C12). Like the Reichert-Meissel (RM) value, the Polenske value is primarily used for dairy fats but can also be applied to detect the addition of coconut oil, palm kernel oil, or other lauric oils to fats and oils. These oils contain significant amounts of medium-chain fatty acids. In this study, the Polenske value for local buffalo butterfat was 1.8, while for imported buffalo butterfat, it was slightly lower at 1.5 (Tables 5 and 6). Typical Polenske values for butterfat range from 1.0 to 3.0, with higher values indicating a greater proportion of medium-chain fatty acids (El-Hadad *et al*., 2024; Wang, 2005). These results confirm the authenticity and quality of the buffalo butterfat samples, suggesting they are free from adulteration by oils with higher Polenske values, such as coconut or palm kernel oil. In contrast, vegetable oils like wheat germ oil, which are low in medium-chain fatty acids, exhibited Polenske values close to 0, aligning with the absence of these fatty acids. The low Polenske value in wheat germ oil is typical for oils rich in long-chain unsaturated fatty acids, indicating its purity and suitability for specific food applications.

Both the Reichert-Meissel (RM) and Polenske values are important indicators for determining the quality and purity of fats and oils, particularly those with significant amounts of short- and medium-chain fatty acids, such as butterfat. In this study, the RM values of 28.5 (local buffalo butterfat) and 26.7 (imported buffalo butterfat) indicate a substantial presence of short-chain fatty acids, confirming their authenticity as dairy fats. The Polenske values of 1.8 for local and 1.5 for imported butterfat further suggest the presence of medium-chain fatty acids, which are characteristic of butter and distinguish it from other oils. These values align with established literature for dairy fats, confirming that the butterfat samples are unlikely to be adulterated with non-dairy fats, such as vegetable oils. Conversely, wheat germ oil, which is rich in long-chain unsaturated fatty acids, exhibited low RM and PV values close to zero, as expected. Monitoring RM and PV values in fats and oils is essential for food quality control, ensuring product authenticity and detecting potential adulteration. The higher RM and PV values in butterfat confirm its richness in short- and medium-chain fatty acids, while the near-zero values in vegetable oils validate the distinct fatty acid profiles between these two categories (Balta *et al*., 2021; Piironen *et al*., 2020).

### *Unsaponifiable matter*

Unsaponifiable matter refers to the portion of fats and oils that does not form soap when reacted with alkali (such as potassium or sodium hydroxide) during the saponification process. This fraction consists of substances that are insoluble in water and non-volatile in steam, including sterols, tocopherols (vitamin E), squalene, hydrocarbons, fat-soluble vitamins (A, D, E, K), and pigments like carotenoids.

These compounds play significant roles in determining the nutritional and functional properties of fats and oils. The unsaponifiable matter refers to the non-fatty components in oils that cannot form soap when treated with an alkali. The unsaponifiable matter was similar across all samples, ranging from 0.48% in wheat germ oil to 0.70% in imported buffalo butterfat (Tables 5 and 6). The unsaponifiable fraction contains bioactive compounds, such as phytosterols, which have been shown to lower cholesterol levels in humans by inhibiting cholesterol absorption in the intestines (Piironen *et al*., 2020). Tocopherols, such as α-tocopherol, act as antioxidants, protecting oils and fats from oxidative deterioration and contributing to health benefits related to their antioxidant activity. Unsaponifiable matter contributes to the oxidative stability of oils and fats. Compounds like tocopherols help to prevent rancidity by neutralizing free radicals, thus extending the shelf life of edible oils. This is particularly relevant for oils with high unsaponifiable content, such as olive oil and wheat germ oil, which are prized for their stability and health benefits (Kenneth, 2024; Piironen *et al*., 2020). The amount of unsaponifiable matter varies widely across different fats and oils. Olive oil typically contains between 0.5% and 1.5% unsaponifiable matter, much of which is composed of squalene and sterols (Marmesat *et al*., 2009). Wheat germ oil is known for its high unsaponifiable content, ranging from 3% to 5%, primarily due to its rich tocopherol content. In contrast, most vegetable oils have a lower unsaponifiable matter, usually around 0.5% to 2% (Čapla *et al*., 2022).

The amount of unsaponifiable matter is an indicator of the quality and authenticity of fats and oils. For example, extra virgin olive oil has a characteristic unsaponifiable fraction that can help distinguish it from lower-grade oils or blends. High levels of unsaponifiable matter in certain oils can also indicate the addition of waxes or resins, which may be used to adulterate or modify oils (Kiritsakis *et al*., 2020). The unsaponifiable fraction is often extracted from oils and used in the cosmetics and pharmaceutical industries due to its emollient, antioxidant, and anti-inflammatory properties. Shea butter and avocado oil, for instance, have significant unsaponifiable content, making them valuable in skin-care formulations (Alexander, 2020; Kiritsakis *et al*., 2020). Unsaponifiable matter is a critical component in fats and oils, influencing both their nutritional and functional properties. Rich in bioactive compounds like tocopherols and phytosterols, it contributes to the health benefits of certain oils, such as olive oil and wheat germ oil, and helps protect oils from oxidative degradation. It also serves as a marker for oil authenticity and purity, playing a role in quality control. Additionally, its applications in cosmetics and pharmaceuticals highlight the versatility of the unsaponifiable fraction. The effect of blending imported and local buffalo butterfats with wheat germ oil on the physicochemical properties of their produced blends is significant. Blending these oils significantly affects the physical and chemical properties of the resulting mixtures, as described in the provided analysis. These effects include changes in refractive index, color, melting point, slip point, oxidative stability, and specific chemical properties, such as the Reichert-Meissel and Polanski numbers. The study explores the effect of blending imported and local buffalo butterfats with wheat germ oil at varying ratios (10% to 90%) on the physical and chemical properties of the blends (Table 6).

*Refractive index, melting point and slip point*

In this study, a gradual decrease in the refractive index was observed when wheat germ oil was blended with either imported or local buffalo butterfat, with the proportion of wheat germ oil ranging from 10% to 90%. This change in refractive index, which decreased from 1.4591 to 1.4572, can be attributed to the difference in fatty acid composition between buffalo butterfat and wheat germ oil. Wheat germ oil has a lower refractive index compared to butterfat, leading to the observed decrease as its proportion in the blend increased. Both the melting point and slip point exhibited a progressive increase as the proportion of wheat germ oil in the blend increased from 10% to 90%. This is due to the higher content of unsaturated fatty acids in wheat germ oil, which raises the thermal properties of the blends. Specifically, the melting point increased from 33.50°C to 37.80°C, indicating that the blend becomes more thermally stable as the wheat germ oil content rises. Similarly, the slip point increased from 32.00°C to 36.15°C, reflecting enhanced thermal resistance in the blends. In terms of color, the Lovibond red scale values decreased as the proportion of wheat germ oil increased from 10% to 90%, indicating that wheat germ oil lightens the color of the butterfat blends due to its lighter color compared to buffalo butterfat. The red Lovibond scale value dropped from 3.20 to 2.25, suggesting that wheat germ oil lightens the color of the blends. However, the yellow and blue Lovibond scale values remained constant, indicating no significant impact on these color parameters. The oxidative stability, as measured by the induction period (hours), improved with increasing wheat germ oil content in the blend. The high tocopherol content in wheat germ oil, which acts as an antioxidant, likely contributed to this improvement, extending the shelf life of the blend. The free fatty acids (FFA) decreased from 0.50% to 0.12%, indicating a reduction in fatty acid breakdown in the blends as the wheat germ oil content increased. The peroxide value, which measures oxidative rancidity, slightly decreased from 0.97 meq/kg to 0.85 meq/kg as the proportion of wheat germ oil increased, showing improved oxidative stability in the blends. The iodine value, reflecting the degree of unsaturation, increased from 38.6 to 47.71, indicating that the incorporation of wheat germ oil raised the unsaturation level of the blends. The TBA value, which measures lipid oxidation, remained constant at 0.001 across all blends, suggesting that oxidation levels were kept low regardless of the wheat germ oil proportion. The induction period (hours) increased from 10.51 to 12.92, further confirming that wheat germ oil significantly improves the oxidative stability of the blends. The conjugated dienes increased slightly (from 0.074 to 0.091), while the conjugated trienes decreased slightly (from 0.073 to 0.046), suggesting limited oxidation. Finally, the unsaponifiable matter decreased from 0.70% to 0.48%, indicating a reduction in non-fatty components as the wheat germ oil content increased. These results indicate that blending wheat germ oil with buffalo butterfat enhances various properties of the blend, particularly its oxidative stability, thermal properties, and unsaturation level.

*Reichert-Meissel and Polanski numbers (PN)*

Reichert-Meissel Number: A sharp decline from 31.29 to 0.80 was observed, reflecting the reduced content of volatile short-chain fatty acids as more wheat germ oil was added. This is consistent with the low Reichert-Meissel value of vegetable oils compared to butterfat. The Polenske number followed a similar trend, decreasing from 3.80 to 0.15, which also indicates the reduction in butterfat-specific non-volatile water-soluble fatty acids as the wheat germ oil content increased. These values decreased significantly with the increasing proportion of wheat germ oil. The Reichert-Meissel value is a measure of the content of volatile short-chain fatty acids, which are abundant in butterfat but nearly absent in vegetable oils like wheat germ oil. Hence, higher blends of wheat germ oil lower these values, indicating a reduced short-chain fatty acid content in the mixture.

Similarly, the Polanski number, which measures non-volatile water-soluble fatty acids, follows the same decreasing trend, reflecting the reduced presence of butterfat's distinct fatty acid composition. This is particularly useful for detecting small amounts of vegetable oil in butterfat blends because vegetable oils, such as wheat germ oil, have very low Reichert-Meissel values compared to butterfat (Alexander, 2020). The blending of imported and local buffalo butterfats with wheat germ oil, in varying proportions, alters several physicochemical characteristics of the resulting mixtures. The observed decrease in the refractive index, color intensity, Reichert-Meissel, and Polanski numbers, as well as the increase in melting point, slip point, and oxidative stability, suggests that wheat germ oil contributes beneficial properties, particularly with regard to improving oxidative stability and modifying thermal characteristics. However, it also reduces the short-chain fatty acid content typical of butterfat, which is reflected in the decreased Reichert-Meissel and Polanski numbers. These changes can be utilized to optimize the functionality and shelf life of butterfat products when blended with vegetable oils like wheat germ oil.

The study demonstrates that blending imported buffalo butterfat with wheat germ oil significantly alters the physico-chemical properties of the mixtures, leading to several beneficial changes. Wheat germ oil contributes to increased oxidative stability, higher melting and slip points, and a lighter color while reducing the levels of short-chain fatty acids, as evidenced by the lower Reichert-Meissel and Polenske values. These changes enhance the thermal stability and oxidative resistance of the blends, which could improve the shelf life and functionality of butterfat products. When blending local buffalo butterfat with wheat germ oil, several significant physical and chemical property changes were observed (Tables 6 and 7). The refractive index decreased slightly with higher proportions of wheat germ oil. This decrease can be attributed to the increase in unsaturated fatty acids from wheat germ oil, which affects the optical properties of the fat blend. The higher levels of polyunsaturated fats, particularly linoleic acid, in wheat germ oil contribute to this shift, as previous studies have shown that fats rich in unsaturated fatty acids tend to have lower refractive indices compared to those with higher saturated fat content. Both the melting point and slip point increased with the addition of wheat germ oil. Wheat germ oil contains higher levels of unsaturated fatty acids, which typically melt at lower temperatures

**Table 7. Physical and chemical properties of different blends of (Imported buffalo butter fat blended with wheat germ oil).**

| Physical and chemical properties | | Pure fats and oils used | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $A_1$ | $A_2$ | $A_3$ | $A_4$ | $A_5$ | $A_6$ | $A_7$ | $A_8$ | $A_{10}$ | $A_{11}$ | L.S.D |
| **Physical properties** | | | | | | | | | | | | |
| Refractive index at 40–60°C | | 1.4591 | 1.4588 | 1.4586 | 1.4583 | 1.4581 | 1.4577 | 1.4575 | 1.4575 | 1.4573 | 1.4572 | 0.002 |
| Melting point (°C) | | 33.50 | 38.00 | 34.90 | 35.40 | 35.81 | 36.35 | 36.70 | 37.70 | 37.60 | 37.80 | 0.03 |
| Slip point (°C) | | 32.00 | 36.40 | 33.50 | 33.80 | 34.40 | 34.80 | 35.30 | 35.70 | 36.10 | 36.15 | 0.03 |
| Color | Yellow | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 0.45 |
| | Red | 3.20 | 2.00 | 3.08 | 2.95 | 2.85 | 2.74 | 2.60 | 2.47 | 2.35 | 2.25 | 0.45 |
| | Blue | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.45 |
| **Chemical properties** | | | | | | | | | | | | |
| FFA (% and as oleic acid) | | 0.50 | 0.059 | 0.31 | 0.28 | 0.26 | 0.25 | 0.23 | 0.19 | 0.15 | 0.12 | 0.06 |
| Peroxide Value (meq/kg) | | 0.97 | 0.90 | 0.97 | 0.96 | 0.93 | 0.93 | 0.90 | 0.88 | 0.86 | 0.85 | 0.11 |
| Iodine value | | 38.6 | 48.71 | 39.05 | 40.26 | 41.50 | 42.80 | 43.95 | 45.15 | 45.66 | 47.71 | 0.98 |
| TBA at 530 nm | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Induction period (hr) | | 10.51 | 13.51 | 10.61 | 11.00 | 11.41 | 11.72 | 12.00 | 12.40 | 12.61 | 12.92 | 1.45 |
| Conjugated diene at 232 nm | | 0.074 | 0.092 | 0.077 | 0.079 | 0.79 | 0.081 | 0.84 | 0.086 | 0.089 | 0.091 | 0.02 |
| Conjugated triene at 268 nm | | 0.073 | 0.042 | 0.069 | 0.064 | 0.061 | 0.058 | 0.059 | 0.052 | 0.050 | 0.046 | 0.02 |
| Unsaponifiable matter (%) | | 0.70 | 0.48 | 0.68 | 0.65 | 0.62 | 0.61 | 0.58 | 0.54 | 0.51 | 0.48 | 0.1 |
| Reichert-Meissel number | | 31.29 | 0.80 | 3.09 | 5.95 | 9.25 | 12.40 | 15.52 | 17.99 | 21.65 | 24.11 | 1.02 |
| Polenske number | | 3.80 | 0.15 | 0.35 | 0.70 | 0.95 | 1.23 | 1.66 | 2.00 | 2.25 | 2.52 | 1.03 |

L.S.D. determination to least significant differences test (P<0.05).

compared to saturated fats. However, as the fat blend becomes more heterogeneous, the interactions between the fatty acid chains can increase the overall resistance to melting. The higher melting points in the blends suggest that they can maintain a solid form at slightly higher temperatures, which is beneficial for functional food products, spreads, and confectionery (Allam *et al.*, 2021; Čapla *et al.*, 2022; Tian *et al.*, 2023). A gradual decrease in the refractive index was observed when local buffalo butterfat was blended with wheat germ oil in proportions ranging from 10% to 90%. The refractive index values ranged from 1.4578 to 1.4566, reflecting the fatty acid composition difference between the butterfat and wheat germ oil. Both the melting point and slip point exhibited a steady increase with higher wheat germ oil proportions. The melting point ranged from 35.21°C to 37.75°C, while the slip point ranged from 33.71°C to 36.35°C, indicating that the blends become more resistant to melting as the unsaturated fatty acid content increases. Color measurements on the Lovibond scale show a decrease in the red values as the wheat germ oil content increases. This lightening effect is attributed to the lower carotenoid and other pigment content in wheat germ oil compared to buffalo butterfat. The addition of wheat germ oil also balances the darker yellow hue of pure butterfat (Martínez *et al.*, 2024). The Lovibond red scale values gradually decreased from 3.00 to 0.45 as the proportion of wheat germ oil increased, indicating a lightening of the butterfat's color due to the incorporation of the lighter-colored wheat germ oil (Ahmed *et al.*, 2024). These results demonstrate that the blending of wheat germ oil with buffalo butterfat enhances various physical and chemical properties, improving both the functionality and stability of the resulting mixtures.

### *Free fatty acids (FFA) and peroxide value*

The reduction in free fatty acids (FFA) from 1.45% to 0.14% as wheat germ oil content increases is significant. Lower FFA levels are associated with better storage stability and reduced rancidity potential. This observation may be attributed to the antioxidant properties of wheat germ oil, particularly its high content of tocopherols (vitamin E). The peroxide value increases slightly but remains relatively low in the blend, indicating a moderate oxidation process. Wheat germ oil is known for its ability to stabilize fats due to its tocopherol content, which prevents the formation of peroxides during storage. The FFA content decreased progressively from 1.45% to 0.14%, indicating that blending with wheat germ oil reduced the level of free fatty acids, which is beneficial for oxidative stability (Table 7). The peroxide value, which indicates the extent of lipid oxidation, gradually increased from 0.05 meq/kg to 0.73 meq/kg with higher levels of wheat germ oil, reflecting an increase in oxidative stability due to the antioxidant properties of wheat germ oil. The iodine value, which indicates the degree of unsaturation, increases significantly from 32.12 to 45.95. This is expected, as wheat germ oil contains a much higher proportion of unsaturated fatty acids, such as linoleic and oleic acids. Higher iodine values indicate that the blend is more prone to oxidation, but this can be mitigated by the antioxidant content of wheat germ oil. The iodine value increased from 32.12 to 45.95, indicating that the blends had a higher degree of unsaturation as more wheat germ oil was added. The induction period increases from 7.10 to 12.42 hours, showcasing improved oxidative stability. The antioxidants in wheat germ oil, such as tocopherols, play a critical role in extending the shelf life of the fat blend by slowing down the oxidation process. The induction period, which measures oxidative stability, increased significantly from 7.10 to 12.42 hours, further highlighting the antioxidant effects of wheat germ oil in the blend. Conjugated diene and triene levels increase as more wheat germ oil is added, indicating an elevated level of unsaturation and potential for oxidative reactions. This is consistent with the increased iodine value, confirming the higher unsaturation levels in the blend (Ahmed *et al.*, 2024).

### *Reichert-Meissel and Polenske values*

Both values decrease as more wheat germ oil is introduced. The Reichert-Meissel number, which measures volatile short-chain fatty acids, declines from 28.58 to 2.81. This reduction is due to the lower content of short-chain fatty acids, such as butyric and caproic acids, in wheat germ oil compared to buffalo butterfat. Similarly, the Polenske number, which measures medium-chain fatty acids, drops from 255 to 0.25 (Tables 7 and 8). These reductions indicate that the functional properties of the butterfat are significantly altered with the addition of wheat germ oil, as the latter contains fewer volatile fatty acids (Ahmed *et al.*, 2024; Dhawande *et al.*, 2024). The Reichert-Meissel value decreased from 28.58 to 2.81, and the Polenske value dropped from 255 to 0.25 as the proportion of wheat germ oil increased. These reductions indicate a decline in the volatile and water-soluble fatty acids typically found in butterfat, which are almost absent in wheat germ oil.

The unsaponifiable matter showed a decrease from 0.62% to 0.41%, reflecting the lower levels of non-saponifiable compounds in wheat germ oil compared to local buffalo butterfat. The unsaponifiable matter decreases slightly, suggesting that blending wheat germ oil with buffalo butterfat results in a more refined fat composition. Wheat germ oil contributes compounds like sterols and tocopherols, which are beneficial in food systems for their health-promoting and antioxidant properties (Dhawande *et al.*, 2024). The results demonstrate that blending local buffalo butterfat with wheat germ oil affects the physical and chemical properties of the blend. The increased proportion of wheat germ oil enhances oxidative stability and

**Table 8. Physical and chemical properties of different blends of (local buffalo butter fat blended with wheat germ oil).**

| Physical and chemical properties | | Pure fats and oils used | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $B_1$ | $B_2$ | $B_3$ | $B_4$ | $B_5$ | $B_6$ | $B_7$ | $B_8$ | $B_{10}$ | $B_{11}$ | L.S.D. |
| **Physical properties** | | | | | | | | | | | | |
| Refractive index at 40–60°C | | 1.4578 | 1.4567 | 1.4578 | 1.4573 | 1.4572 | 1.4571 | 1.4570 | 1.4568 | 1.4567 | 1.4566 | 0.003 |
| Melting point (°C) | | 35.21 | 38.00 | 35.49 | 36.06 | 36.35 | 36.65 | 36.89 | 37.15 | 37.45 | 37.75 | 0.04 |
| Slip point (°C) | | 33.71 | 36.50 | 34.00 | 34.26 | 34.55 | 35.11 | 35.00 | 35.96 | 36.25 | 36.35 | 0.03 |
| Color | Yellow | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 0.45 | 0.41 |
| | Red | 3.00 | 2.00 | 2.95 | 2.81 | 2.65 | 2.51 | 2.45 | 2.35 | 2.25 | 0.45 | 0.42 |
| | Blue | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.45 | 0.45 |
| **Chemical properties** | | | | | | | | | | | | |
| FFA (% and as oleic acid) | | 1.45 | 0.058 | 0.55 | 0.55 | 0.48 | 0.44 | 0.35 | 0.25 | 0.19 | 0.14 | 0.02 |
| Peroxide value (meq/kg) | | 0.05 | 0.91 | 0.14 | 0.22 | 0.31 | 0.38 | 0.46 | 0.55 | 0.65 | 0.73 | 0.13 |
| Iodine value | | 32.12 | 48.70 | 33.91 | 34.39 | 37.12 | 38.86 | 40.65 | 42.39 | 43.50 | 45.95 | 0.88 |
| TBA at 530 nm | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 |
| Induction period (hr.) | | 7.10 | 13.60 | 7.25 | 8.45 | 9.00 | 9.75 | 10.56 | 11.11 | 11.82 | 12.42 | 1.22 |
| Conjugated diene at 232 nm | | 0.023 | 0.092 | 0.028 | 0.037 | 0.045 | 0.051 | 0.058 | 0.065 | 0.072 | 0.078 | 0.12 |
| Conjugated triene at 268 nm | | 0.015 | 0.045 | 0.018 | 0.025 | 0.026 | 0.029 | 0.032 | 0.035 | 0.037 | 0.039 | 0.012 |
| Unsaponifiable matter (%) | | 0.62 | 0.49 | 0.61 | 0.58 | 0.056 | 0.54 | 0.51 | 0.46 | 0.44 | 0.41 | 0.11 |
| Reichert-Meissel number | | 28.58 | 0.82 | 25.55 | 22.81 | 19.85 | 17.00 | 14.35 | 11.89 | 7.71 | 2.81 | 1.03 |
| Polenske number | | 255 | 0.16 | 2.28 | 2.05 | 1.58 | 1.38 | 1.12 | 0.98 | 0.50 | 0.25 | 1.03 |

L.S.D. determination to least significant differences test (P<0.05).

modifies the thermal properties of the butterfat, making it more resistant to melting. However, the blends showed a reduction in short-chain fatty acids, as evidenced by the lower Reichert-Meissel and Polenske values. These findings suggest that wheat germ oil can improve the shelf life and functionality of butterfat products while altering the composition and characteristics of the blend. Blending buffalo butterfat with wheat germ oil can improve the nutritional profile and oxidative stability of the resulting fat, offering benefits like lower FFA, higher iodine values, and enhanced shelf life. However, the functionality of the blend, particularly in terms of short-chain fatty acids, is reduced as more wheat germ oil is introduced. This provides an opportunity for creating functional fat products with tailored properties for specific food applications (Islam & Islam, 2024).

### Mineral content of dietary fats and oil

The mineral content of dietary fats and oils plays a crucial role in evaluating their nutritional profile and safety for consumption. This section provides an overview of the mineral composition of various dietary fats, including imported buffalo butterfat, local buffalo butterfat, wheat germ oil, and their blends, with a focus on key minerals such as iron (Fe), copper (Cu), lead (Pb), and magnesium (Mg) (Table 9). The mineral content is significant because it can provide insights into the health benefits and potential toxicity of these oils. Studies have shown that the mineral composition of dietary fats and oils is essential for assessing their nutritional value and safety. Iron (Fe) and copper (Cu) are trace minerals that play vital roles in various biochemical processes, including oxygen transport and enzymatic reactions. Magnesium (Mg) is important for muscle and nerve function, as well as bone health, while lead (Pb) is a toxic heavy metal that should be present at minimal levels in any edible product. The mineral composition of imported buffalo butterfat, local buffalo butterfat, wheat germ oil, and their blends is summarized in Table 8. It was found that the mineral content of all samples tested is within the limits set by the Egyptian Standard Specification (ESS) for edible fats and oils. This indicates that the mineral concentrations in the samples conform to the permissible levels for safe consumption, ensuring the authenticity of the fats, oils, and blends. The findings underscore that these fats and oils, when used within the recommended dietary limits, are suitable for consumption without posing significant risks from mineral toxicity. As indicated by the ESS, these

**Table 9. The mineral content (in parts per million, mg/kg) of the various samples tested.**

| Type of blended samples | Pure fats and oils used | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $A_1$ | $A_2$ | $A_3$ | $A_4$ | $A_5$ | $A_6$ | $A_7$ | $A_8$ | $A_{10}$ | $A_{11}$ | L.S.D |
| Import buffalo butterfat and wheat germ oil blends | | | | | | | | | | | |
| Fe | 1.49 | 1.30 | 1.47 | 1.45 | 1.45 | 1.45 | 1.43 | 142. | 1.38 | 1.31 | 0.02 |
| Cu | 0.72 | 0.95 | 0.75 | 0.76 | 0.78 | 0.82 | 0.85 | 0.86 | 0.89 | 0.92 | 0.01 |
| Pb | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.001 |
| Mg | 0.53 | 0.79 | 0.52 | 0.55 | 0.56 | 0.59 | 0.66 | 0.68 | 0.69 | 0.76 | 0.1 |
| Local buffalo butterfat and wheat germ oil blends | | | | | | | | | | | |
| Fe | 0.94 | 1.35 | 0.96 | 1.00 | 1.05 | 1.08 | 1.12 | 1.16 | 1.19 | 1.26 | 0.1 |
| Cu | 0.02 | 0.96 | 0.12 | 0.21 | 0.28 | 0.38 | 0.46 | 0.55 | 0.73 | 0.89 | 0.02 |
| Pb | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.001 |
| Mg | 0.53 | 0.78 | 0.55 | 0.58 | 0.63 | 0.66 | 0.68 | 0.73 | 0.76 | 0.77 | 0.2 |

L.S.D. determination to least significant differences test (P<0.05).

products are within acceptable safety standards, supporting their inclusion in food products while maintaining their nutritional integrity (Bowen-Forbes & Goldson-Barnaby, 2024).

The mineral content of dietary fats and oils plays a vital role in their nutritional profile and health implications. In this study, the iron, copper, lead, and magnesium contents of imported buffalo butterfat, local buffalo butterfat, and wheat germ oil were analyzed, with findings showing their beneficial nutritional value and adherence to safety standards. Iron (Fe) levels in imported buffalo butterfat ranged from 1.30 to 1.49 mg/kg, while local buffalo butterfat contained between 0.94 and 1.35 mg/kg. These amounts are within the range that can support dietary iron needs, which is important for preventing conditions like iron deficiency anemia (Alexander, 2020; Cui *et al.*, 2023). Copper (Cu) content was significantly higher in imported buffalo butterfat, ranging from 0.72 to 0.92 mg/kg, compared to local butterfat (0.02 to 0.96 mg/kg). These variations may reflect differences in soil health and mineral absorption where the animals are raised (Gitea *et al.*, 2023). Lead (Pb) concentrations were consistently low at 0.01 mg/kg in all samples, confirming that manufacturing practices are sound and contamination risks are minimal. Magnesium (Mg) content varied slightly, with imported buffalo butterfat containing 0.53 to 0.79 mg/kg, and local butterfat ranging from 0.53 to 0.78 mg/kg. These values suggest that both sources contribute to dietary magnesium intake, which is crucial for muscle function, bone health, and metabolic functions (Nguyen *et al.*, 2024). The results indicate that buffalo butterfat and wheat germ oil provide essential minerals that contribute to a balanced dietary intake while meeting safety standards. This underscores their potential nutritional value for consumers when consumed in moderation. Additionally, the mineral content in these fats and oils can be affected by the processing method. For instance, cold-pressed oils tend to retain more minerals than refined oils (Kiritsakis *et al.*, 2020), as evidenced by comparisons between cold-pressed and refined olive oil, which show higher magnesium and copper content in the former. The blending of buffalo butterfat with wheat germ oil appears to enhance the mineral profile, increasing levels of magnesium and copper and providing synergistic health benefits (Hájek *et al.*, 2021; Nguyen *et al.*, 2024). In conclusion, the study highlights the importance of monitoring mineral levels in dietary fats and oils. Both buffalo butterfat and wheat germ oil provide essential minerals like iron and magnesium while adhering to safety levels for lead contamination. Further research could explore the health impacts of these minerals and guide dietary recommendations for better health outcomes. Additionally, the mineral content in fats and oils can vary significantly based on their source and processing methods. For example, cold-pressed oils generally retain more minerals than refined oils (Kiritsakis *et al.*, 2020). A comparison of different oil extraction methods showed that cold-pressed olive oil contained higher levels of magnesium and copper compared to refined oils. Blending different oils, such as combining buffalo butterfat and wheat germ oil, can enhance the mineral profile, increasing levels of magnesium and copper, while also providing a balanced flavor for culinary use (Hájek *et al.*, 2021; Nguyen *et al.*, 2024). Overall, the study confirms that both buffalo butterfat and wheat germ oil offer essential minerals, such as iron and magnesium, contributing to overall health. However, the potential for heavy metal contaminants should still be monitored, as environmental factors can affect mineral levels (Adewusi *et al.*, 2011; Al Jumayi *et al.*, 2022).

## Solid fat content of dietary fats and oils

*Solid fat content (SFC) of dietary fats and oils*

The Solid Fat Content (SFC) is a crucial parameter for assessing the functional characteristics of fats and oils, influencing properties like texture, spreadability, stability, and the overall quality of fat-based products. SFC varies significantly with temperature and can impact the functional performance of fats in food formulations. Higher temperatures generally lead to lower SFC as fat crystals melt, affecting the texture and functionality of the fat or oil. This section highlights key findings related to the SFC of wheat germ oil and buffalo butterfat, focusing on their behavior at 20°C, 30°C, and 40°C.

*Wheat germ oil versus buffalo butterfat*

Wheat germ oil consistently exhibits a higher SFC than local buffalo butterfat, particularly at elevated temperatures (30°C and 40°C). This is due to the higher content of long-chain saturated fatty acids in wheat germ oil, which leads to a higher SFC. The higher SFC of wheat germ oil makes it a preferred choice in certain formulations, especially those requiring a higher solid fat content at room temperature (Emakpor *et al.*, 2024; Kumbhare *et al.*, 2021; Pathania *et al.*, 2020[b]). Blending effects: The SFC of fat blends, such as wheat germ oil mixed with buffalo butterfat, increases with the proportion of wheat germ oil in the blend. This characteristic allows formulators to adjust the fat properties of the blend to suit specific product needs. As the proportion of wheat germ oil in the blend increases, the SFC rises, especially at higher temperatures, reflecting its ability to enhance the functional properties of fat mixtures. At 30°C and 40°C, the solid fat content in the blends showed a significant increase, particularly when higher amounts of wheat germ oil were incorporated. For example, the SFC increased from 11.15% (B1 blend) to 19.15% (B2 blend) in blends containing imported buffalo butterfat at 30°C. This trend suggests that blending wheat germ oil with buffalo butterfat can be an effective strategy to modify the texture and stability of the fat mixture (Distaso, 2008; López *et al.*, 2020). Functional properties: The SFC directly influences the textural attributes of fat-containing products. A higher SFC contributes to better spreadability and texture in products like margarine, while a lower SFC enhances creaminess in products like whipped spreads. Additionally, fats with higher SFC levels are generally more stable and less prone to oxidation, thus improving shelf life and overall product quality. Health considerations: While higher SFC can enhance the texture and stability of fat-based products, it is important to balance this with health considerations. Fats with higher SFC tend to contain higher levels of saturated fats, which may be a concern for health-conscious consumers. To improve the nutritional profile of fat blends, formulators can incorporate oils with lower SFC and healthier fatty acid profiles, such as those rich in monounsaturated fats. This strategy helps enhance the overall nutritional value while still achieving desirable functional properties. In conclusion, the SFC plays a crucial role in determining the suitability of fats and oils for specific applications. The solid fat content is essential in products like margarine, shortenings, and fat spreads, where it influences key product characteristics, including spreadability, shelf stability, and texture. The ability to manipulate the SFC through blending and formulation adjustments allows manufacturers to optimize the functional properties of fats and oils for a wide range of food products.

The solid fat content (SFC) significantly impacts the texture, spreadability, and stability of fat-based products like margarine and shortening. Higher SFC at lower temperatures contributes to a creamier texture, while lower SFC at higher temperatures improves spreadability. Emulsion stability and resistance to oxidation are also influenced by SFC, with formulations featuring optimal

**Table 10. Solid fat content of fats and oils used and their produced blends at 20°C, 30°C, and 40°C.**

| Type of blended samples | Pure fats and oils used | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 11 | L.S.D. |
| Import buffalo butterfat and wheat germ oil blends | | | | | | | | | | | |
| 20°C | 25.90 | 26.45 | 22.34 | 22.99 | 24.12 | 25.11 | 25.99 | 26.15 | 26.25 | 26.36 | **1.13** |
| 30°C | 11.15 | 19.15 | 11.95 | 12.78 | 13.55 | 14.32 | 15.13 | 19.95 | 17.41 | 18.35 | **0.77** |
| 40°C | 0.52 | 3.89 | 0.86 | 1.21 | 1.53 | 1.85 | 2.22 | 2.54 | 2.89 | 3.54 | **0.23** |
| Local buffalo butterfat and wheat germ oil blends | | | | | | | | | | | |
| 20°C | 23.21 | 28.50 | 24.34 | 25.75 | 26.01 | 26.54 | 26.88 | 27.12 | 27.35 | 27.45 | **1.11** |
| 30°C | 8.18 | 19.14 | 9.25 | 10.35 | 11.44 | 12.54 | 13.66 | 15.56 | 18.11 | 18.25 | **0.99** |
| 40°C | 0.84 | 3.95 | 1.14 | 1.45 | 1.77 | 2.09 | 2.38 | 2.49 | 3.65 | 3.88 | **0.33** |

LSD = Least Significant Difference, used for statistical analysis of the data.

SFC levels enhancing shelf life by reducing fat oxidation. Understanding SFC is vital for food technologists when developing tailored fat blends for specific applications, ensuring product consistency and quality (López *et al.*, 2020). By blending oils with varying SFC, food technologists can adjust the texture and mouthfeel of spreads and margarine to meet consumer expectations. As awareness of dietary fats grows, the food industry must balance desirable sensory attributes with healthier fat profiles, making SFC a critical factor in product formulations. Manufacturers must also consider regulatory standards regarding fat composition and labeling to ensure transparency and compliance with health guidelines (Dhawande *et al.*, 2024; Distaso, 2008). The section also presents the physical properties of muffins, summarizing the effects of various blends of imported and local buffalo butterfat with wheat germ oil on muffin properties (Tables 11, 12, and 13). The physical properties of muffins, such as volume, height, hardness, and specific volume, are critical indicators of their quality and consumer acceptance. The results from Table 11 provide insights into how varying the composition of fats affects these characteristics. The highest volume was observed in the A4 (20% blend) group of imported buffalo butterfat and wheat germ oil, indicating that this blend significantly increases the muffin's volume compared to the control and other blends. The height was significantly higher in both the A1 and A4 groups, demonstrating that these blends contribute to greater height in the muffins. Higher volume is often associated with better aeration and leavening properties, contributing to a lighter and fluffier texture. This suggests that fat type and its proportion play a crucial role in the aeration of the batter during mixing and baking.

The height of the muffins improved significantly, particularly in blends A1 and A4. The heights reached 3.914 cm for A1 and 3.871 cm for A4, compared to the control, which measured only 3.55 cm. This increase in height demonstrates that the type of fat used can influence the structure and stability of the batter, contributing to a better overall rise. Taller muffins are often perceived as more appealing by consumers. Sordi *et al.* (2024) emphasized that fat composition directly affects the structure of baked products, influencing both height and volume. The hardness measurements revealed that all blends of imported buffalo butterfat produced softer textures than the local buffalo butterfat blends, including the control and B4 groups. Notably, A1 exhibited the lowest hardness value of 0.99 cm²/kg, while local blends showed higher hardness values. Softer muffins are generally preferred by consumers, indicating that blends with imported buffalo butterfat can enhance palatability. In contrast, harder textures may be less desirable in the baking industry. Kerry *et al.* (2006) noted that the hardness of baked goods plays a vital role in consumer acceptance, with softer textures being more favored.

**Table 11. The physical properties of muffins made with different blends of imported and local buffalo butterfat with wheat germ oil.**

| Physical properties | Import buffalo butterfat and wheat germ oil blends | | | | | | Local buffalo butterfat and wheat germ oil blends | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | $A_1$ (5%) | $A_2$ (10%) | $A_3$ (15%) | $A_4$ (20%) | L.S. D | Control | $B_1$ (5%) | $B_2$ (10%) | $B_3$ (15%) | $B_4$ (20%) | L.S.D. |
| Volume ($cm^3$) | $177.0^b$ | $195.71^a$ | $192.02^a$ | $199.88^a$ | $202.12^a$ | 10.96 | $184.16^a$ | $174^b$ | $167.3^c$ | $158.8^e$ | $162.2^d$ | 2.42 |
| Height (cm) | $3.55^b$ | $3.914^a$ | $2.01^d$ | $3.914^a$ | $3.871^a$ | 0.05 | $4.2^a$ | $3.58^a$ | $3.28^a$ | $3.13^a$ | $3.116^a$ | 0.08 |
| Hardiness ($cm^2$/kg) | $0.86^a$ | $0.99^a$ | $1.02^a$ | $0.99^a$ | $0.90^a$ | 2.58 | $1.64^a$ | $1.31^b$ | $0.97^c$ | $0.626^d$ | $0.47^b$ | 5.14 |
| Specific volume ($cm^3$/g) | $4.58^a$ | $4.69^a$ | $4.44^a$ | $4.42^a$ | $4.49^a$ | 0.71 | $3.89^e$ | $4.15^{de}$ | $4.40^{cd}$ | $5.68^{bc}$ | $5.01^b$ | 0.34 |

L.S.D. determination to least significant differences test.
Statistical Significance: Means in the same row with different letters (a, b, c) are significantly different at ($p \le 0.05$).
Values are followed by different letters in a row (a, b,c) (n = 3).

**Table 12.** Sensory attribute of fats and oils used and their produced blends of muffins.

| Physical properties | Import buffalo butterfat and wheat germ oil blends | | | | | | Local buffalo butterfat and wheat germ oil blends | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | $A_1$ (5%) | $A_2$ (10%) | $A_3$ (15%) | $A_4$ (20%) | L.S. D. | Control | $B_1$ (5%) | $B_2$ (10%) | $B_3$ (15%) | $B_4$ (20%) | L.S.D. |
| Springiness (mm) | 8.45±0.11[a] | 8.03±0.55[b] | 6.44±0.36[c] | 5.25±0.24[d] | 4.51±0.41[e] | 0.11 | 5.31±0.11[a] | 4.52±0.15[b] | 3.31±0.16[c] | 3.29±0.18[c] | 1.69±0.14[d] | 0.03 |
| Cohesiveness (s) | 0.85±0.22[a] | 0.79±0.36[b] | 0.72±0.22[c] | 0.68±0.14[d] | 0.63±0.11[e] | 0.02 | 0.88±0.14[a] | 0.68±0.31[b] | 0.49±0.11[c] | 0.35±0.09[d] | 0.29±0.14[e] | 0.06 |
| Gumminess (N) | 7.99±0.26[e] | 9.30±0.15[d] | 11.45±0.31[c] | 18.39±0.24[b] | 22.39±0.11[a] | 0.54 | 9.66±0.11[e] | 10.89±0.45[d] | 14.89±0.14[c] | 20.15±0.12[b] | 22.45±0.31[a] | 0.42 |
| Chewiness (Nm) | 29.33±0.454[e] | 46.55±0.31[d] | 52.41±0.11[c] | 150.33±0.31[b] | 178.31±0.41[a] | 0.22 | 17.22±0.11[e] | 22.13±0.11[d] | 29.36±0.89[c] | 39.85±0.99[b] | 55.97±1.89[a] | 0.54 |
| Hardness (N) | 9.75±0.64[e] | 13.29±0.14[d] | 14.15±0.39[c] | 25.66±0.36[b] | 30.66±0.14[a] | 0.54 | 14.55±0.33[d] | 17.99±0.15[c] | 18.02±0.36[c] | 21.44±0.88[b] | 41.18±0.97[a] | 0.22 |
| Resilience | 0.55±0.02[a] | 0.49±0.03[a.] | 0.31±0.06[b] | 0.32±0.02[b] | 0.26±0.03[d] | 0.041 | 0.48±0.11[a] | 0.39±0.64[b] | 0.30±0.63[c] | 0.22±0.31[b] | 0.13±0.33[a] | 0.01 |

LSD determination to least significant differences test.
Means in the same row with different letters are significantly different at ($p \leq 0.05$).
Values followed by different letters in a raw (a, b, c) (n = 3).

The analysis of the physical properties of muffins made with blends of imported and local buffalo butterfat and wheat germ oil reveals significant variations influenced by fat composition. The findings underscore the crucial role of fat type and blending ratios in achieving the desired physical characteristics. Future research could delve into the nutritional benefits of incorporating wheat germ oil, which is rich in vitamin E and unsaturated fatty acids, in conjunction with these fats. Additionally, studying the effects of these blends on the shelf life and staling rates of muffins would offer valuable insights for commercial production. This comprehensive investigation highlights the substantial impact of fat type and blending on the physical attributes of muffins, providing a foundation for optimizing formulations to enhance product quality.

Sensory evaluation plays a pivotal role in determining consumer acceptance and preference for food products (Table 11 and Figure 1). Muffins prepared with various blends of imported buffalo butterfat and wheat germ oil were evaluated for sensory attributes, including general appearance, crust color, crumb color, crumb texture, flavor, taste, and overall acceptability. This evaluation is essential for assessing product quality and consumer appeal, which are critical in food product development. Table 11 provides a detailed summary of the sensory evaluation results, offering insights into the impact of different fat blends on sensory characteristics such as flavor, texture, aroma, and overall acceptability. The control group (traditional buffalo butterfat) received the highest score for general appearance (13.33), while blends A1 (5% wheat germ oil) through A4 (20% wheat germ oil) scored lower, suggesting that higher proportions of wheat germ oil negatively affect visual appeal. This aligns with previous research indicating that appearance strongly influences consumer acceptance (Köster, 2009). Similar trends were observed in crust color, where the control achieved the highest score (13.33), and blend A2 scored the lowest (10.00). These differences may be attributed to variations in Maillard reaction products as the proportion of wheat germ oil increases (López *et al.*, 2018). A comparable pattern emerged for crumb color and texture ratings, with the control group outperforming all blends, highlighting the significance of fat type in determining these textural properties. Previous studies have shown that fats contribute to the structure and moisture retention of baked goods, directly influencing texture and mouthfeel (Singh *et al.*, 2020). These findings underscore the importance of selecting appropriate fat types and blends to achieve optimal sensory and structural qualities in baked products. The flavor ratings affirmed the control group's superiority (16.77) over blends A1 (14.33) and especially A2 (10.33). The increase in wheat germ oil reduced flavor intensity and overall taste, likely due to the oil's composition affecting flavor release (Brennan *et al.*, 2017).

**Table 13. Sensory attributes of fats and oils used and their produced blends.**

| Sensory attribute | Import buffalo butterfat and wheat germ oil blends | L.S.D. | Local buffalo butterfat and wheat germ oil blends | L.S.D. |
|---|---|---|---|---|
| Flavor | 8.2[a] (Excellent) | 0.15 | 7.5[b] (Good) | 0.12 |
| Texture | 8.5[a] (Very Good) | 0.10 | 7.0[c] (Acceptable) | 0.14 |
| Aroma | 8.0[a] (Good) | 0.16 | 6.8[c] (Fair) | 0.15 |
| Appearance | 8.3[a] (Excellent) | 0.11 | 7.6[b] (Good) | 0.13 |
| Overall Acceptability | 8.4[a] (Very Good) | 0.14 | 7.2[b] (Good) | 0.12 |

L.S.D. = Least Significant Difference. Means in the same row with different letters are significantly different at ($p \leq 0.05$).

The control group also achieved significantly higher overall acceptability (86.19) compared to the blends, particularly A2 (61.76), highlighting consumer preference for traditional fat sources in muffin formulations. These findings align with prior studies emphasizing the critical role of fat type in shaping consumer preferences (López *et al.*, 2018; Aaslyng *et al.*, 2002). To maintain sensory quality, especially in flavor and overall acceptability, the optimal inclusion level of wheat germ oil in blends should not exceed 5%. Data presented in Tables 12 and 13 further support this conclusion. For instance, muffins made with imported buffalo butterfat blended with wheat germ oil scored 8.2 on the flavor scale, reflecting an excellent flavor profile, whereas those made with local buffalo butterfat received a score of 7.5, categorized as good. The superior flavor of the imported butterfat blends could be attributed to their fatty acid composition, which enhances flavor release during baking. This supports findings by Aaslyng *et al.* (2002), who noted that fat type significantly impacts flavor perception in baked goods. The texture of muffins made with imported butterfat was rated 8.5 (very good), compared to 7.0 (acceptable) for local butterfat blends.

This suggests that imported butterfat improves mouthfeel and tenderness, likely due to its higher levels of unsaturated fatty acids, which contribute to a more desirable texture. Kerry *et al.* (2006) also emphasized the role of fat types in influencing texture. Aroma scores further highlight the superiority of imported butterfat blends, scoring 8.0 versus 6.8 for local blends. A higher aroma rating enhances sensory appeal and contributes to consumer satisfaction. Ferguson *et al.* (2004) noted the strong connection between aroma and overall acceptability in food products. Appearance ratings were similarly higher for imported butterfat blends (8.3) compared to local blends (7.6), suggesting that imported butterfat contributes to a more attractive product color and finish. As Bourne (2002) stated, appearance plays a critical role in consumer choice and product acceptance. Overall acceptability scores were 8.4 for imported butterfat blends and 7.2 for local blends, reflecting a well-rounded sensory experience due to favorable flavor, texture, aroma, and appearance. Guinard *et al.* (1997) emphasized the importance of overall acceptability, which encompasses multiple sensory attributes. The control sample scored highest in sensory evaluations, achieving 13.33 in general appearance, outperforming blends A1, A2, and A3. Similarly, the control excelled in crust color and crumb color, with scores of 13.33 in both categories, while A2 scored the lowest in both (10.00 for crust color and 10.11 for crumb color). These findings suggest that higher proportions of wheat germ oil negatively affect appearance. In terms of texture, the control also excelled with a score of 12.77, compared to A2, which was the least favored at 9.77. This trend extended to flavor, where the control scored 16.77, while A2 achieved only 10.33. The dilution effect of increased wheat germ oil percentages likely diminishes flavor attributes. Overall acceptability was highest for the control (86.19), significantly outperforming A2 (61.76). Increasing wheat germ oil percentages corresponded to a notable decline in sensory appeal, reinforcing consumer preference for traditional butterfat blends. These findings demonstrate that fat type and composition are critical to the sensory qualities of muffins. Blends with imported buffalo butterfat consistently outperformed local butterfat blends, indicating that careful selection of fat sources is essential for optimizing consumer satisfaction. The results suggest that using up to 10% wheat germ oil in blends with buffalo butterfat significantly diminishes the sensory attributes of the muffins. Therefore, it is recommended to limit the proportion of wheat germ oil to maintain desirable sensory characteristics. The choice of fats and oils in baked goods plays a crucial role in determining sensory properties, which directly influence consumer acceptance (Table 13). This analysis reviews the sensory attributes of various fat blends, particularly those of local buffalo butterfat and wheat germ oil, and their effects on the overall quality of muffins. Sensory properties, such as general appearance, crust color, crumb color, crumb texture, flavor, taste, and overall acceptability, are essential for ensuring consumer satisfaction and acceptance. The sensory evaluation focuses on how these different fat blends impact the sensory qualities of the final product. The following table summarizes the sensory attributes evaluated for the

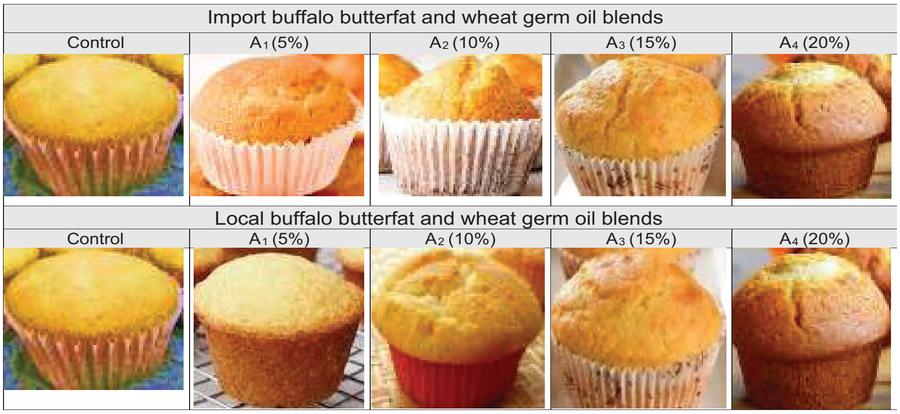


**Figure 1. The image shows muffins made with different blends of buffalo butterfat and wheat germ oil, divided into two main sections: imported and local buffalo butterfat blends. Each section includes a control muffin (no added oil blend) and four experimental groups with varying proportions of wheat germ oil in the butterfat: Control: No added wheat germ oil blend; $A_1$ (5%): 5% wheat germ oil blend; $A_2$ (10%): 10% wheat germ oil blend; $A_3$ (15%): 15% wheat germ oil blend $A_4$ (20%): 20% wheat germ oil blend.**

different fat and oil blends in muffins, providing scores for key sensory aspects such as general appearance, crust color, crumb color, crumb texture, flavor, taste, and overall acceptability. The control group achieved the highest score (13.55), indicating superior visual appeal compared to the blends. Studies indicate that the appearance of baked goods plays a significant role in consumer acceptance, with a preference for traditional fats often correlating with better visual attributes (Aaslyng *et al*., 2002; Raghavan *et al*., 2015). The control group also excelled in crust color (13.55) and crumb color (13.88). The significant reduction in scores for higher wheat germ oil blends suggests that blending alters the Maillard reaction, affecting browning and, subsequently, color perception (Köster, 2009; Wang *et al*., 2020). The control muffins scored highest for crumb texture (12.44). Texture is a crucial factor in consumer satisfaction, and traditional fats often provide a more desirable mouthfeel compared to alternative oils (Brennan *et al*., 2017). The flavor (18.11) and taste (18.00) of the control were markedly higher than those of the blends, particularly A4 (13.33 for flavor and 13.66 for taste). The reduction in sensory scores suggests that higher wheat germ oil proportions may dilute the flavor complexity that consumers expect in muffins (Baldwin *et al*., 2011; Pérez *et al*., 2018). The control group scored significantly higher (89.53) than all the blends, particularly A1 (63.42), indicating that consumers prefer the sensory attributes associated with traditional buffalo butterfat. This aligns with previous studies demonstrating that traditional fats often provide superior overall acceptability in baked products (Vlachos *et al*., 2019). The analysis of sensory attributes in muffins made with different fat blends indicates that local buffalo butterfat significantly enhances visual appeal, flavor, and overall consumer acceptance. Blending with wheat germ oil, while potentially beneficial for nutritional enhancement, may compromise critical sensory qualities, particularly at higher concentrations. Future research could explore strategies to balance health benefits with sensory quality, such as flavor enhancement techniques or novel emulsifiers.

The control group (using only buffalo butterfat) received the highest score (13.55), indicating a visually appealing product (Table 13). In contrast, the highest wheat germ oil blend (A4, 20%) received the lowest score (10.22), suggesting that excessive substitution may detract from appearance. Studies show that appearance significantly influences consumer preferences (Köster, 2009). The control samples also led in crust color (13.55), while the A1 blend (5% wheat germ oil) received the lowest score (10.11). The diminishing scores across blends may indicate changes in the Maillard reaction due to different oil compositions, affecting browning (López *et al*., 2018). Crumb color followed a similar trend, with the control scoring the highest (13.88) and the A4 blend scoring the lowest (10.77). The perception of crumb color can be attributed to the inherent properties of the fats used, with traditional fats usually providing richer coloration (Singh *et al*., 2020). Texture scores indicated that the control (12.44) was preferred over most blends, particularly A4 (10.00). Textural integrity is crucial in baked products, with fats impacting moisture retention and structural properties (Brennan *et al*., 2017).

The control showed significantly higher flavor (18.11) and taste (18.00) scores compared to the blends, especially A4, which scored considerably lower (13.33 for flavor and 13.66 for taste). The reduction in scores with higher oil percentages may relate to the flavor profile of wheat germ oil, which can be subtle compared to traditional fats (Aaslyng *et al.*, 2002). The control group had the highest overall acceptability (89.53), while the A1 blend received the lowest (63.42). This emphasizes that consumer preference leans heavily toward traditional fat sources for optimal sensory quality. To maintain quality attributes in muffins, it is advisable to limit wheat germ oil to a maximum of 5%. This ratio preserves essential sensory characteristics while offering potential health benefits associated with wheat germ oil.

The selection of fats and oils, as well as their blends, plays a crucial role in determining the sensory attributes of muffins. Understanding how different fats interact during baking can help optimize product formulation to enhance texture, flavor, and overall consumer acceptance. Future studies should further explore the sensory impacts of emerging fats, such as plant-based oils and modified fats, to cater to evolving consumer preferences for health and sustainability. The control group (using only buffalo butterfat) achieved significantly higher flavor (18.11) and taste (18.00) scores compared to the blends, particularly the A4 blend, which scored considerably lower (13.33 for flavor and 13.66 for taste). The reduction in scores with higher wheat germ oil proportions could be attributed to the more subtle flavor profile of wheat germ oil, which might not provide the same depth and richness as traditional fats like buffalo butterfat (Aaslyng *et al.*, 2002). The control group also scored highest for overall acceptability (89.53), while the A1 blend (5% wheat germ oil) received the lowest (63.42).

This highlights that consumer preferences are strongly inclined toward traditional fat sources, especially in terms of achieving optimal sensory quality in baked products. To maintain the desired sensory characteristics in muffins, it is advisable to limit wheat germ oil to a maximum of 5%. This ratio allows for the preservation of key sensory attributes while still potentially offering the health benefits associated with wheat germ oil. The selection of fats and oils plays a pivotal role in determining the sensory attributes of baked goods. By understanding how different fats interact during the baking process, product formulations can be optimized to enhance texture, flavor, and overall consumer acceptance. As consumer preferences evolve toward healthier and more sustainable options, future research should explore the sensory impacts of emerging fats, such as plant-based oils or modified fats, to meet the growing demand for nutritious yet enjoyable baked products. This analysis underscores the critical role of fat composition in product formulation and its direct effect on sensory quality, consumer preference, and product success.

## Conclusion

This study evaluated the physicochemical properties, oxidative stability, fatty acid composition, and sensory attributes of buffalo butterfat blended with wheat germ oil (WGO). The incorporation of WGO significantly enhanced the nutritional value and oxidative stability of the butterfat blends due to its high unsaturated fatty acid content and antioxidant properties. Blends with higher WGO proportions exhibited improved peroxide and thiobarbituric acid values, indicating enhanced resistance to oxidation. Sensory evaluation revealed that blends containing up to 30% WGO maintained acceptable sensory characteristics, making them suitable for various applications in food formulations. The findings underscore the potential of WGO as a functional ingredient to improve the health profile of traditional fats without compromising quality. The study also highlights the need for further research to optimize blending ratios for broader applications and extended shelf life. These results provide valuable insights for the development of innovative dairy products with enhanced health benefits, aligning with consumer demands for functional and nutritious food products. Future studies could explore the bioavailability of WGO components in these blends and their long-term impact on human health.

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## Data Availability

Data will be made available on request.

## Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

## Conflict of Interest

The authors declare no conflict of interest.

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