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Effect of Replacing Vegetable Oil with Anhydrous Milk Fat on the Quality Properties of Laboratory-Produced Nutella

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ABSTRACT

This study aimed to prepare a healthy Nutella rich in specific fatty acids (e.g., oleic, palmitic, and stearic acids) and fat-soluble vitamins (A, E, K, and D) by replacing sunflower oil with anhydrous milk fat (ghee) at substitution levels of 10%, 20%, and 30%. The effect of this substitution on the physicochemical and sensory properties of the laboratory-produced Nutella was investigated. The chemical composition analysis revealed significant differences ($p < 0.05$) in fatty acid profiles and vitamin content among the treatments, while some other compositional parameters showed no significant differences. The percentages of oleic, palmitic, stearic, and linolenic fatty acids increased significantly with increasing substitution levels of anhydrous milk fat, whereas the percentages of linoleic and arachidonic acids decreased significantly. The results also indicated a significant increase in the concentrations of fat-soluble vitamins (A, E, K, and D) as the substitution level increased. Viscosity analysis showed a statistically significant increase in the viscosity of the Nutella samples with increasing substitution levels of anhydrous milk fat. Sensory evaluation revealed that the Nutella treatments containing anhydrous milk fat (ghee) scored higher than the control treatment (100% sunflower oil) in all sensory properties. Among the substitution levels, the 20% anhydrous milk fat treatment showed the highest consumer acceptance.

1-Introduction

Nutella is a fat emulsion sweet food spread. It is achieved by homogenizing oil or fat with hazelnuts, sugar, cocoa, and lecithin [1]. Nutella has a high calorie value due to its high fat and oil content. Over the years, consumer demand for Nutella has increased, and this has prompted changes in product formulations to make the product more appealing to consumers [2]. Because some oils and fats carry health risks when consumed in high quantities, especially those high in trans fats or with an imbalanced omega-6 to omega-3 ratio, many consumers are cutting back on fat or swapping unhealthy fats for healthier options. Good fats are beneficial to overall health and also beneficial for enhancing the nutritional value of foods. Anhydrous milk fat (commonly known as ghee) is being considered as a potential substitute for vegetable oils in spreadable products because of its peculiar flavor and nutritional advantages. Ghee is the end product of the concentration of milk fat, wherein milk, cream, or butter is heated at a temperature above 100°C to evaporate the water and solids, not fat [3].

Ghee is produced on a large scale in India, Ethiopia, Sudan, and the Middle East [4]. Over the last ten years, ghee production has also increased in the United States, Argentina, and Paraguay, with annual production ranging from 3,000 to 12,000 tons. With the globalization of the West, animal ghee consumption in Western countries has somewhat increased, partly due to concerns regarding artificially trans fatty acids in vegetable ghee [5]. Studies have proven that artificially trans fatty acids have a much more negative impact on cardiovascular diseases, diabetes, and depression than the naturally occurring saturated fatty acids in animal fats [6].

In laboratory mice, ghee (homemade, traditional, or commercial) was found to have better effects on total fats and triglycerides in comparison with vegetable shortening and sunflower oil [7]. Thus, the potential adverse health effects of each fatty acid individually may not have much impact in comparison to maintaining a good balance between different fatty acids [8]. Saturated fatty acids raise blood cholesterol; however, they may have positive effects when consumed in moderation. Animal ghee contains 99-99.5% fat, where saturated fat constitutes 46-47.8%, monounsaturated fat 36%, and polyunsaturated fat 18% [9]. Ghee is a rich source of fat-soluble vitamins [10], particularly vitamins A and E [11]. Essential fatty acids such as linolenic acid, linoleic acid, and arachidonic acid are also present in ghee [12].

Goghrita in animals is one of the oldest medicines in its systems [13]. It is said to be helpful in stress and bloating, improving cognitive functions, stimulating gastric secretion, and enhancing digestion [14]. It also has anti-inflammatory properties that can be useful in reducing the risk of tumors, improving the immune system, and relieving psychological stress. Research has proven that alpha-linolenic acid, a constituent of ghee, reduces cholesterol and triglyceride levels and vision; it is also anti-inflammatory and reduces weight [15].

Considering the above nutritional and therapeutic values of anhydrous milk fat (animal ghee), very limited studies have been conducted on its utility in manufactured food products, especially in sweet spreads like Nutella. Therefore, the present work was undertaken to replace sunflower oil with anhydrous milk fat (animal ghee) at three substitution levels of 10%, 20%, and 30% in the production of Nutella to develop a healthier product enriched with beneficial fatty acids and fat-soluble vitamins.

2- Materials and Methods

2.1. Raw Materials

The raw materials necessary for the manufacture of Nutella, namely cocoa, sunflower oil, anhydrous milk fat (animal ghee), milk, sugar, milk powder, hazelnuts, lecithin, vanilla, and salt, were purchased from local markets in Baghdad. The standard recipe followed by Tlay & Al-Bidhani (2023) was adopted with some modifications [14]. Table 1 shows the components of the standard recipe.

Table 1. Ingredients of the standard Nutella mix

Ingredient	Percentage (%)
Cocoa	7.40
Sunflower oil	27.00
Milk powder	8.70
Hazelnuts	13.00
Sugar	43.80
Salt	0.018
Vanilla	0.015
Lecithin	0.07
Total	**100.00**

2.2. Nutella Production Method

Four Nutella formulations were prepared:

- **Treatment A (Control):** Standard Nutella recipe (100% sunflower oil, no anhydrous milk fat)
- **Treatment B:** Replacement of 10% of sunflower oil with anhydrous milk fat (ghee)
- **Treatment C:** Replacement of 20% of sunflower oil with anhydrous milk fat (ghee)
- **Treatment D:** Replacement of 30% of sunflower oil with anhydrous milk fat (ghee)

Nutella was prepared according to the technique described in Tlay & Al-Bidhani (2023) [14]. Dry components (cocoa, sugar, milk powder, hazelnuts, salt, and vanilla) were mixed to obtain a homogeneous mixture. The mass was placed in an electrically driven cutter; then, vegetable oil (sunflower oil or its mix with anhydrous milk fat) and lecithin were slowly incorporated until the desired consistency was reached. The product was then packed in sterile, airtight glass jars for further testing.

2.3. Chemical Analysis

2.3.1. Fat Extraction from the Sample

Fat was extracted from Nutella samples as described in the A.O.A.C. (1995) report using a Soxhlet fat extraction apparatus [15].

2.3.2. Esterification of Fats

The sample was prepared according to the method adopted by A.O.A.C. (1995), which is based on the esterification of fats by reacting them with methanolic potassium hydroxide. The methanolic potassium hydroxide solution was prepared by dissolving 11.2 g of potassium hydroxide in 100 mL of methanol. Esterification was carried out by taking 1 g of fat in a 10 mL test tube and adding 5 mL of methanolic potassium hydroxide, then shaking well for 5 minutes. Subsequently, 5 mL of hexane was added, and the contents of the tube were mixed well again for 30 seconds. The mixture was then left to separate into two layers. The upper layer contained the fatty acid esters in hexane, while the lower layer contained the saponified materials from the reaction.

2.3.3. Fatty Acid Determination Using Gas Chromatography

The fatty acids of the Nutella samples were determined using a gas chromatograph (GC-2010, Shimadzu, Japan) equipped with a flame ionization detector (FID) and a capillary column (SE-30, 30 m length × 0.25 mm diameter). The following conditions were used: injection port temperature 280°C, detector temperature 310°C, and column temperature programmed from 120°C to 290°C at 10°C/min. Helium was used as the carrier gas at a flow rate of 30 mL/min. One microliter of pre-esterified fat (the top layer containing hexane and fatty acid esters) was injected into the GC using a syringe.

2.3.4. Determination of Fat-Soluble Vitamins

Fat-soluble vitamins (A, E, K, and D) were determined according to the method described by **A.O.A.C. (2016)** using a SYKAM HPLC analyzer (Germany) [16]. A C-18 column (250 cm × 4.6 mm) was used with a mobile phase consisting of 75% acetonitrile and 25% deionized water at a flow rate of 0.7 mL/min. The wavelength was set at 280 nm using a UV-Vis detector. The injection volume was 100 µL.

Sample preparation for vitamin analysis: A 0.4 M aqueous solution of lithium perchlorate at pH 2.4 (Solution 1) and a 0.1% butylhydroxytoluene solution in methanol (Solution 2) were prepared. Twenty mL of Solution 2 and 8 mL of Solution 1 were added to 5 g of the sample and placed in a sealed container in the dark for two hours. The mixture was then stirred on a magnetic mixer for 20 minutes at room temperature. The samples were centrifuged before being injected into the column and analyzed within one hour.

2.4. Viscosity Measurement

Viscosity was measured using a Brookfield viscometer with spindle No. 4 at 60 rpm and 28°C [17].

2.5. Sensory Evaluation

The laboratory-produced Nutella samples were evaluated by ten specialists from the Department of Home Economics, College of Education for Women, University of Baghdad, using the sensory evaluation form developed by Salman et al. (1987) [18]. The samples were evaluated after being spread on slices of toast.

2.6. Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, version 26, 2019) was used to analyze the data. A completely randomized design (CRD) was employed to study the effect of different treatments on the measured traits [19]. Significant differences between means were compared using the Least Significant Difference (LSD) test at a probability level of $p < 0.05$.

3- Results and Discussion

3.1. Fatty Acid Composition

Table 2 shows the fatty acid profiles (oleic, palmitic, stearic, linoleic, linolenic, and arachidonic acids) of the laboratory-produced Nutella samples. The results of the statistical analysis indicated significant differences ($p \leq 0.05$) in fatty acid values among the different treatments. The values of oleic, palmitic, stearic, and linolenic acids increased with increasing replacement levels of sunflower oil with anhydrous milk fat (ghee). Treatment D (30% replacement) recorded the highest values **for oleic (30.30%), palmitic (19.12%), stearic (12.03%), and linolenic (4.22%) acids, respectively,** while the control treatment

(A, 0% replacement) recorded the lowest values **for the same fatty acids (20.67%, 6.65%, 3.63%, and 2.64%, respectively).** In contrast, the values of linoleic and arachidonic acids decreased with increasing replacement levels of sunflower oil with anhydrous milk fat. Treatment D recorded the lowest values **for linoleic (53.22%) and arachidonic (0.33%) acids, respectively,** while the control treatment (A) recorded the highest values **(65.45% and 0.67%, respectively).**

Table 2. Effect of replacing sunflower oil with anhydrous milk fat (ghee) on fatty acid composition (%) of different Nutella treatments (mean ± SD, n=3)

Fatty Acid	Treatment A (0% ghee)	Treatment B (10% ghee)	Treatment C (20% ghee)	Treatment D (30% ghee)	LSD (p≤0.05)
Oleic acid	20.67 ± 0.94 c	22.86 ± 1.32 bc	25.42 ± 1.68 b	30.30 ± 2.05 a	4.071
Palmitic acid	6.65 ± 0.35 d	11.37 ± 0.57 c	15.48 ± 0.76 b	19.12 ± 0.84 a	3.680
Stearic acid	3.63 ± 0.16 c	5.52 ± 0.32 bc	8.12 ± 0.52 b	12.03 ± 0.67 a	2.750
Linoleic acid	65.45 ± 4.07 a	60.25 ± 3.29 b	56.14 ± 0.78 bc	53.22 ± 2.36 c	4.390
Linolenic acid	2.64 ± 0.11 c	3.08 ± 0.17 bc	3.98 ± 0.14 ab	4.22 ± 0.25 a	1.042
Arachidonic acid	0.67 ± 0.04 a	0.45 ± 0.02 ab	0.40 ± 0.02 ab	0.33 ± 0.01 b	0.317

Different superscript letters within the same row indicate significant differences at $p \leq 0.05$.

3.2. Fat-Soluble Vitamins

Table 3 shows the **concentrations** of fat-soluble vitamins (A, E, D, and K) in the laboratory-produced Nutella samples. Statistical analysis indicated significant differences ($p \leq 0.05$) in vitamin content among the treatments. Treatment D (30% replacement) recorded the highest values for all vitamins, reaching 51.6, 88.7, 30.2, and 14.80 **ppm/100 g** for vitamins A, E, D, and K, respectively. In contrast, the control treatment (A) recorded the lowest values at 42.6, 75.0, 24.6, and 8.47 **ppm/100 g**, respectively.

Table 3. Effect of replacing sunflower oil with anhydrous milk fat (ghee) on fat-soluble vitamin content (ppm/100 g) of different Nutella treatments (mean ± SD, n=3)

Vitamin	Treatment A (0% ghee)	Treatment B (10% ghee)	Treatment C (20% ghee)	Treatment D (30% ghee)	LSD (p≤0.05)
Vitamin A	42.6 ± 2.17 c	45.9 ± 2.66 bc	48.0 ± 2.70 ab	51.6 ± 1.95 a	5.157

Vitamin	Treatment A (0% ghee)	Treatment B (10% ghee)	Treatment C (20% ghee)	Treatment D (30% ghee)	LSD (p≤0.05)
Vitamin E	75.0 ± 3.47 c	81.5 ± 4.52 bc	84.6 ± 4.68 ab	88.7 ± 5.02 a	7.078
Vitamin D	24.6 ± 1.26 b	26.5 ± 1.64 ab	27.8 ± 1.07 ab	30.2 ± 2.24 a	4.810
Vitamin K	8.47 ± 0.52 c	10.25 ± 0.71 bc	11.98 ± 0.66 b	14.80 ± 0.76 a	2.794

Different superscript letters within the same row indicate significant differences at p ≤ 0.05.

3.3. Viscosity

Table 4 shows the viscosity of Nutella samples in which sunflower oil was replaced with anhydrous milk fat (ghee). Significant differences ($p \leq 0.05$) were observed among the treatments. Viscosity increased progressively with increasing replacement levels: Treatment A (control) showed the lowest viscosity (34.42%), followed by Treatment B (38.23%), Treatment C (43.82%), and Treatment D (the highest viscosity at 50.05%).

Table 4. Effect of replacing sunflower oil with anhydrous milk fat (ghee) on viscosity (%) of different Nutella treatments (mean ± SD, n=3)

Treatment	
A (0% ghee)	
B (10% ghee)	
C (20% ghee)	
D (30% ghee)	50.05 ± 1.89 a
LSD (p≤0.05)	**6**

Different superscript letters within the same column indicate significant differences at p ≤ 0.05.

3.4. Sensory Evaluation

Table 5 presents the sensory evaluation results for the different Nutella treatments. Significant differences ($p \leq 0.05$) were observed among treatments for appearance, texture and consistency, flavor, and total sensory score, while no significant differences were detected for color ($p > 0.05$).

Treatment C (20% replacement) received the highest scores across most sensory attributes, with a total sensory score of 72.2, followed closely by Treatment B (70.2). Treatment D (30% replacement) received the lowest total sensory score (65.5), indicating that excessive replacement with ghee negatively affected consumer acceptability.

Table 5. Effect of replacing sunflower oil with anhydrous milk fat (ghee) on sensory properties of different Nutella treatments (mean ± SD, n=10)

Treatment	Appearance (10)	Color (10)	Texture & Consistency (40)	Flavor (40)	Total (100)
A (0% ghee)	7.2 ± 0.38 ab	7.2 ± 0.45 a	25.5 ± 1.29 b	29.4 ± 1.33 ab	69.4 ± 2.86 ab
B (10% ghee)	7.7 ± 0.43 a	6.8 ± 0.35 a	25.5 ± 1.29 b	30.2 ± 1.08 ab	70.2 ± 3.53 ab
C (20% ghee)	7.9 ± 0.55 a	7.3 ± 0.44 a	32.8 ± 1.87 a	32.2 ± 2.53 a	72.2 ± 3.64 a
D (30% ghee)	6.6 ± 0.28 b	6.8 ± 0.31 a	26.1 ± 1.47 b	28.3 ± 1.47 b	65.5 ± 2.26 b
LSD (p≤0.05)	**1.037**	**0.791 (NS)**	**3.041**	**3.277**	**5.164**

*Different superscript letters within the same column indicate significant differences at $p \leq 0.05$. NS = not significant. *

4- Discussion

4.1. Fatty Acid Composition

The increase in oleic, palmitic, stearic, and linolenic acids with increasing replacement levels of sunflower oil by anhydrous milk fat (ghee) can be attributed to the higher natural content of these fatty acids in ghee compared to sunflower oil. In anhydrous milk fat, palmitic acid ranges from 24% to 28.8%, stearic acid from 9.4% to 14% [20], and oleic acid approximately 28.7% [21]. In contrast, sunflower oil contains approximately 8.12% palmitic acid, 5.06% stearic acid [22], and 30% oleic acid [23]. Hassan (2016) reported that unsaturated fatty acids (oleic and linoleic) constitute approximately 85-91% of total fatty acids in sunflower oil [24].

The decrease in linoleic and arachidonic acids with increasing ghee replacement is due to the inherently low content of these fatty acids in milk fat compared to sunflower oil. Sunflower oil is characterized by a high linoleic acid content (approximately 59%) [23], while milk fat contains only about 0.66% linoleic acid. Jeyarani et al. (2015) found that commercial hazelnut spread contained a higher percentage of oleic acid compared to chocolate made from soybean oil, while linolenic acid was higher in soybean oil-based chocolate [25].

4.2. Fat-Soluble Vitamins

The significant increase in fat-soluble vitamins (A, E, D, and K) with increasing ghee replacement is attributed to the higher vitamin content of anhydrous milk fat compared to sunflower oil. Nour (2019) stated that ghee is a good source of vitamins A and D compared to vegetable oils [7]. One tablespoon (12 g) of ghee contains approximately 87.12 µg of vitamin A and 3.12 µg of vitamin D, while sunflower oil contains only 3.96 µg of vitamin A and no vitamin D. Ulambayar et al. (2024) reported that the average vitamin A content in ghee was 606 µg/100 g, vitamin E was 1650 µg/100 g, and beta-carotene was 392 µg/100 g [12].

4.3. Viscosity

The gradual increase in viscosity at each level of ghee replacement could be explained by the fact that anhydrous milk fat has more saturated fatty acids than sunflower oil. The melting point of saturated fatty acids increases with molecular weight (chain length) — which directly relates to the increase in viscosity of the final product. The high viscosity of ghee-containing treatments may also affect spreadability — an

important quality parameter for consumer preference.

4.4. Sensory Evaluation

Treatment C (20% replacement) was observed to give the highest sensory scores, meaning that moderate substitution of sunflower oil with ghee (20%) is most preferred by the consumer. Lower scores for Treatment D (30% replacement) show that high ghee content does not favor flavor, texture, or overall palatability, perhaps due to the stronger characteristic flavor of ghee or the increased viscosity that affects spreadability.

These findings are in partial agreement with Noshe & Abboud (2018), who earlier reported that Nutella made from white chia seeds received an unacceptable flavor rating due to certain compounds that affect taste [26]. Nutella made from black chia seeds had the same problem in spreadability due to gelling agents. According to Alhusyne (2015), flavor-enhanced yolk nut with free fat was significantly better than the control treatment on sensory properties during storage [27].

5- Conclusions

It has been proven that the substitution of sunflower oil by anhydrous milk fat (ghee) in Nutella production enhances the nutritional profile and sensory properties of the product. The best results were observed at the 20% substitution level (Treatment C). This substitution increased the percentages of beneficial fatty acids while decreasing linoleic and arachidonic acids. It also enhanced fat-soluble vitamin concentrations. Viscosity was observed to increase with higher ghee content. Sensory evaluation confirmed that Treatment C received the highest total acceptance score. Therefore, anhydrous milk fat (ghee) is recommended as a favorable functional ingredient for improving the nutritional quality of chocolate spreads. A substitution level of 20% is recommended to balance nutritional improvement and consumer acceptability. Further study is recommended to assess the oxidative stability and shelf life of Nutella formulations with ghee and to carry out consumer acceptance studies on a larger and more diverse scale.

Conflict of interest

The authors have no conflicts of interest to disclose that are compatible with the subject matter of this article.

Consent to participate

All authors have expressed their authorization to engage in this publication.

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Ethical Statement

Except for sensory evaluation, we have not conducted any human or animal investigations during this study. Regarding the sensory case.

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