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RESEARCH ARTICLE

DEVELOPMENT OF OMEGA-3 ALPHA-LINOLENIC ACID (ALA)-RICH BUTTER USING PLANT SEEDS: INTEGRATION OF FLAX, CHIA, AND SESAME SEEDS AND ASSESSMENT OF ITS QUALITY AND FUNCTIONAL CHARACTERISTICS

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Abstract

Peanut butter is globally renowned for its popularity, yet alternative plant seeds might offer superior nutritional and therapeutic benefits for developing plant seed-based butter. This study aimed to develop a peanut-based butter enriched with Flax (FS), Chia (CS), and Sesame Seeds (SS), evaluating its sensory, physicochemical, and therapeutic properties. Various seed ratios were tested, while the control comprised 100% peanut seed. Physicochemical parameters, radical scavenging, and phenolic activities were assessed using standard methods. A sensory evaluation by 30 semi-trained panelists on a 5-point hedonic scale identified the optimal ratio: 70% peanut, 15% FS, 10% CS, and 5% SS (T_2). T_2 exhibited significantly higher crude fiber (15.63±0.14%) and protein (24.20±0.10%), with lower fat (26.34±0.10%) and ash (1.32±0.36%) compared to the control. The addition of FS, CS, and SS significantly increased alpha-linolenic acid content, total phenolic content, and antioxidant activities (5.16 ± 0.06%, 2.66±0.09 GAE mg/g, and 42.96±0.78%) respectively. Texture attributes improved compared to the control (hardness: 11.01±0.03 g, cohesiveness: 0.11±0.02 g, adhesiveness: 1.1±0.17 mJ, and chewiness: 0.16 ±0.12 mJ). This investigation highlights the potential of formulating a spread based on peanuts, enriched with FS, CS, and SS. The resulting product not only exhibits appealing sensory attributes and texture but also demonstrates promise for enhanced functional properties.

Keywords: Alpha linoleic acid content, antioxidant activity, functional foods, peanut-based spread, phenolic content

INTRODUCTION

Peanut spread, commonly referred to as peanut butter, and enjoys widespread popularity as a breakfast staple globally. Its usage in various morning meals owes to its unique taste and texture. Moreover, peanut butter is acknowledged for its potential health benefits, such as its ability to mitigate the risk of gastric cancer (Yu *et al.*, 2021). Peanuts are rich in omega-6 linoleic acid (LA) but lack substantial quantity of omega-3 alpha-linolenic acid (ALA), crucial for maintaining the balance of essential fatty acids necessary for optimal health and development (Brenna *et al.*, 2015). This disproportion, frequently marked by an elevated ratio of omega-6 to omega-3, may affect cardiovascular well-being because mammalian cells lack the enzyme omega-3 desaturase, preventing the conversion of omega-6 to omega-3 fatty acids (Simopoulos *et al.*, 2002). Epidemiological evidence suggests a correlation between higher omega-6 levels and increased cardiovascular mortality risk (Bill, 2014).

In today's world, people encounter an increased susceptibility to heart conditions like cardiovascular diseases (CVDs), which remain the leading cause of worldwide fatalities. Statistics reveal that in 2019 alone around 17.9 million deaths were linked to cardiovascular diseases (CVDs), making up roughly 32% of the total worldwide mortality. Among these deaths, strokes and heart attacks comprised 85%, according to McFarlane *et al.* (2012). Chronic conditions, including cardiovascular disease, diabetes, obesity, asthma rheumatoid arthritis, cancer, autoimmune diseases, and depression, are

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linked to heightened levels of leukotriene B4 (LTB4), thromboxane A2 (TXA2), interleukin-6 (IL-6), IL-1β, tumor necrosis factor (TNF), and C-reactive protein. These factors tend to rise with increased consumption of omega-6 fatty acids and decrease with higher intake of omega-3 fatty acids (Simopoulos, 2002). Elevated intake of ALA has been linked to enhanced cardiovascular well-being, decreased levels of anxiety and depression, and lower incidences of cancer, multiple sclerosis, type 1 diabetes, Alzheimer's disease, and overall mortality (Dempsey *et al*., 2023). However, as LA and ALA are not biosynthesized in animals, they are deemed essential fatty acids primarily sourced from plant-based foods, highlighting the significance of a diet rich in plant-derived sources (Djuricic and Calder, 2021).

Flax seeds contain approximately 56%-60% alpha-linolenic acid as part of their total fatty acid composition, while chia seeds contain an appreciable amount of omega-3, comprising around 63%-65% alpha-linolenic acid. Notably, chia seeds are recognized for their high levels of this essential fatty acid (Nitrayová *et al*., 2014). Moreover, flaxseed, renowned for its abundance of functional compounds including lignans, and fiber, has emerged as a valuable crop and functional food (McCullough *et al*., 2011). In addition, both flax seeds and chia seeds are optimal choices for maintaining a balanced serum lipid profile, although it's important to note that flax seeds must be ground to unleash their nutrients. Additionally, despite containing a small amount of alpha-linolenic acid, sesame seeds contribute to the distinctive aroma and flavor of various foods (Hadipour *et al*., 2023). Therefore, the current study aims to develop a peanut-based plant seed butter rich in omega-3 alpha-linolenic acid through the incorporation of sesame, chia, and flax seeds to fulfill the demand for plant-based omega-3 fatty acids.

MATERIALS AND METHODS

Raw material collection

Peanuts (*Arachis hypogaea L*), flax seeds (*Linum usitatissimum L*) chia seeds (*Salvia hispanica L*), and sesame seeds (*Sesamum indicum*. L) were purchased from a supermarket in Badulla, Sri Lanka.

Preparation of plant seed-based butter samples

Peanut butter was made using the procedure by Mulindwa *et al*. (2019), with adjustments. Peanuts were subjected to heating at 100 °C in a hot air oven (GX125B, Faithful Instrument, Hebei, China) for 8-10 minutes, followed by cooling to achieve a uniformly roasted product. The peanuts were then peeled, and any discolored, or unwanted seeds were removed. The visual representation of seeds samples is given in Figure 1.

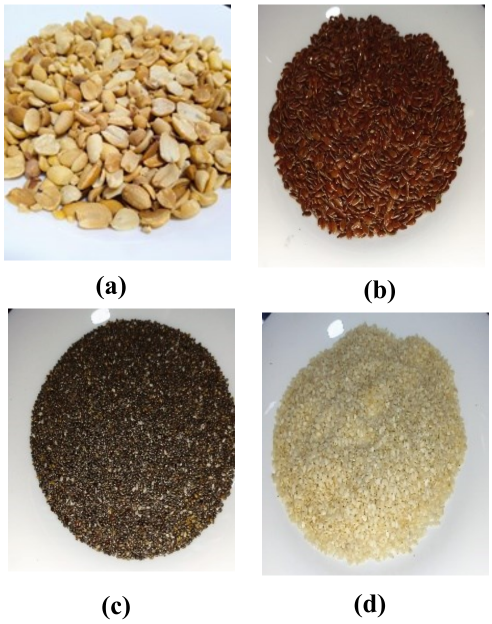

Figure 1: Visual representation of plant seeds (a) Peanut, (b) Flax seed, (c) Chia seed, (d) White sesame seed

To prepare the experimental samples, roasted and cooled peanuts were mixed with flaxseeds, chia seeds, and sesame seeds in different ratios (Table 1). The optimum ratios of chia, flax, sesame, and peanut seeds for making plant seed-based butter were established through preliminary investigations, which included sensory analysis and physicochemical testing. The control sample consisted of 100% peanuts. The seed samples were ground at a lower speed in a mixer (SF1365SM, Sanford, Dubai, UAE). This seed mixture was spread onto vessels and left undisturbed for 4 to 5 hours until the de-oiling of the peanut powder occurred. Finally, the samples were stored in airtight containers and maintained in cold conditions (4°C).

Table 1: Plant seeds ratios for making butter samples (w/w)

Sample	Peanut Seed (PS)	Flax Seed (FS)	Chia Seed (CS)	Sesame Seed (SS)
C (control)	100	-	-	-
T_1	75	5	10	10
T_2	70	15	10	5
T_3	70	10	5	15

C: control sample, T1: 75% PS, 5% FS, 10% CS, and 10% SS butter, T2: 70% PS, 15% FS, 10% CS, and 5% SS butter, and T3: 70% PS, 10% FS, 5% CS, and 15% SS butter

Sensory evaluation of butter samples

The organoleptic properties of four plant seed -based spread samples were evaluated using a five-point hedonic scale ranging from 5 for 'like very much' and 1 for 'dislike very much'. The evaluation was based on six attributes, including color, taste, aroma, appearance, mouthfeel, and overall acceptability, with assessments conducted by thirty semi-trained panelists. Individual samples were prepared and presented on distinct plates alongside a cracker, each identified by a unique three-digit random number code. The analysis was performed according to the guidelines provided by the International Organization for Standardization (ISO 6658:2017).

Determination of omega-3 fatty acid profile (Alpha-linolenic acid) of butter samples

The Omega-3 fatty acid composition of the sample was assessed through Gas Chromatography following the ISO 5508:2014 & ISO 12966-2:2011 standards. In summary, fats and oils were extracted from the butter samples' matrix. The fatty acids attached to the triacylglycerols (TAGs), along with the free fatty acids (FFA) and other lipids depending on the esterification process, were converted into fatty acid methyl esters (FAMEs). These esters were subsequently analyzed using capillary gas chromatography (ISO 12966-1:2014).

Determination of the antioxidant activity of butter samples

The antioxidant capacity of the samples was evaluated according to the procedure outlined by Kariyawasam *et al*. (2023), with modifications. Methanol extracts of the butter samples were prepared at a concentration of 10 µg/mL, and then mixed with 1 mL of 100 µM 2,2-diphenyl-2-picrylhydrazyl radical (DPPH) solution. Following a 30-minute incubation period in the dark at room temperature, the absorbance was measured at 517 nm using a UV/Vis spectrophotometer. Ethanol and DPPH solution served as controls. All measurements were conducted in triplicate. The DPPH radical scavenging activity was calculated using the below equation.

DPPH radical scavenging activity (%) = [A control – A sample / A control] × 100%

Determination of total phenolic compounds of butter samples

The total phenolic content (TPC) of the peanut spreads was assessed following the methodology outlined by Thakaeng *et al.* (2020), with modifications. Initially, 2.5 g of peanut spread was vortexed with methanol (3 mL) for 2 minutes. The resultant mixture was then subjected to microcentrifuge centrifugation at 2,058×g for 10 min at 4°C. After centrifugation, the supernatant was decanted into 50 mL conical tubes, repeating this process twice. The combined supernatant was utilized, with 200 µL transferred into a test tube and then 1 mL of Folin–Ciocalteu solution was added, and the mixture was allowed to stand for 5 minutes. Following this, 0.5 mL of sodium carbonate (Na_2CO_3) solution (1% w/v) was added. The reaction mixture was incubated at room temperature at dark for 30 min. The absorbance was measured at 760 nm using a spectrophotometer. A calibration curve was made using gallic acid standards at varying concentrations, and the TPC was expressed as mg GAE/g.

Texture profile analysis of butter samples

The butter samples were evaluated for firmness, cohesiveness, adhesiveness, and chewiness at 22°C with a Brookfield texture analyzer (CT3, DKSH Technology Limited, Bangkok, Thailand). The analyzer was calibrated, and the conical probe was descended at 1.0 mm/s for to a penetration distance of 2 mm (He *et al*., 2023).

Proximate analysis of butter samples

The proximate compositions of the butter samples were analyzed including moisture content following AOAC 930.15, crude fat analysis according to AOAC 920.39, crude fiber analysis based on AOAC 978.10, and crude protein analysis using the Kjeldahl method (method 991.20; AOAC, 2006). Additionally, ash content was determined through AOAC 942.05 (AOAC, 2006).

Statistical data analysis

Statistical analysis was performed utilizing SPSS Statistics 25 (IBM, USA). The dataset underwent assessment employing either one-way analysis of variance (ANOVA) or T-test for simple comparisons between two means, with statistical significance set at a 5% level (P<0.05). The Tukey HSD All-Pairwise Comparisons Test was utilized to determine significance. Sensory data were analyzed using the Kruskal-Wallis one-way analysis of variance.

RESULTS AND DISCUSSION

Sensory properties of butter samples

The sensory evaluation involved 30 semi-trained panelists (Table 2, Figure 2). Results indicated that the 100% peanut spread (control) was the most preferred, receiving the highest overall preference score (P<0.05). This preference might be attributed to the distinctive aroma of peanuts, which garnered the highest acceptance rates at 4.57±0.50. Interestingly, the second most preferred sample was T_2, containing 70% peanut, 15% flax seed, 10% chia seed, and 5% sesame seed. Despite not being purely like C it ranked second among participants (4.17±0.38) (P<0.05). Previous research has suggested that incorporating seeds with their unique properties can enhance the sensory attributes of value-added butter samples compared to regular peanut spreads (Azhari *et al*., 2015). It is plausible that the combination of flax seed, chia seed, and sesame seed in sample T_2 contributed positively to its overall sensory experience, possibly enriching its flavor profile and texture. This was supported by evaluations of aroma, color, and mouthfeel, where sample T_2 received strong scores across all categories (P<0.05).

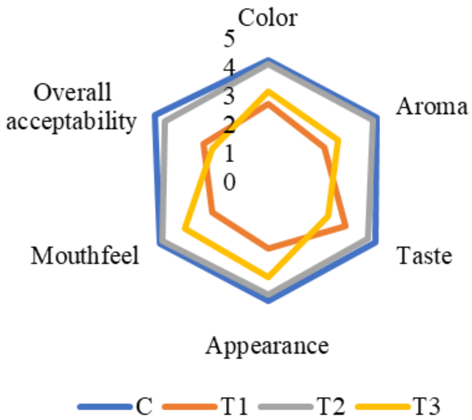

Figure 2: Radar graph for sensory analysis median score values for butter samples

C: control sample, T1: 75% PS, 5% FS, 10% CS, and 10% SS butter, T2: 70% PS, 15% FS, 10% CS, and 5% SS butter, and T3: 70% PS, 10% FS, 5% CS, and 15% SS butter.

Table 2: Sensory properties of butter samples

Sample	Color	Aroma	Taste	Appearance	Mouthfeel	Overall acceptability
C	4.20±0.41^{a}	4.40±0.50^{a}	4.33±0.48^{a}	4.20±0.41^{a}	4.37±0.49^{a}	4.57±0.50^{a}
T_1	2.70±0.47^{b}	2.30±0.47^{b}	3.17±0.38^{b}	2.37±0.49^{b}	2.23±0.43^{b}	2.60±0.50^{b}
T_2	4.07±0.25^{a}	4.23±0.43^{a}	4.07±0.45^{a}	4.00±0.53^{a}	4.23±0.43^{a}	4.17±0.38^{c}
T_3	3.13±0.35^{c}	2.83±0.38^{c}	2.43±0.50^{c}	3.37±0.49^{c}	3.33±0.48^{c}	2.23±0.43^{d}

a-d Means within a column labeled with different superscripts indicate significant differences (P< 0.05).

C: control sample, T1: 75% PS, 5% FS, 10% CS, and 10% SS butter, T2: 70% PS, 15% FS, 10% CS, and 5% SS butter, and T3: 70% PS, 10% FS, 5% CS, and 15% SS butter

Omega-3 fatty acid profile (Alpha-linolenic acid) of butter samples

Reducing omega-6 intake while increasing omega-3 is essential for preventing and managing chronic diseases (Simopoulos, 2002). The control sample had an omega-3 fatty acid (FA) content of 0.11±0.07%, whereas the plant seed-enriched butter product (T_2) contained 5.16±0.06% omega-3 FA (P<0.05), primarily due to the high omega -3 FA content in flax and chia seeds. Previous studies have identified flax and chia seeds as rich sources of omega-3 fatty acids, with chia and flax seeds containing 59.76% and 63.79% of Linolenic acid (C18:3–ω-3), respectively (Kulczyński *et al*., 2019). Our results align with findings reported in prior studies; Gong *et al*. (2018) reported lower omega-3 (0.03%) compared to omega-6 (13.88%) in peanuts, while Timbadiya *et al*. (2017) also found higher omega-6 (18.74%) than omega-3 (0.03%) in peanut butter. Therefore, enriching plant-based butter with flax and chia seeds can enhance the omega-3 FA content and functional benefits of these products.

Antioxidant activity of butter samples

The antioxidant activity of butter samples was assessed using DPPH assay. The antioxidant activity of the plant seed-enriched butter sample (T_2) showed higher antioxidant activity (42.96±0.78%) than the control butter sample (8.56±0.98%) (P<0.05). The observed rise in antioxidant activity in samples enriched with plant seeds could be attributed to the potent antioxidant properties of chia seeds and flaxseeds, owing to their rich content of phenolic compounds (Orona-Tamayo *et al*., 2015). Gupta *et al*. (2016) also reported the high antioxidant activity in flax and chia seeds. Moreover, sesame seeds have a nutritionally dense profile abundant in antioxidants and distinctive bioactive compounds like lignans, which encompass phytosterols, tocopherols, sesamin, and sesamolin (Oboulbiga *et al*., 2023). These results indicate that adding these plant seeds to butter could provide health advantages because of their abundance of antioxidants, thus rendering them valuable components for functional foods.

Phenolic activity of butter samples

Phenolic compounds, classified into non-flavonoids and flavonoids, provide health-promoting benefits and are involved in mitigating various chronic illnesses (Lin *et al*., 2017). Regarding chia seeds, existing literature generally reports TPC values spanning from 0.5 to 3.9 mg (GAE)/g chia. As for the composition of phenolic compounds in chia seeds, various phenolic components, tannins, and flavonoids have been consistently identified across studies (Fernández-López *et al*., 2020). Flaxseed is also recognized as a rich source of phenolic components, typically reported at 500 mg/100 g of flaxseed (Anwar and Przybylski, 2012). Previous research indicates that the total phenolic content of sesame seeds ranges from 129.7 to 355.3 mg GAE/100 g. In the present study, the phenolic content of the butter sample was 2.66±0.09 GAE mg/g and 1.57±0.08 GAE mg/g for the T_2 and C samples, respectively. The observed high phenolic activity (P<0.05) in T_2 is attributed to the presence of phenolic compounds derived from flax, chia, and sesame seeds.

Textural properties of butter samples

The textural attributes play a pivotal role in determining the quality of both liquid and semi-solid food products. The textural properties of the butter samples were changed as in Table 3.

Table 3: Textural Properties of butter samples

Parameter	Control	T_2 Sample
Hardness (g)	24.33±0.58^a	11.01±0.03^b
Cohesiveness	0.76±0.01^a	0.11±0.02^b
Adhesiveness(mj)	1.23±0.06^a	1.10±0.17^b
Chewiness (mj)	0.66±0.06^a	0.16±0.12^b

a-b Means within a row labeled with different superscripts indicate significant differences (P< 0.05).

All values presented are the means of three replicates (mean ± SD).

C: control sample, T1: 75% PS, 5% FS, 10% CS, and 10% SS butter, T2: 70% PS, 15% FS, 10% CS, and 5% SS butter, and T3: 70% PS, 10% FS, 5% CS, and 15% SS butter.

Incorporating chia, flax, and sesame seeds have notably influenced the hardness, cohesiveness, adhesiveness, and chewiness of the butter samples (P<0.05). Among these attributes, hardness and spreadability are particularly crucial for consumer perception. These qualities, along with spreadability, are largely affected by the compositional characteristics, specifically the solid fat content. An increase in solid fat content typically results in increased hardness and decreased spreadability (Glibowski *et al.*, 2008). The introduction of flax, sesame, and chia seeds led to a decrease in the hardness of the T_2 sample (11.01±0.028 g), which aligns with the observed reduction in fat content (26.34±0.10%) compared to the control sample ($P<0.05$). Furthermore, the control sample exhibited higher adhesiveness ($P<0.05$) than the T_2 sample, possibly due to its higher fat content. Adhesiveness refers to the effort required to counteract the pulling force between the surface of food items and external objects (Bourne, 2002). Therefore, the incorporation of sesame, flax, and chia seeds can enhance the desirable textural characteristics of plant seed-based butter.

Proximate composition of butter samples

Incorporating plant seeds into the mixture notably boosts crude protein (24.20±0.10%) and fiber content (15.63±0.14%), while simultaneously leading to a significant reduction in fat (26.34±0.10%) and ash content ($P<0.05$) (Table 4). This lower fat content suggests a potential reduction in the risk of cardiovascular disease.

Table 4: Proximate composition of butter samples

Parameter	Control	T_2 Sample
Moisture (%)	3.48±0.09^a	4.24±0.04^b
Crude Protein (%)	21.73±0.25^a	24.20±0.10^b
Crude Fiber (%)	10.65±0.43^a	15.63±0.14^b
Crude Fat (%)	30.91±0.64^a	26.34±0.10^b
Ash (%)	2.63±0.24^a	1.32±0.36^b

a-b Means within a row labeled with different superscripts indicate significant differences (P< 0.05).

All values presented are the means of three replicates (mean ± SD).

C: control sample, T1: 75% PS, 5% FS, 10% CS, and 10% SS butter, T2: 70% PS, 15% FS, 10% CS, and 5% SS butter, and T3: 70% PS, 10% FS, 5% CS, and 15% SS butter

Previous research indicates that the fat content of dairy butter is around 82.2%, peanut butter is 56.37%, and soy butter is 38.75% (Gorrepati *et al.*, 2014). Therefore, this plant-seed butter could serve as a healthier alternative to peanut and dairy butter. Moreover, the higher protein and fiber contents suggest a robust nutritional profile for this developed butter.

CONCLUSIONS

The focus of this study was to develop a plant seed-based butter enriched with omega-3 fatty acids. Based on sensory data, a formulation consisting of 70% peanuts, 15% flax seeds, 10% chia seeds, and 5% sesame seeds was identified as the optimal blend for butter preparation. The enriched peanut spread displayed notably higher levels of alpha-linolenic acid (ALA), total phenolic content, and antioxidant activity compared to the control peanut spread. Additionally, the developed butter exhibited improved textural properties, lower fat content, and higher fiber and protein content compared to the control suggesting enhanced nutritional value. This indicates that incorporating seeds not only boosted the spread's nutritional profile but also increased its potential health benefits, particularly concerning cardiovascular health. Consequently, this study highlights the potential of chia, flax, and sesame seeds in producing vegan butter with significant therapeutic and nutritional attributes.

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AUTHOR CONTRIBUTION

KMGMM and WAS designed the study. WAS conducted the experiments and analyzed the data. KMGMM and WAS wrote the original draft. KMGMM revised the manuscript.

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