International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Amino Acid and Mineral Estimation of Gluten-Free Cupcakes with *Lepidium sativum L.* Seed Mucilage as a Fat Replacer

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Abstract

Background: Growing demand for gluten-free bakery products has stimulated the development of nutritionally enhanced formulations that address both gluten intolerant people and consumers interested in healthier food alternatives. However, many gluten-free baked products are often characterized by high fat content and limited nutritional value. Plant-derived hydrocolloids have gained attention as a natural fat replacer because of their functional , technological and nutritional properties. The present study investigated the potential of *Lepidium sativum* seed mucilage (LSM) as a natural oil substitute in gluten-free cupcakes and evaluated its influence on amino acid and mineral composition.

Methodology: Gluten-free cupcakes were formulated using composite flour blend of amaranth, barnyard and soybean. Commercial refined oil was progressively substituted with LSM and completely oil-substituted formulation (LSM100) was compared to control (LSM0). Amino acid profiling was performed using liquid chromatography-mass spectrometry (LC-MS), while mineral estimation was carried out through atomic absorption spectrometry (AAS) and flame photometry following wet digestion.

Results: The incorporation of LSM significantly affected the nutritional composition of the cupcakes. Among the essential amino acids, phenylalanine, isoleucine, leucine, lysine increased significantly ($p<0.05$) in LSM100 compared to LSM0. Non-essential amino acids showed significant improvement, particularly arginine, glycine, glutamic acid and proline. Mineral analysis revealed significant increase in calcium, phosphorus, magnesium, potassium and iron content following complete oil substitution ($p<0.05$). In contrast sodium, zinc, copper, manganese and selenium remained statistically unchanged indicating good mineral retention during baking.

Conclusion: Result showed that *Lepidium sativum* seed mucilage can be used as an effective oil-substitute in gluten-free cupcakes along with improving overall nutritional profile. Beyond its technological role as a fat replacer, LSM incorporation was associated with changes in the measured amino acid and mineral composition, supporting its application as a clean-label functional ingredient in the development of nutritionally improved gluten-free bakery products.

Keywords: *Lepidium sativum* seed mucilage, gluten-free cupcakes, fat replacer, amino acids, functional foods.

Introduction

Gluten-related disorders (GRDs) compromise a heterogeneous group of autoimmune, allergic and non-allergic conditions and have been estimated to affect approximately 5% of global population (Taraghikhah et al. 2020), global seroprevalence of celiac disease has reached 1.4% and biopsy-confirmed is 0.7% (Singh et al. 2018). 1.2% of population is affecting from Ig-E mediated wheat allergy (Leonard et al. 2017). Among various dietary concerns, gluten sensitivity and celiac disease have led to an increased demand for gluten-free bakery products (Dharani Raja Sree B and Sindhu S 2024). Gluten is a network forming protein component and texture to baked products. For individuals affected by celiac disease, non-celiac gluten sensitivity, or related gluten conditions must rigorously follow a gluten-free diet to avoid negative health effects. As a result, creating gluten-free bakery products with enhanced nutritional value has recognized as a significant study area in the field of contemporary food science and technology (Das et al. 2024). Growing awareness of nutrition, lifestyle-related disorders and food intolerances has led to an increased demand for functional and health-promoting foods worldwide.

Although commercially available gluten-free baked products offer alternatives to conventional bakery items, most of the formulations are nutritionally inadequate due to the lower protein content (Alzaben et al. 2023), reduced dietary fiber (De Aguiar et al. 2023) and limited micronutrient availability (El Khoury et al. 2018; Nakov et al. 2025). Additionally, commercial gluten-free products frequently contain elevated levels of fat, refined starches and sugars to restore texture and sensory properties resulting from gluten removal (Naqash et al. 2017; Conte et al.). Consumers nowadays creating need for healthier bakery formulations with reduced fat while maintaining desirable quality attributes (Denton 2012).

Fat replacers have become a focal point as a functional ingredient that can reduce calorie density while maintaining essential sensory attributes including taste, texture, mouthfeel (Nayani 2020). Hydrocolloids sourced from natural plant have shown promising effect as a fat substitutes due to their ability to retain water, emulsify and maintain structural integrity (Cakmak et al. 2023). Hydrocolloids derived from *Lepidium sativum* seeds, commonly known as garden cress show promising effect as a fat substitute due to their ability to retain water, emulsify and maintain structural integrity (Akbar 2020; Tyagi et al. 2025).

Beyond its technological application, *Lepidium sativum* seeds hold significant nutritional value. These ingredients are characterized by their high content of protein (22.47±0.78 g/100g), total dietary fiber (30±0.47 g/100g), crude fiber (7.01±0.08 g/100g), amino acids, essential minerals and bioactive components all of which that enhance nutritional quality. Amino acids are crucial for physiological functions such as tissue growth, enzyme production, immune regulation and metabolic activities. Since essential amino acids cannot be synthesized by human body and they must be consumed through diet, assessing their composition in new food products is vital. Significantly substituting fat with garden cress seed mucilage elevates products nutritional value. Garden cress seeds inherently have significantly high level of essential minerals; potassium, magnesium, copper, iron, manganese, zinc and moderate levels of calcium (Doke and Guha 2014) along with essential amino acids; leucine, arginine, lysine and moderate levels of valine, phenyl alanine, isoleucine (Doke and Guha 2014). Consequently, fat replacement by garden cress seed hydrocolloids improves textural mimetics while simultaneously compensating the micronutrient and amino acid deficiency (Archana and Singh 2022).

Gluten-free bakery products formulated using composite flours derived from nutritionally dense ingredients such as amaranth, barnyard and soybean flour have demonstrated improved nutritional quality and functional properties. Incorporation of hydrocolloid-based fat replacer in such products may further enhance health benefits by reducing fat content while preserving desirable product characteristics (Fernandes et al. 2021). However, limited studies have been investigated the application of *Lepidium sativum* seed mucilage as a fat replacer specifically in gluten-free cupcake formulations, particularly with regards to amino acids and mineral composition.

Therefore, the present study was undertaken to develop gluten-free cupcakes incorporating *Lepidium sativum* mucilage as natural fat substitute and further evaluated amino acid and mineral composition of the developed products. The findings of this study may help in development of nutritionally enhanced gluten-free bakery products for consumers and has the ability to be commercialized at industrial level.

Raw material sourcing

Raw material employed in the current investigation compromised barnyard millet (VL172), amaranth grain (RMA120), soyabean (VL Soya99), *Lepidium sativum* seeds, powdered sugar, sunflower oil, yogurt and leavening agents. All ingredients and materials necessary for the product formulation and analysis were sourced from the retail outlets located in Ghaziabad, India.

Materials and Methods

Composition of Composite Flour Blend.

For preparation of gluten-free cupcakes, a composite flour mixture was formulated using equal portions of amaranth grain (200 g), barnyard millet (200 g), soyabean (200 g). Prior to processing, soyabean and barnyard millet grains were undergone thoroughly to cleaning to eliminate impurities and were subsequently soaked in distilled water at 25°C for 8 h. After soaking, the grains were drained and adequately dried before milling. Similarly, amaranth seeds underwent through cleaning to remove impurities and unwanted materials before being grounded. The milling of these raw materials was conducted out using a disc pulverizer (SCP20/2HP, India), and the resulting flours were sieved to achieve a uniform particle size distribution. The processed flours were then combined in equal proportions to create the composite gluten-free flour blend which was stored in airtight containers until further analysis and product development (Tyagi et al. 2024).

Preparation Procedure of *Lepidium sativum* seed mucilage (LSM)

The seeds were weighed precisely 20g, were suspended in 200 mL of distilled water within a beaker continuous stirring with a glass rod was employed to achieve uniform dispersion. The mixture subsequently incubated in a metabolic shaker at 80°C for one hour. During the heating process, the seeds developed a translucent mucilaginous coating on their surfaces. Post-extraction, the hydrated seeds were separated through sieving and then ground for two minutes using a 750W Philips grinder to produce slurry. The resulting suspension underwent centrifugation at 8000 rpm for ten minutes using a Remi R4C centrifuge (India). Centrifugation facilitated suspension of the soluble mucilaginous polysaccharide fractions into the supernatant, while insoluble seed residue settled as sediment at the bottom. The extracted *Lepidium sativum* seed mucilage is carefully collected, transferred into airtight containers and stored under refrigerated conditions (4°C) until further use (Tyagi et al. 2025; Chadha et al. 2026).

Formulation of Gluten-free Cupcakes (GFCs)

Initially, 30% of the sieved gluten-free flour blend was placed in a moisture-free mixing vessel and then combined with the chemical leavening agents (1%) namely baking soda and baking powder were added plus thoroughly mixed using spatula to obtain a uniform dry mixture. In a separate clean bowl, *Lepidium sativum* seed mucilage (20%), (29%) yogurt, and powdered sugar (20%) was combined and whisked manually for about 1 min until a uniform mixture was obtained. The prepared dry ingredients were gradually incorporated to the wet mixture gently with a spatula until a uniform smooth batter is obtained. Subsequently the batter was portioned in lined cupcake moulds and subjected to baking in a preheated (Morphy Richards OTG-60-RCSS-Luxe) oven for 30 min at 175°C. Gluten-free cupcakes were formulated by replacing oil with varying proportion of LSM, while maintaining all remaining ingredient quantities constant as detailed in **Table 1**.

Table 1: Formulation of Gluten-Free Cupcake Samples (GFCs)

Ingredient list	**LSM0**	**LSM25**	**LSM50**	**LSM75**	**LSM100**
CF	30	30	30	30	30
Yogurt	29	29	29	29	29
Sugar	20	20	20	20	20
Oil	20	15	10	5	0
LSM	0	5	10	15	20
Leavening Agent	1	1	1	1	1

Amino Acid Profiling

GFCs sample (1g) was transferred into individual 15 mL Falcon tubes. A methanol-acetonitrile solvent mixture (2 mL) was subsequently added to each tube for the extraction. The mixtures were vortexed for 1 min to obtain uniform sample dispersion, followed by ultrasonication at 37°C for 1 h to improve the extraction of amino acids from the samples. The resulting extracts were filtered using a 0.45 µm syringe membrane filter and collected into chromatographic vials prior to analysis. Amino acid profiling was carried out using an Agilent 6460 liquid chromatography-mass spectrometry (LC-MS) system equipped with triple quadrupole detector operated in positive ionization mode. Separation of amino acids was achieved using an Agilent EC-C18 column (4.6×50mm, 2.7 µm). Quantification of amino acids was performed through external calibration curves generated using reference standards (Chadha et al. 2026).

Mineral Estimation

The total ash content was obtained prior to mineral quantification by incineration samples in muffle furnace, after which the resulting ash was wet acid-digested using a concentrated acid mixture (HNO_3 and $HClO_4$) on a hot plate until clear. Wet acid-digested samples were subjected to mineral analysis for essential elements including calcium, iron, manganese, copper, zinc, phosphorus, magnesium and selenium. The digestion mixture was heated at 70°C for 6h until a clear solution was obtained. Following cooling the digested samples were diluted with 50 mL of deionized water and analyzed using an atomic absorption spectrometer (Perkin Elmer, NJ, USA). Calibration of the instrument was performed using element-specific hollow cathode lamps along with appropriate standard solutions. Dilution factors were adjusted according to the mineral being analyzed with the dilution levels of 10,000 for magnesium, 2500 for phosphorus and 100 for the remaining minerals. The obtained mineral values were expressed as mg/100 g based on the analytical measurements.

Potassium and Sodium contents were determined separately using a flame photometer (D Haridas and Company, Pune, India). Standard solutions with concentrations of 25, 50 and 100 ppm were employed to establish calibration curves prior to analysis. The concentrations of these minerals were calculated from the respective emission intensity values and the final results were expressed in mg/100 g (Choudhary et al. 2024).

Statistical Analysis

All analyses were performed in three independent replicates and the results were expressed as mean ± standard deviation (SD). Statistical difference between LSM0 and LSM100 were analyzed using student t test. Differences were considered statistically significant at $p<0.05$. Statistical analyses were performed using ANOVA 2.0 GraphPad Software.

Result and Discussion

Amino Acid Profiling

Amino acid profile of LSM0 and LSM100 is presented in Table 2. A total of nine essential and eight non-essential amino acids were detected and quantified using LC-MS. Several amino acids exhibited significant differences ($p<0.05$). Indicating that complete substitution of oil with LSM altered the amino acid composition of the cupcakes.

Among essential amino acids, phenylalanine, isoleucine, leucine, lysine showed a significant increase that is from 0.3525±0.023 to 0.5029±0.034 mg/100g protein (42.7%), 0.0201±0.014 to 0.2352±0.059 mg/100g protein, 0.2487±0.002 to 0.3018±0.001 mg/100g protein, 0.1831±0.006 to 0.2519±0.004 mg/100g protein ($p<0.05$) respectively. These amino acids are nutritionally important because they contribute to the protein synthesis, muscle maintenance and growth. The increase may have resulted because of the proteinaceous components naturally present in *Lepidium sativum* seeds (Singh and Paswan 2017)and to enhanced release of amino acids from the protein matrix due to the hydrocolloid nature of mucilage (Türker and İşleroğlu 2023). Garden cress seeds provide good amount of essential amino acids, especially leucine and lysine which may explain the elevated concentrations observed in the LSM-enriched formulation (Akbar 2020; Azene et al. 2022).

While methionine showed significant reduction from 1.2221±0.035 to 0.7866±0.003 mg/100g protein ($p<0.05$).The reduction in methionine may be associated with heat-induced changes during baking, although the present study did not directly determine the mechanism responsible for this reduction. Valine, histidine, tryptophan and threonine showed no statistical difference ($p>0.05$), suggesting that replacement of oil with mucilage had minimal influence on their stability and availability (Tomić et al. 2022; Syan et al. 2024).

Non- essential amino acids, significant increase were observed in proline, glutamic acid, arginine, and glycine. Arginine exhibited the greatest increase, rising from 0.5675±0.008 to 0.9521±0.004 mg/100g protein (67.8%) and past studies have seen that garden cress seeds have substantial amount of arginine (Azene et al. 2022). Glycine increased by approximately 159% that is from 0.2463±0.003 to 0.6389±0.002 mg/100g protein, while proline by 134%, 0.1098±0.032 to 0.2565±0.017 mg/100g protein and glutamic acid by 89% from 0.2395±0.031 to 0.4532±0.041 mg/100g protein. Higher concentration of these amino acids may be due to garden cress seeds as they are considered good source of non-essential amino acids (Akbar 2020) and improved protein hydrolysis and liberation of free amino acids in presence of mucilage network.

In contrast, serine and tyrosine decreased significantly in LSM100. Tyrosine showed reduction from 0.9761±0.003 to 0.5612±0.032 mg/100g protein (42.5%) ($p<0.05$) and serine from 0.4817±0.001 to 0.3427±0.012 mg/100g protein. These reductions may be related to their active participation in Maillard reactions, Strecker degradation and other thermal transformations during baking (Troise et al. 2015). The reduction in serine may be associated with heat-induced transformations during baking, potentially including dehydration and Maillard-related reactions (Friedman). Conversely, aspartic acid and cystine showed no significant change ($p \geq 0.05$). The acidic chain of aspartic acid reduces its reactivity towards reducing sugars, making it less likely to participate in initial stages of Maillard reaction (Martins et al. 2000). Likewise, cystine the absence of a significant difference in cystine may indicate that the processing conditions used in the present study did not substantially alter its measured concentration (Volkin and Klibanov 1987).

Finally, incorporating LSM as a complete fat replacement modified the amino acid composition of developed gluten-free cupcakes by increasing several nutritionally desirable amino acids, particularly lysine, leucine, isoleucine, arginine, glutamic acid, glycine. The enhancement of these amino acids makes LSM a good fat replacer and also helps improving nutritional quality. Therefore, the use of LSM as a fat replacer may improve both functional and nutritional characteristics of gluten-free bakery products (Tyagi et al. 2025).

Table 2 Amino acid analysis of LSM0 and LSM100

Amino Acids (mg/100g protein)	LSM0	LSM100	p-value
Essential Amino Acids			
Phenylalanine	0.3525±0.023	0.5029±0.034	p<0.05
Histidine	0.1301±0.076	0.2104±0.041	p≥0.05
Methionine	1.2221±0.035	0.7866±0.003	p<0.05
Valine	0.7653±0.023	0.6712±0.051	p≥0.05
Tryptophan	0.0326±0.012	0.0609±0.056	p≥0.05
Isoleucine	0.0201±0.014	0.2352±0.059	p<0.05
Leucine	0.2487±0.002	0.3018±0.001	p<0.05
Lysine	0.1831±0.006	0.2519±0.004	p<0.05
Threonine	0.0791±0.012	0.0701±0.009	p≥0.05
Non-essential Amino Acids			
Serine	0.4817±0.001	0.3427±0.012	p<0.05
Proline	0.1098±0.032	0.2565±0.017	p<0.05
Aspartic Acid	0.6865±0.015	0.7010±0.031	p≥0.05
Cystine	0.8115±0.016	0.7664±0.049	p≥0.05
Glutamic Acid	0.2395±0.031	0.4532±0.041	p<0.05
Tyrosine	0.9761±0.003	0.5612±0.032	p<0.05
Arginine	0.5675±0.008	0.9521±0.004	p<0.05
Glycine	0.2463±0.003	0.6389±0.002	p<0.05

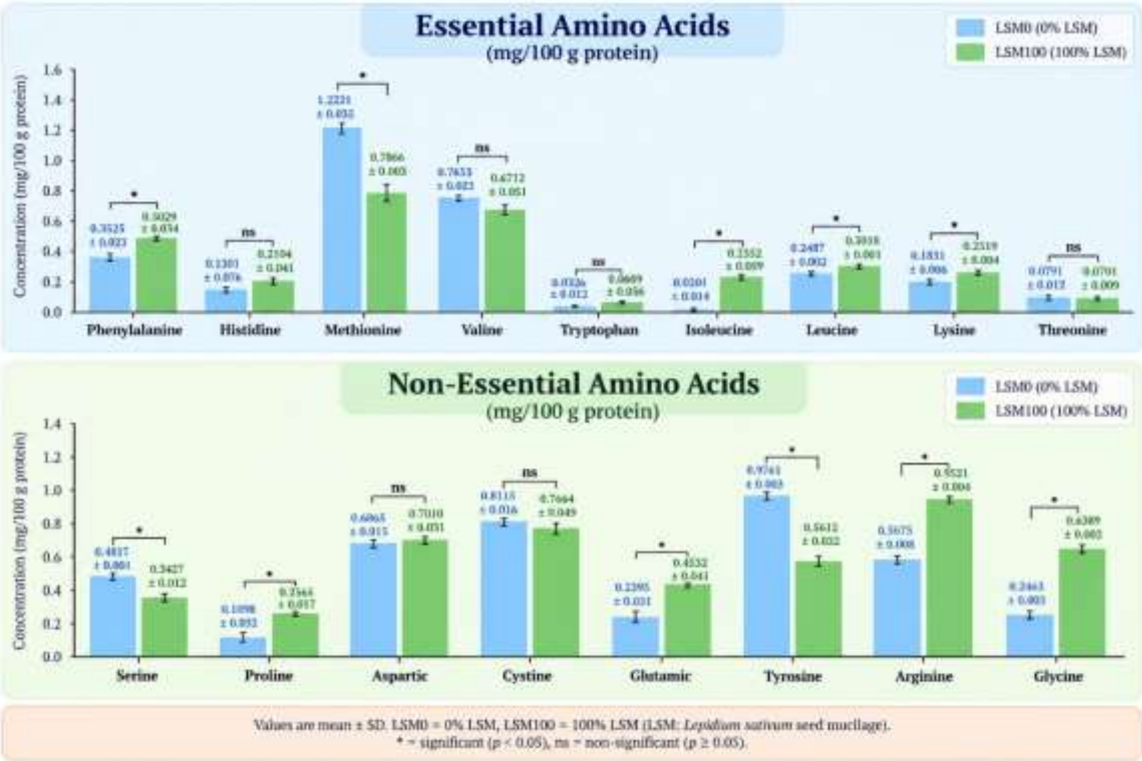

Figure 1: Comparative amino acid profile of LSM0 and LSM100

Mineral estimation

The mineral composition of gluten-free cupcakes prepared with complete oil replacement using *Lepidium sativum* seed mucilage (LSM100) was compared with the control formulation prepared with conventional oil (LSM0). The results demonstrated that incorporation of seed mucilage significantly influenced the mineral content of formulated GFCs.

Among analyzed minerals calcium, phosphorus, magnesium, potassium and iron contents, increased significantly (p<0.05) following complete fat replacement. Calcium increased from 88.81±0.36 to 90.19±0.39 mg/100g, phosphorus from 196.25±0.49 to 199.21±0.51 mg/100g, magnesium from 69.17±0.27 to 71.41±0.22 mg/100g, potassium from 420.19±0.41 to 430.16±0.49 mg/100g and iron increased from 4.49±0.01 to 4.61±0.03 mg/100g. This increase in these minerals highlights the nutritional advantage of replacing oil with plant-derived hydrocolloid. Commercially available oil has almost zero ash content, which means very low mineral content while *Lepidium sativum* seed mucilage may contribute to the mineral content of the formulated product; calcium phosphorus, magnesium, potassium, iron and selenium (Al-Snafi 2019). Furthermore, hydrocolloids form, gel-like network that holds water during baking, which reduces moisture loss and heat damage. This helps retain minerals efficiently that conventional oils (Hollingworth 2010; Archana and Singh 2022). These findings indicate the replacement of oil with *Lepidium sativum* seed mucilage contributed positively toward improving micronutrient density, reducing added oil content and also as a functional bio-Fortificant in the gluten-free cupcake formulation.

Sodium, zinc, copper, manganese and selenium showed no significant differences between LSM0 and LSM100 (p≥0.05) indicating that their measured concentration were not significantly affected by complete replacement of oil with LSM. These findings were also observed in previous researches where seed mucilage based fat replacers were used; chia seed (Fernandes et al. 2021) and flaxseed mucilage helped retain minerals in baked products by holding water, minimizing mineral loss during thermal processing (Puligundla and Lim 2022) The improved mineral profile may be attributed to the nutritional composition of *Lepidium sativum* (Archana and Singh 2022) and the nutrient-retention ability of hydrocolloids during baking (Hollingworth 2010). These findings indicate that *Lepidium sativum* seed mucilage functions not only as an effective fat replacer but also as a nutritionally beneficial ingredient capable of improving mineral composition of gluten-free cupcakes.

Table 3 Mineral analysis of LSM0 and LSM100

Parameters	LSM_0	LSM_{100}	*p*-value
Major minerals (mg/100g)			
Calcium	88.81±0.36	90.19±0.39	(<0.05)*
Phosphorus	196.25±0.49	199.21±0.51	(<0.05)**
Magnesium	69.17±0.27	71.41±0.22	(<0.05)***
Potassium	420.19±0.41	430.16±0.49	(<0.05)***
Sodium	250.63±0.52	251.78±0.51	(≥0.05)^{ns}
Trace minerals (mg/100g)			
Iron	4.49±0.01	4.61±0.03	(<0.05)**
Zinc	1.65±0.05	1.72±0.03	(≥0.05)^{ns}
Copper	0.33±0.04	0.34±0.08	(≥0.05) ^{ns}
Manganese	0.89±0.01	0.92±0.03	(≥0.05) ^{ns}
Selenium (µg/100g)	4.3±0.04	4.4±0.05	(≥0.05) ^{ns}

LSM_0 =0% LSM, LSM_{100}=100% LSM, LSM (*Lepidium sativum* seed mucilage), *-significant, **-highly significant, *-very highly significant, ns-non-significant**

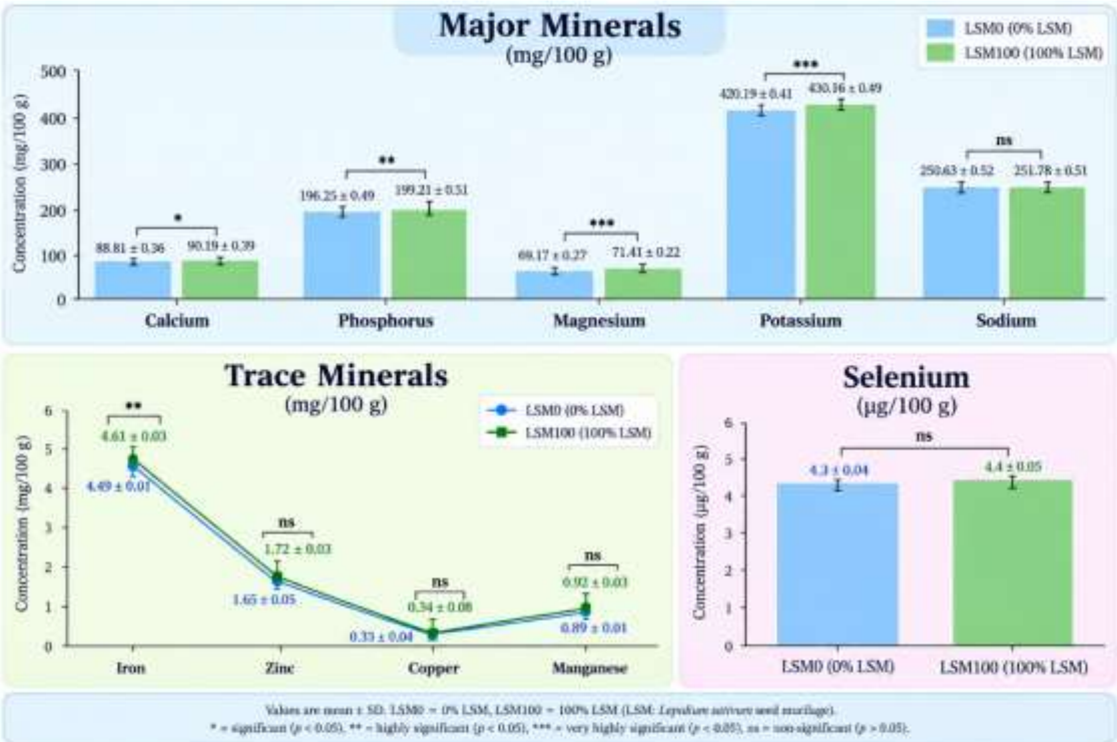

Figure 2: Comparative mineral profile of LSM0 and LSM100

Conclusion

This present investigation confirms that *Lepidium sativum* seed mucilage can be successfully utilized as a plant-derived fat replacer in gluten-free cupcakes without altering the sensory attributes. Replacement of 100% oil with mucilage significantly improved the amino acid profile by improving the levels of certain amino acids particularly lysine, leucine, isoleucine, arginine, glutamic acid and glycine. In addition to this incorporation of mucilage positively influenced mineral composition also increased calcium, iron, magnesium, phosphorus and potassium. Most minerals remain stable, showing good mineral retention during processing. These findings depicted dual technological and nutritional functionality of LSM. LSM is also a promising clean-label alternative to conventional fats and a potential functional food with a potential to enhance nutritional quality. Further investigations on storage stability, consumer acceptance are to be facilitated for its commercial application.

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