INSIGHTS-JOURNAL OF
LIFE AND SOCIAL SCIENCES

INSIGHTS-JLSS
Insights-Journal of Life and Social Sciences

EXPLORING THE PCOS MANAGEMENT POTENTIAL OF A BARLEY FLOUR-BASED COOKIES WITH SUNFLOWER, PUMPKIN, FLAX, AND SESAME SEEDS

Original Article

Bushra Ghulam[1], Nida Iqbal[1]*, Tayyba Eman[1], Laiba Tariq[1], Aleena Bibi[1], Samavia Rashid Saleem[1], Saadia Manzoor[1], Anum Obaid[1], Fakiha Mehak[1]
[1]Department of Human Nutrition and Dietetics, Riphah International University, Faisalabad Campus, Pakistan.
Corresponding Author: Nida Iqbal, Department of Human Nutrition and Dietetics, Riphah International University, Faisalabad Campus, Pakistan, dr.nida@riphahfsd.edu.pk

Conflict of Interest: None
Grant Support & Financial Support: None
Acknowledgment: The authors gratefully acknowledge the Department of Human Nutrition and Dietetics, Riphah International University, Faisalabad, for their support during this study.

ABSTRACT

Background: polycystic ovarian syndrome (PCOS) is a prevalent endocrine disorder in women of reproductive age, associated with metabolic and reproductive disturbances including insulin resistance, hormonal imbalance, and infertility. Nutritional interventions incorporating functional ingredients rich in bioactive compounds may play a supportive role in managing PCOS. Seeds such as flax, pumpkin, sesame, and sunflower, alongside barley flour, are valuable sources of protein, fiber, healthy fats, and antioxidants, which can contribute to improving metabolic and hormonal health.

Objective: To develop and evaluate barley flour–based cookies enriched with polyunsaturated fatty acid–rich seeds (PSSF) for their nutritional composition, antioxidant activity, and sensory acceptability, with potential application in dietary management of PCOS.

Methods: Cookies were formulated with varying proportions of barley flour (65–90%) and PSSF seed powders (0–25%). Proximate analysis measured moisture, ash, crude protein, crude fiber, crude fat, and nitrogen-free extract (NFE). Antioxidant activity was determined through total phenolic content (TPC, mg GAE/100 g) and DPPH free radical–scavenging activity (%) over 0, 15, and 30 days of storage. Sensory evaluation for texture, taste appearance, and overall acceptability was performed using a nine-point hedonic scale. Data were analyzed using a completely randomized design.

Results: Barley recorded the highest moisture (11.54 ± 0.3%) and NFE (63.82 ± 0.2%), sunflower seeds had the highest ash (7.38 ± 0.34%) and crude protein (37.9 ± 0.51%), flax seeds showed the highest crude fiber (20.22 ± 0.45%), and sesame seeds exhibited the highest fat content (56.55 ± 0.61%). TPC ranged from 29.28 to 888.52 mg GAE/100 g, with T4 maintaining the highest values during storage. DPPH activity was highest in T3 (75.40%), with T3, T4, and T5 retaining greater antioxidant activity over time. Sensory scores declined slightly during storage, with T0 and T1 maintaining superior texture and overall acceptability, while T1 achieved the highest taste scores.

Conclusion: Seed-enriched barley cookies demonstrated enhanced nutritional and antioxidant properties, with favorable sensory attributes at moderate seed inclusion, offering potential as a functional dietary option for PCOS management.

Keywords: Antioxidant activity, barley flour, cookies, PCOS, polyunsaturated fatty acids, seeds, sensory evaluation.

INTRODUCTION

Polycystic ovarian syndrome (PCOS), first described in 1935 and historically referred to as Stein–Leventhal syndrome, is a prevalent endocrine disorder affecting approximately 4% to 12% of women of reproductive age (1). It is characterized by a spectrum of clinical features, including anovulation, infertility, hirsutism, hyperandrogenism, acne, and hair loss. Functional ovarian hyperandrogenism is observed in nearly 70% of affected women, and polycystic ovaries typically exhibit multiple immature follicles that rarely yield viable eggs (2). Endocrine abnormalities such as elevated estrogen, testosterone, and luteinizing hormone (LH) levels, alongside reduced follicle-stimulating hormone (FSH) secretion, underpin the pathophysiology. These hormonal imbalances are closely linked to disruptions in the hypothalamic–pituitary–ovarian axis and may also be associated with androgen-secreting neoplasms (3,4). The presentation and severity of PCOS vary, with distinct phenotypes demonstrating different risks for metabolic dysfunction and reproductive complications. Both environmental factors—such as socioeconomic conditions, geography, toxic exposures, lifestyle, and diet—and genetic influences, including specific gene variants, epigenetic modifications, and ethnicity, contribute to its epidemiology and phenotypic expression (5,6). Nutritional interventions have gained attention as adjunctive strategies for managing PCOS, particularly through dietary sources rich in bioactive compounds that support hormonal balance and metabolic health. Pumpkin (Cucurbita spp.), a member of the Cucurbitaceae family, has long been cultivated worldwide for its nutritional and medicinal value. Widely grown varieties include *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata*. Pumpkin seeds, traditionally used in regions such as China, Pakistan, India, Yugoslavia, Argentina, Mexico, the United States, and Brazil, are valued for their distinctive nutty flavor and high nutrient density. They provide an abundant source of polyunsaturated fatty acids (PUFAs), iron, protein, manganese, magnesium, zinc, potassium, copper, phosphorus, carotenoids, and tocopherols, and are increasingly processed into protein isolates and specialty seed oils with emerging nutraceutical potential (7-9).

Similarly, barley (*Hordeum vulgare*), particularly highland barley, is rich in minerals, vitamins, phytochemicals, flavonoids, β-glucan, and other polyphenols. Its high β-glucan content is associated with a wide array of health benefits, including anti-inflammatory, anti-cancer, antidiabetic, antibacterial, anti-obesity, and antioxidant effects, as well as the ability to modulate blood glucose and lipid profiles. Highland barley, owing to its elevated concentration of bioactive constituents, demonstrates superior preventive and therapeutic potential against chronic diseases such as cardiovascular disorders, cancer, and neurodegenerative conditions compared to other barley cultivars (10-12). Given the shared bioactive potential of nutrient-dense seeds and barley, functional food formulations offer an innovative dietary approach for individuals with PCOS. Incorporating PUFA-rich seeds—such as pumpkin, flax, sunflower, and sesame—into barley-based food products may not only enhance palatability and nutrient intake but also confer metabolic and hormonal benefits relevant to PCOS management. In addition to their role in promoting digestive health, such combinations may improve antioxidant defenses and reduce oxidative stress, which is a contributing factor in PCOS pathophysiology. The present study aims to formulate and develop barley-based cookies enriched with selected seed powders as functional ingredients, assess their sensory qualities including taste, texture, aroma, and overall acceptability, and evaluate the impact of varying storage conditions on their shelf life. By addressing the gap in functional food applications targeted at PCOS, this research seeks to explore the potential of seed–barley combinations as a dietary strategy for improving reproductive and metabolic health outcomes in affected women.

METHODS

The present experimental study was conducted in the Department of Human Nutrition and Dietetics, Riphah International University, Faisalabad, Pakistan, following approval from the Institutional Ethical Review Committee. All procedures adhered to established ethical guidelines, and informed consent was obtained from all participants involved in the sensory evaluation phase. The primary aim was to formulate barley-based cookies enriched with polyunsaturated fatty acid (PUFA)-rich seed powders, assess their nutritional composition and antioxidant properties, and evaluate their sensory acceptability under different storage conditions.

Procurement of raw materials: Flax, sesame, sunflower, and pumpkin seeds were procured from the Ayub Agriculture Research Center, Faisalabad, ensuring uniform quality and maturity. Barley flour and other baking ingredients, including sugar, vegetable oil, and eggs, were sourced from local commercial suppliers.

Preparation of seed powder: The seeds were cleaned thoroughly to remove visible contaminants and foreign matter. An electronic blending machine (Ranker, Model: GMO 1 grinder) was used to grind the seeds until a homogenous fine powder was obtained. The powder was sieved through a 250 µm mesh to remove residual seed coats. The sieved powder was stored in airtight, food-grade plastic bags under controlled conditions until use. For food safety, the powder was heat-treated at 60°C to inactivate potential anti-nutritional factors before incorporation into the product.

Proximate analysis: The nutritional composition of the seed powder was determined according to the standard methods described by the American Association of Cereal Chemists (AACC, 2016), including assessments of moisture, ash, crude protein, crude fat, crude fiber, and nitrogen-free extract (NFE).

Moisture content: Moisture content was measured by drying 10 g of each sample in a forced-draft oven at 105 ± 5°C until a constant weight was achieved, as per AACC (2016) guidelines.

Ash: Ash content was determined by initially charring 5 g of sample in crucibles over a flame until smoke emission ceased. Samples were then incinerated in a muffle furnace at 500–600°C for 6–7 hours until white or grey ash was obtained, and ash percentage was calculated using the standard AACC formula.

Crude protein: Crude protein was quantified using the Kjeldahl method (AACC Method No. 46-10). Two grams of sample were digested with 20 mL of concentrated sulfuric acid (98%) and digestion tablets (catalyst) for 3–4 hours until a clear solution was obtained. The digest was cooled, diluted to 50 mL, and distilled with 40% sodium hydroxide. Released ammonia was trapped in 4% boric acid and titrated against 0.1 N sulfuric acid, with nitrogen percentage calculated and multiplied by 6.25 to determine crude protein.

Crude fiber: Fat-free samples were sequentially digested with 1.25% sulfuric acid and 1.25% sodium hydroxide. Residues were filtered, washed, dried, and ignited in a muffle furnace at 500–600°C until white ash was obtained. Crude fiber percentage was calculated as per AACC (2016).

Crude fat: Crude fat was determined by Soxhlet extraction using petroleum ether (boiling range 40–60°C) as the solvent. Five grams of seed powder were extracted for approximately 15 hours at a condensation rate of 3–4 drops per second. The solvent was removed by distillation, and the residue was dried at 100°C for 30 minutes to constant weight.

Nitrogen free extracts (NFE): NFE was calculated using the expression: NFE (%) = 100 – (% moisture + % ash + % crude protein + % crude fiber + % crude fat).

Assay of total antioxidant activity: Total antioxidant activity was determined following the method of Lovatto.

Sample Preparation and Extraction: Seed powder extracts were prepared using the method of Kazemi with four sample-to-solvent ratios (5:1, 10:1, 15:1, 20:1). Deionized water (40–50°C) was added to 25–100 mg of seed powder, shaken for 5 minutes, and refrigerated at 4°C for 30 minutes before centrifugation at 10,000×g for 10 minutes. Supernatants were pooled, re-extracted, and stored at –20°C until analysis.

Powder: The prepared powders from the four seed types were individually assessed for their bioactive potential.

Total phenolic content (TPC): TPC was determined using the Folin–Ciocalteu method (13). Fifty microliters of extract were mixed with 250 µL of Folin–Ciocalteu reagent and 750 µL sodium bicarbonate, diluted to 5 mL with distilled water, covered with aluminum foil, and incubated in the dark for 2 hours. Absorbance was measured at 765 nm, and results were expressed as gallic acid equivalents (mg GAE/g).

Diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity: DPPH radical scavenging activity was assessed as per Bhebhe. Sample extracts (0.0256 mL) were mixed with 10 mL ethanol and 3 mL of freshly prepared DPPH solution (1 mg/100 mL methanol) and incubated in the dark for 15 minutes. Absorbance was recorded at 517 nm, and radical scavenging activity (%) was calculated using standard formulas.

Product development: Cookies were prepared according to AACC with varying proportions of barley flour and seed powder blends (PSSF: pumpkin, sesame, sunflower, flax) as detailed in the treatment plan (T0–T5: 0–25% seed powder substitution). Standardized baking protocols included ingredient weighing, mixing for 3–5 minutes, kneading for 5 minutes, rolling dough to 8 mm thickness, cutting with a circular scone cutter, and baking at 160°C for 15 minutes. Cookies were cooled to ambient temperature (28°C) before storage in polyethylene bags.

Sensory evaluation of cookies: Sensory evaluation followed the methodology of Hooda and Jood. Thirty untrained panelists from the university community assessed taste, aroma, color, crispiness, and overall acceptability using a nine-point hedonic scale (1 = dislike extremely; 9 = like extremely). Evaluations were conducted at designated storage intervals.

Statistical analysis: Data were analyzed using a completely randomized design (CRD) as described by Montgomery (2008). Mean values and standard deviations were calculated, and statistical significance was determined at $p < 0.05$.

RESULTS

The proximate analysis of flax, pumpkin, sesame, sunflower, and barley seeds revealed notable variations in their nutritional composition. Moisture content ranged from 3.54 ± 0.3% in sesame seeds to 11.54 ± 0.3% in barley seeds, with sunflower seeds (9.42 ± 0.9%) and barley exhibiting higher moisture levels, while sesame and pumpkin seeds demonstrated lower values. Ash content was highest in pumpkin seeds (6.80 ± 0.13%) and sunflower seeds (7.38 ± 0.34%), moderate in sesame seeds (4.37 ± 0.13%), and lowest in flax seeds (1.87 ± 0.1%) and barley seeds (2.46 ± 0.3%). Crude protein content was highest in sunflower seeds (37.9 ± 0.51%), followed by pumpkin seeds (28.8 ± 0.13%), flax seeds (21.1 ± 0.2%), sesame seeds (15.56 ± 0.17%), and barley seeds (12.84 ± 0.2%). Crude fiber content was maximum in flax seeds (20.22 ± 0.45%) and sunflower seeds (21.49 ± 0.42%), moderate in barley (7.04 ± 0.3%) and sesame (6.81 ± 0.41%), and lowest in pumpkin seeds (4.58 ± 0.3%). Fat content was highest in sesame seeds (56.55 ± 0.61%), followed by flax (43.16 ± 0.88%) and pumpkin (31.74 ± 0.44%), while sunflower (0.67 ± 0.3%) and barley (2.3 ± 0.2%) recorded minimal values. Nitrogen-free extract (NFE) was greatest in barley (63.82 ± 0.2%), moderate in sunflower (23.14 ± 0.4%) and pumpkin (22.56 ± 0.7%), low in sesame (13.17 ± 0.5%), and very low in flax (5.2 ± 0.3%). Analysis of total phenolic content (TPC) across storage intervals (0, 15, and 30 days) showed that treatment T4 (70% barley + 20% seed powder) maintained the highest mean value (888.52 ± 44 mg GAE/100 g) with minimal decline, followed by T1 (606.33 ± 5.20 mg GAE/100 g) and T2 (100.50 ± 3.63 mg GAE/100 g). The control (T0) decreased from 83.02 ± 4.17 mg GAE/100 g at day 0 to 76.8 ± 4.14 mg GAE/100 g at day 30. T3 exhibited the lowest mean TPC (29.28 ± 0.73 mg GAE/100 g) with a progressive decline during storage.

DPPH free radical-scavenging activity followed a different pattern. T3 (75% barley + 15% seed powder) recorded the highest mean antioxidant activity (75.40 ± 0.3%), followed by T5 (65% barley + 25% seed powder) at 61.55 ± 0.7% and T1 at 54.80 ± 0.7%. The control (T0) had the lowest mean value (24.90 ± 0.3%). Across most treatments, DPPH values decreased over storage time, although T3 and T5 showed relatively stable antioxidant retention. Sensory evaluation for texture indicated that the highest mean score was observed in the control group (7.33 ± 0.15), followed by T1 (7.23 ± 0.13). The lowest texture score was recorded in T5 (6.43 ± 0.05). Texture scores decreased slightly across all treatments over the 30-day storage period. Taste assessment showed the highest mean score in T1 (7.73 ± 0.5), followed closely by the control and T2 (both 7.33 ± 0.3). The lowest taste score was recorded for T5 (6.95 ± 0.2). A gradual decline in taste scores was observed with extended storage. Appearance scores were highest in T5 (8.70 ± 0.4) and T1/T2 (both 8.46 ± 0.3–0.5), with the lowest in T3 (8.20 ± 0.5). Slight reductions in appearance were noted over time across all treatments. Overall acceptability was highest in the control group (7.91 ± 0.2), followed by T1 (7.65 ± 0.4) and T2 (7.50 ± 0.5), with the lowest in T5 (7.20 ± 0.4). A small but consistent decline in acceptability was observed during storage across all formulations.

Table 1: Proximate Composition of Flax, Pumpkin, Sesame, Sunflower, and Barley Seeds

Parameters	Flax seed	Pumpkin seed	Sesame seed	Sunflower seed	Barley seed
Moisture	8.45 ± 0.8	5.42 ± o.5	3.54 ± 0.3	9.42 ± 0.9	11.54 ± 0.3
Ash	1.87 ± 0.1	6.8 ± 0.13	4.37 ± 0.13	7.38 ± 0.34	2.46± 0.3
Crude protein	21.1 ± 0.2	28.8 ± 0.13	15.56 ± 0.17	37.9 ± 0.51	12.84± 0.2
Crude fiber	20.22 ± 0.45	4.58 ± 0.3	6.81 ± 0.41	21.49 ± 0.42	7.04± 0.3
Fat	43.16 ± 0.88	31.74 ± 0.44	56.55 ± 0.61	0.67 ± 0.3	2.3 ± 0.2
NFE	5.2±0.3	22.56±0.7	13.17±0.5	23.14±0.4	63.82±0.2

Table 2: Total Phenolic Content (mg GAE/100 g) of Barley–Seed Cookies Across Treatments and Storage Durations

Treatment	Storage (days) 0	Storage (days) 15	Storage (days) 30	Means
T_0	83.02 ± 4.17	80.82 ± 4.16	76.8 ± 4.14	80.22 ± 4.15
T_1	608 ± 5.50	606 ± 5.20	605 ± 4.90	606.33 ± 5.20
T_2	102.79 ± 3.67	100 ± 3.63	98.70 ± 3.61	100.50 ± 3.63
T_3	30.20 ± 0.90	29.85 ± 0.70	27.80 ± 0.60	29.28 ± 0.73
T_4	900.21 ± 45	880.20 ± 44	885.15 ± 43	888.52 ± 44
T_5	75.09 ± 0.11	70.07 ± 0.10	68.60 ± 0.11	71.07 ± 0.10
Overall mean	299.85 ± 9.89	294.49 ± 9.63	293.67 ± 9.39	

Notes: T_0 = control group 90% barley; T_1 = 85% barley, 5% seeds; T_2 = 80% barley, 10% seeds; T_3 = 75% barley, 15% seeds; T_4 = 70% barley, 20% seeds; T_5 = 65% barley, 25% seeds.

Table 3: DPPH Free Radical–Scavenging Activity (%) of Barley–Seed Cookies Across Treatments and Storage Durations

Treatment	Storage (Days) 0	Storage (Days) 15	Storage (Days) 30	Mean
T_0	25.20 ± 0.4	24.98 ± 0.3	24.70 ± 0.2	24.90 ± 0.3
T_1	55.90 ± 0.8	54.80 ± 0.7	53.07 ± 0.6	54.80 ± 0.7
T_2	32.83 ± 0.5	28.80 ± 0.6	31.70 ± 0.3	31.11 ± 0.4
T_3	76.50 ± 0.2	75.40 ± 0.4	74.30 ± 0.5	75.40 ± 0.3
T_4	55.50 ± 0.8	54.40 ± 0.8	53.20 ± 0.1	54.36 ± 0.5
T_5	61.21 ± 0.6	62.23 ± 0.7	61.21 ± 0.9	61.55 ± 0.7
Overall mean	51.10 ± 0.5	50.10 ± 0.5	49.80 ± 0.4	

Notes: T_0 = control group 90% barley; T_1 = 85% barley, 5% seeds; T_2 = 80% barley, 10% seeds; T_3 = 75% barley, 15% seeds; T_4 = 70% barley, 20% seeds; T_5 = 65% barley, 25% seeds.

Table 4: Texture Scores of Barley–Seed Cookies Across Treatments and Storage Durations

Treatment	Storage (Days) 0	Storage (Days) 15	Storage (Days) 30	Mean
T_0	7.05 ± 0.12	7.03 ± 0.15	7.02 ± 0.18	7.33 ± 0.15
T_1	7.04 ± 0.11	7.02 ± 0.14	7.01 ± 0.15	7.23 ± 0.13
T_2	7.00 ± 0.01	6.98 ± 0.09	6.95 ± 0.08	6.97 ± 0.06
T_3	6.96 ± 0.07	6.95 ± 0.06	6.94 ± 0.05	6.95 ± 0.06
T_4	6.93 ± 0.08	6.97 ± 0.05	6.93 ± 0.07	6.94 ± 0.06
T_5	6.05 ± 0.06	6.45 ± 0.08	6.35 ± 0.03	6.43 ± 0.05
Overall mean	7.04 ± 0.07	6.97 ± 0.09	6.91 ± 0.09	

Notes: T_0 = control group 90% barley; T_1 = 85% barley, 5% seeds; T_2 = 80% barley, 10% seeds; T_3 = 75% barley, 15% seeds; T_4 = 70% barley, 20% seeds; T_5 = 65% barley, 25% seeds.

Table 5: Taste Scores of Barley–Seed Cookies Across Treatments and Storage Durations

Treatment	Storage (Days) 0	Storage (Days) 15	Storage (Days) 30	Mean
T_0	7.2 ± 0.2	7.5 ± 0.3	7.6 ± 0.4	7.43 ± 0.3^{ab}
T_1	7.9 ± 0.7	7.8 ± 0.5	7.5 ± 0.5	7.73 ± 0.5^{a}
T_2	7.5 ± 0.4	7.3 ± 0.2	7.2 ± 0.3	7.33 ± 0.3^{a}
T_3	7.3 ± 0.4	7.2 ± 0.2	7.1 ± 0.3	7.20 ± 0.3^{ab}
T_4	7.1 ± 0.5	6.9 ± 0.7	6.9 ± 0.4	7.00 ± 0.5^{b}
T_5	6.9 ± 0.5	6.9 ± 0.1	6.9 ± 0.2	6.95 ± 0.2^{b}
Overall Mean	7.3 ± 0.45	7.2 ± 0.33	7.2 ± 0.35	

Notes: T_0 = control group 90% barley; T_1 = 85% barley, 5% seeds; T_2 = 80% barley, 10% seeds; T_3 = 75% barley, 15% seeds; T_4 = 70% barley, 20% seeds; T_5 = 65% barley, 25% seeds.

Table 6: Appearance Scores of Barley–Seed Cookies Across Treatments and Storage Durations

Treatment	Storage (Days) 0	Storage (Days) 15	Storage (Days) 30	Mean
T_0	8.5 ± 0.4	8.4 ± 0.3	8.2 ± 0.1	8.36 ± 0.2
T_1	8.7 ± 0.5	8.5 ± 0.3	8.2 ± 0.2	8.46 ± 0.3
T_2	8.6 ± 0.6	8.5 ± 0.5	8.3 ± 0.4	8.46 ± 0.5
T_3	8.3 ± 0.7	8.3 ± 0.5	8.1 ± 0.4	8.20 ± 0.5
T_4	8.4 ± 0.7	8.3 ± 0.6	8.1 ± 0.5	8.26 ± 0.6
T_5	8.8 ± 0.5	8.7 ± 0.4	8.6 ± 0.3	8.70 ± 0.4
Overall Mean	8.5 ± 0.56	8.4 ± 0.43	8.3 ± 0.31	

Notes: T_0 = control group 90% barley; T_1 = 85% barley, 5% seeds; T_2 = 80% barley, 10% seeds; T_3 = 75% barley, 15% seeds; T_4 = 70% barley, 20% seeds; T_5 = 65% barley, 25% seeds.

Table 7: Overall Acceptability Scores of Barley–Seed Cookies Across Treatments and Storage Durations

Treatment (A)	Storage (Days) 0	Storage (Days) 15	Storage (Days) 30	Overall Acceptability
T_0	8.0 ± 0.3	7.95 ± 0.3	7.8 ± 0.2	7.91 ± 0.2^{a}
T_1	7.7 ± 0.6	7.65 ± 0.4	7.6 ± 0.2	7.65 ± 0.4^{ab}
T_2	7.55 ± 0.7	7.50 ± 0.5	7.45 ± 0.3	7.50 ± 0.5^{ab}
T_3	7.4 ± 0.8	7.35 ± 0.6	7.3 ± 0.4	7.35 ± 0.6^{ab}
T_4	7.25 ± 0.2	7.20 ± 0.4	7.15 ± 0.3	7.25 ± 0.3^{b}
T_5	7.15 ± 0.4	7.10 ± 0.2	7.5 ± 0.6	7.20 ± 0.4^{b}
Overall Mean	7.50 ± 0.5	7.45 ± 0.4	7.46 ± 0.3	

Notes: T_0 = control group 90% barley; T_1 = 85% barley, 5% seeds; T_2 = 80% barley, 10% seeds; T_3 = 75% barley, 15% seeds; T_4 = 70% barley, 20% seeds; T_5 = 65% barley, 25% seeds.

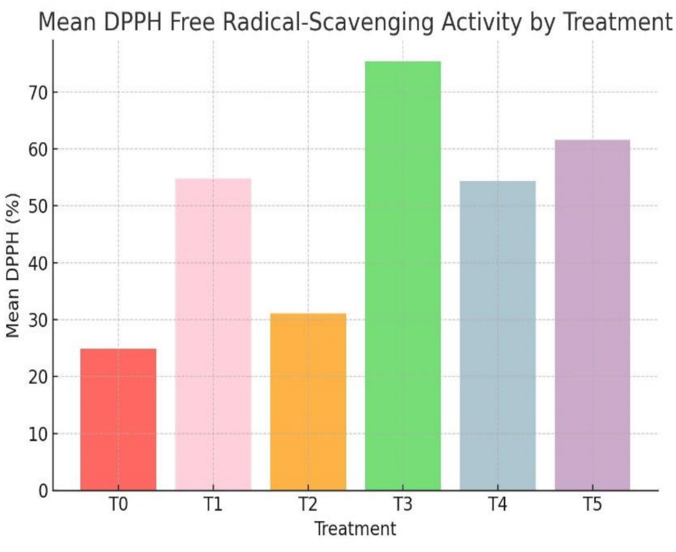

Figure 1 Mean DPPH Free Radical-Scavenging Activity by Treatment

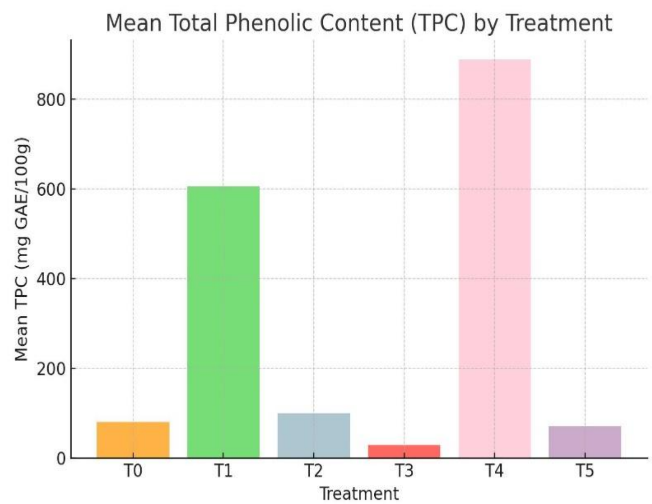

Figure 2 Mean Total Phenolic Content (TPC) by Treatment

DISCUSSION

The present study demonstrated that incorporating polyunsaturated fatty acid–rich seeds, including flax, pumpkin, sesame, and sunflower, into barley-based cookies enhanced the nutritional profile and antioxidant activity of the final product, with notable variations among treatments. The proximate analysis revealed substantial diversity in macronutrient composition across the seeds, aligning closely with previously reported values for moisture, protein, fat, fiber, and ash contents. For example, the high protein concentration in sunflower and pumpkin seeds and the elevated fat content in sesame and flax seeds corroborated earlier reports, confirming their potential as nutrient-dense functional ingredients (14,15). The low moisture content observed in sesame and pumpkin seeds was consistent with previous findings and suggested extended shelf stability, while higher moisture levels in barley and sunflower seeds indicated a greater water-binding capacity, which could influence textural characteristics during storage. The study's findings on nitrogen-free extract (NFE) highlighted barley as the richest source of carbohydrate-based energy among the evaluated ingredients, a factor contributing to the desirable texture and structure of cookies when blended with seeds (16,17). The balance between macronutrient contributions from seeds and barley was essential in developing a composite flour capable of delivering both functional health benefits and consumer-acceptable sensory qualities. Antioxidant profiling through total phenolic content (TPC) and DPPH free radical–scavenging activity provided insight into the bioactive potential of the formulations. Treatments with higher seed inclusion, particularly T4 for TPC and T3 for DPPH, demonstrated superior antioxidant retention across storage periods (18-20). This confirmed the contribution of phenolic compounds, lignans, and tocopherols naturally present in seeds to the oxidative stability of the product. The minimal decline in TPC in T4 over 30 days suggested that certain formulations could maintain bioactivity despite prolonged storage, whereas the sharper decrease in TPC in T3 indicated a less stable phenolic profile. The observation that T3 retained the highest DPPH activity suggested that antioxidant mechanisms may not always parallel total phenolic content, reflecting the complexity of antioxidant systems and the potential contribution of non-phenolic compounds such as unsaturated fatty acids and peptides (21-24).

Sensory evaluation demonstrated that formulations with moderate seed inclusion, such as T1 and T2, achieved higher scores for taste, texture, and overall acceptability compared to higher inclusion levels such as T5. This could be attributed to the balance between the nutty flavor of seeds and the structural integrity contributed by barley flour. While T5 achieved high appearance scores, its lower taste and overall acceptability indicated that visual appeal did not fully translate into consumer preference. Storage-induced declines in sensory parameters were consistent with known effects of lipid oxidation, moisture migration, and textural changes in baked goods. These results reinforced the potential of seeds as functional food ingredients for managing conditions such as polycystic ovarian syndrome (PCOS), owing to their high fiber, protein, and omega-3 fatty acid content (25,26). The study's strength lay in its integration of compositional, bioactive, and sensory evaluations under controlled conditions, allowing for a holistic assessment of product feasibility. However, certain limitations were evident. The study did not evaluate microbiological safety or specific oxidative rancidity markers, which would be essential for defining actual shelf life. Additionally, only one storage condition was tested, limiting the applicability of findings to varied climatic environments. The absence of glycemic index measurement or in vivo assessments restricted conclusions regarding the direct metabolic impact of these formulations in PCOS management. Future research should address these limitations by incorporating multi-environment storage trials, microbial stability assessments, and advanced oxidative stability testing. Furthermore, clinical trials involving women with PCOS would provide valuable data on the physiological effects of regular consumption of these functional cookies. Optimization of seed–barley ratios to balance sensory acceptability with maximal bioactive retention remains an important area for further development. Overall, the study highlighted the nutritional and functional promise of seed-enriched barley cookies while underscoring the need for expanded research to fully translate these findings into therapeutic dietary interventions.

CONCLUSION

The study concluded that incorporating nutrient-rich seed powders into barley-based cookies successfully enhanced their functional and sensory qualities, offering a promising approach for developing healthier snack options. While all formulations provided nutritional benefits, moderate seed inclusion achieved the most favorable balance between taste, texture, appearance, and overall acceptability, demonstrating that functional ingredients can be integrated without compromising consumer appeal. The findings support the potential of such products as convenient dietary interventions, particularly for individuals seeking nutrient-dense foods that may aid in the management of conditions like PCOS, and highlight their viability for broader application in functional food development.

AUTHOR CONTRIBUTION

Author	Contribution
Bushra Ghulam	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Nida Iqbal*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Tayyba Eman	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Laiba Tariq	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Aleena Bibi	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Samavia Rashid Saleem	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Saadia Manzoor	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Anum Obaid	Writing - Review & Editing, Assistance with Data Curation
Fakiha Mehak	Writing - Review & Editing, Assistance with Data Curation

REFERENCES

1. Abbas, S., Sharif, M. K., Sibt-E-Abbas, M., Teferra, T. F., Sultan, M. T., & Anwar, M. J. (2022). Nutritional and therapeutic potential of sesame seeds. *Journal of Food Quality,* 2022, (1) 1–9.
2. Adeleke, B. S., & Babalola, O. O. (2020). Oilseed crop sunflower (*Helianthus annuus*) as a source of food: Nutritional and health benefits. *Food Science & Nutrition*, *8*(9), 4666–4684.
3. Agurla, V. (2023). Effect of incorporation of chia seed oil on the physicochemical and nutritional characteristics of cookies: *A research. Energy, 388*(3.528), 395-17.
4. Al-Madhagy, S., Ashmawy, N. S., Mamdouh, A., Eldahshan, O. A., & Farag, M. A. (2023). A comprehensive review of the health benefits of flaxseed oil in relation to its chemical composition and comparison with other omega-3-rich oils. *European Journal of Medical Research, 28*(1),1269
5. Aly, A. A., Refaey, M., Hameed, A. M., Sayqal, A., Abdella, S. A., Mohamed, A. F., MAA, H., & Ismail, H. A. (2020). Effect of addition sesame seeds powder with different ratio on microstructural and some properties of low fat Labneh. *Arabian Journal of Chemistry, 13*(10), 7572–7582.
6. Batool, M., Ranjha, M. M. a. N., Roobab, U., Manzoor, M. F., Farooq, U., Nadeem, H. R., Nadeem, M., Kanwal, R., AbdElgawad, H., Jaouni, S. K. A., Selim, S., & Ibrahim, S. A. (2022). Nutritional Value, Phytochemical Potential, and Therapeutic Benefits of Pumpkin (Cucurbita sp.). *Plants*, *11*(11), 1394.
7. Beshaw, T., Demssie, K., Tefera, M., & Guadie, A. (2022). Determination of proximate composition, selected essential and heavy metals in sesame seeds (*Sesamum indicum L.*) from the Ethiopian markets and assessment of the associated health risks. *Toxicology Reports, 9*, 1806–1812.
8. Bilal, M., Shabbir, M. A., Zhang, X., Arslan, M., Usman, M., Azam, M., Aadil, R. M., & Ahmad, N. (2020). Characterization of peanut seed oil of selected varieties and its application in the cereal-based product. *Journal of Food Science and Technology/Journal of Food Science and Technology*, *57*(11), 4044–4053.
9. Boyd, C. E., D'Abramo, L. R., Glencross, B. D., Huyben, D., Juárez, L. M., Lockwood, G. S., McNevin, A. A., Tacon, A. G. J., Teletchea, F., Tomasso, J. R., Tucker, C. S., & Valenti, W. C. (2020). Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. *Journal of the World Aquaculture Society, 51*(3), 578–633.

10. Bull, C., Belobrajdic, D., Hamzelou, S., Jones, D., Leifert, W., Ponce-Reyes, R., Terefe, N. S., Williams, G., & Colgrave, M. (2022). How healthy are Non-Traditional dietary proteins? The effect of diverse protein foods on biomarkers of human health. *Foods, 11*(4), 528.

11. deOliveira Filho, J. G. and M. B. Egea (2021). "Sunflower seed byproduct and its fractions for food application: An attempt to improve the sustainability of the oil process." *Journal of Food Science 86*(5): 1497-1510.

12. Tahzeeb, Saiyyadah & Shabbir, Hamza & Fatima, Iraj & Adeel-ur-Rehman, & Fatima, Seerat & Aziz, Abdul & khalid, Filza. (2025). RADIOGRAPHIC ASSESSMENT OF LUMBOSACRAL ANGLE AND ITS CORRELATION WITH LOW BACK PAIN IN SEDENTARY WORKERS-A CROSS-SECTIONAL STUDY. Insights-Journal of Health and Rehabilitation. 3. 779-785. 10.71000/v2mq0h49.

13. Devi, N. M., Prasad, R., & Palmei, G. (2020). Physico-chemical characterisation of pumpkin seeds. *International Journal of Chemical Studies*, *6* (5), 828-831.

14. Dhakad, G. G., Nannaware, M. S., Wagh, G. V., Shrirao, A. V., Kochar, N., & Chandewar, A. (2023). Review on medicinal Use of Pumpkin Seed on Human Health. *Research Journal of Pharmacology and Pharmacodynamics, 15*(2), 59-65.

15. Dong, X., Chen, J., Chen, S., Li, Y., & Zhao, X. (2021). The composition and anti-inflammatory properties of pumpkin seeds. *Journal of Food Measurement and Characterization*, *15*(2), 1834–1842.

16. Dravie, E. E., Kortei, N. K., Essuman, E. K., Tettey, C. O., Boakye, A. A., & Hunkpe, G. (2020). Antioxidant, phytochemical and physicochemical properties of sesame seed (Sesamum indicum L). *Scientific African, 8*(10), 349-350.

17. Eid, O., Elkady, W. M., Ezzat, S. M., Sayed, A. E., & Abdel-Sattar, E. (2023). Comprehensive Overview: The Effect of Using Different Solvents for Barley Extraction with Its Anti-Inflammatory and Antioxidant Activity. *Chemistry & Biodiversity*, *20*(3), 11-12.

18. Emamat, H., Najafpour Boushehri, S., Eslami, M. A., Saneei Totmaj, A., & Ghalandari, H. (2023). The effects of flaxseed or its oil supplementations on polycystic ovary syndrome: A systematic review of clinical trials. *Phytotherapy Research, 37*(10), 20-25.

19. Gagour, J., Ahmed, M. N., Bouzid, H. A., Oubannin, S., Bijla, L., Ibourki, M., Hajib, A., Koubachi, J., Harhar, H., & Gharby, S. (2022). Proximate Composition, Physicochemical, and Lipids Profiling and Elemental Profiling of Rapeseed (*Brassica napus L.*) and Sunflower (*Helianthus annuus L.*) Grown in Morocco. *Evidence-based Complementary and Alternative Medicine,* 2022, 1–12.

20. Ghosal, P., & Sen, B. (2022). Multivariate ranks and quantiles using optimal transport: Consistency, rates and nonparametric testing. *The Annals of Statistics*, *50*(2), 1012-1037.

21. Hossain, M. S., Kader, M. A., Goh, K. W., Islam, M., Khan, M. S., Rashid, M. H., Ooi, D. J., Coutinho, H. D. M., Al-Worafi, Y. M., Moshawih, S., Lim, Y. C., Kibria, K. M. K., & Ming, L. C. (2022). Herb and spices in Colorectal Cancer Prevention and Treatment: A Narrative review. *Frontiers in Pharmacology, 13*(20), 1-23.

22. Irfan, T., Seher, K., Rizwan, B., Fatima, S., & Sani, A. (2021). Role of seed cycling in polycystic ovarian syndrome: Seed cycling in PCOS. *Pakistan BioMedical Journal, 4*(2), 21-25.

23. Irshad, Z., Aamir, M., Akram, N., Asghar, A., Saeed, F., Ahmed, A., Afzaal, M., Ateeq, H., Shah, Y. A., Faisal, Z., Khan, M. R., Busquets, R., & Asres, D. T. (2023). Nutritional profiling and sensory attributes of sesame seed-enriched bars. *International Journal of Food Properties, 26*(2), 2978–2994.

24. Ishag, O. a. O., Khalid, A. A., Abdi, A., Erwa, I. Y., Omer, A. B., & Nour, A. H. (2020). Proximate composition, physicochemical properties and antioxidant activity of flaxseed. *Annual Research & Review in Biology, 34*(2), 1–10.

25. Masood, Ateeqa & Mansoor, Aymah & Shabbir, Hamza & Nouman, Talha & Mughal, Summan & Gul, Sajjal. (2025). KNOWLEDGE AND ADOPTION OF AI-ENHANCED REHABILITATION TECHNOLOGIES AMONG PHYSICAL THERAPISTS: A CROSS-SECTIONAL STUDY. Insights-Journal of Life and Social Sciences. 3. 105-111. 10.71000/8h5pxj13.

26. Wei, P., Zhao, F., Wang, Z., Wang, Q., Chai, X., Hou, G., & Meng, Q. (2022). Sesame (*Sesamum indicum L.):* A Comprehensive Review of Nutritional Value, Phytochemical Composition, Health Benefits, Development of Food, and Industrial Applications. *Nutrients, 14*(19), 4079.

