



Nutritional and Therapeutic Potential of Gelatin-Based Functional Cocofee Gummies Against High Fat Diet-Induced Non-Alcoholic Fatty Liver Disease

Tooba Arif¹, Mahnoor Saleh², Ramisa Maryam¹, Ayesha Saddiq¹, Javaria Ashfaq¹, Maheen Sabir¹, Baila Ahmad³, Eisha Arif¹, Muhammad Asif^{1,4*}, Anum Urooj^{1*}

¹National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan.

²Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing, China.

³Department of Food Science and Technology, The Islamia University of Bahawalpur, Pakistan.

⁴Department of Food Science and Technology, UAF-Constituent College, Burewala, Pakistan.

Article Info

Keywords:

Functional confectionery;
Chewable nutraceuticals;
Chlorogenic acid

Date:

Received: 17 June 2026

Accepted: 02 August 2026

Published: 05 September 2026

Volume: 04 (02), 2026



Copyright: © 2026 by the authors. This article is distributed under the Creative Commons Attribution (CC BY) license, allowing for open access and sharing under specified terms and conditions. (<https://creativecommons.org/licenses/by/4.0/>).

Abstract

Non-alcoholic fatty liver disease (NAFLD), an umbrella term, a growing global epidemic with limited pharmacological interventions. The study aimed to develop and characterized a gelatin-based functional "Cocofee" gummies enriched with bioactive botanicals and evaluated its therapeutic potential against high-fat diet (HFD)-induced NAFLD in Wistar albino rats. Physicochemical profiling revealed a 57.84 ± 0.98% nitrogen-free extract, 27.28 ± 0.12% moisture, 5.90 ± 0.95% crude fat, 3.60 ± 0.05% crude protein, and 2.60 ± 0.18% ash. Phytochemical screening demonstrated high total phenolic content (24.1 mg GAE/100 g), total flavonoid content (4.9 mg ECE/100 g), and radical scavenging activity (22.06 mM TE/100 g). Major essential minerals, indicating high potassium (334.00 ± 8.71 mg/100 g), calcium, magnesium, and essential trace elements (iron, boron, and manganese). Colorimetric (CIE L a* b*) and sensory evaluations (9-point hedonic scale) confirmed desirable visual appeal (L* = 28.9, a* = 5.8, b* = 11.01) and high overall acceptability (7.2 ± 0.4). In vivo trials, daily oral administration of Cocofee gummies to HFD-induced NAFLD rats over 30 days significantly (p < 0.05) mitigated body weight gain in a dose-dependent manner (3.7% reduction at 10 g/day). Crucially, high-dose gummy supplementation exerted strong hepatoprotective effects by reducing alanine aminotransferase (ALT) by 20.6% and alkaline phosphatase (ALP) by 14.5%. Overall, these findings showed cocofee gummies as a novel, highly palatable, and bioactive rich nutraceutical delivery system capable of attenuating hepatic dysfunction associated with NAFLD.*

1. Introduction

In recent years, non-alcoholic fatty liver disease (NAFLD) has attracted considerable attention. NAFLD is an umbrella term characterized by the accumulation of fat in the liver and is particularly prevalent among overweight and obese individuals. It develops in the absence of excessive alcohol consumption and is associated with multiple metabolic risk

factors (Del Bo' et al., 2023; Shabbir et al., 2026; Arif et al., 2026a). A recent meta-analysis encompassing 479 studies and 78,001,755 participants from 38 countries estimated the global prevalence of NAFLD at 30.2%, indicating that nearly one in three individuals worldwide is affected (Maqsood et al., 2025). Burden of NAFLD varies considerably across geographical regions, highest

Corresponding Author: Muhammad Asif & *, Anum Urooj

Email: asifrajab3251@gmail.com & anumurooj.uaf@gmail.com

prevalence in South America (34.0%), followed by Asia (30.9%), Europe (30.2%), and North America (29.0%), whereas Australia showed the lowest prevalence (16.1%). At the country level, Guatemala (60.7%), Qatar (58.5%), and Austria (51.6%) showed the highest prevalence, while Norway (5.0%), England (11.2%), and France (14.1%) reported the lowest prevalence (Amini-Salehi et al., 2024). Sex-specific global analyses revealed a greater disease burden among males (36.6%) than females (25.5%), while in children prevalence reached 16.2% in boys and 11.7% in girls (Bayram et al. 2021; Fazal et al., 2026). Burden of NAFLD among individuals is 57.5% in adults with obesity and 38.0% in children with obesity. Disease severity analysis among affected individuals, 57.3% had Grade 1 steatosis, 27.3% had Grade 2, and 8.9% had Grade 3 (Urooj et al., 2026a).

NAFLD does not have any FDA approved pharmacological treatments until 2020, hence, there is an immense unmet therapy need for it (Pathak et al., 2023). Moreover, to existing treatment options, some of the recent treatment strategies used to treat NAFLD involve the use of lifestyle modifications, which involve consumption of healthy food and exercises, as well as using the conventional drugs that could have anti-NAFLD properties when treating the metabolic syndromes (Campbell et al., 2021; Butt et al., 2026). According to recent findings, during the 18th century, apothecaries would be prescribing herbal medicines with sugar content in the form of candies to treat various diseases including digestive issues, while during the mid-19th century, pharmacist Oliver Chase came up with the first machine that was used to manufacture medicated candies (Thomas, 2023).

Now a days, concerning the consumers' desire for healthful food items that offer good quality and functionality (Naqvi et al., 2025); therefore, formulating food products to serve their particular nutritional and health needs (; Urooj et al., 2026b). Called as a skeleton structure of food tasty, convenient, and effective functional gummies that are regarded as a sweet delicacy are among the most rapidly emerging trends of dietary supplements in Europe, North America, and Asia (Chaudhry et al., 2026). Global jellies and gummies market size is estimated at USD 6665 million by 2023, registering a compound annual growth rate of 5.6% from 2023 to 2033 (Aiello et al., 2024; Arif et al., 2026). A dynamic evolution over the years can be ascribed to multiple factors such as aesthetically pleasing, unlimited

flavor possibilities and easy to chew and swallow (Székelyhidi et al., 2024; Arif et al., 2026b).

Stevia is a natural sweetener obtained from *Stevia rebaudiana* plant (Munteanu & Schwartz, 2023), whose influence on development of NASH needs to be determined in a study (Peteliuk et al., 2021). Rebaudioside A of Stevia showed significant improvement in liver enzymes, liver steatosis and liver fibrosis. As a result, use of Rebaudioside A instead of fructose and sucrose can guarantee the protection of liver, better glucose tolerance, lowering of liver fibrosis and reduction of inflammation by way of decreased oxidative stress (Iqbal et al., 2026). Overall, stevia has several benefits for NAFLD patients, which can be explained mainly due to the decrease of oxidative stress and hypoglycemic changes of gut microbiota (Casas-Grajales et al., 2019).

Coffee is one of the major sources of caffeine that acts as an appetite suppressant and is composed of bioactive ingredients in its composition (Domingues et al., 2023), high in nitrogen compounds, which include chlorogenic acid, caffeine, and trigonelline (Analinasari et al., 2024). Coffee consists of 50-60% carbohydrates, 15-20% lipids, 10-15% proteins, 3-5% minerals, and around 1% of caffeine (Yeager et al., 2023). Regular consumption of conventional coffee greatly decreases the incidence of progressive liver fibrosis in NAFLD patients (Bosso et al., 2023) through improved liver functions due to attenuation of serum liver parameters such as ALT and GGT. This is due to the reduced oxidative stress and inflammatory pathways (Freitas et al., 2024).

Gummies can be considered an effective and innovative way of administering drugs and active components (Herawati et al., 2024). The purpose of this research is the selection of functional ingredients for the development of gummies. Gummies replace the usual methods of oral drug delivery like tablets and capsules (Wang et al., 2024), as they provide greater possibilities to produce more palatable products (Nascimento et al., 2024). As for the inclusion of water-soluble functional ingredients into food delivery systems, gummy candy could prove extremely helpful (Vojvodić Cebin et al., 2024).

This study aimed to formulate and characterize a gelatin-based functional “Cocofee” gummy incorporating coffee, coconut, black pepper, stevia, orange juice, and lime, with particular emphasis on its nutritional, phytochemical, antioxidant, mineral, physicochemical, and sensory

Table 1. Experimental Composition of orange juice, coffee, black pepper, stevia and gelatin in cocofee gummies

Ingredients	Standardized quantity	Composition (% w/w)
Fresh Sweet Orange Juice (<i>Citrus sinensis</i>)	250.0 mL	87.52
Bovine Gelatin (Food Grade)	25.0 g	8.75
Fresh Lime Extract (<i>Citrus aurantifolia</i>)	10.0 mL	3.50
Roasted Coffee Bean Powder (<i>Coffea arabica</i>)	500.0 mg	0.17
Black Pepper Powder (<i>Piper nigrum</i>)	100.0 mg	0.04
Coconut Endosperm Flakes (<i>Cocos nucifera</i>)	50.0 mg	0.02
Stevia Leaf Powder (<i>Stevia rebaudiana</i>)	2.0 mg	0.001

attributes. Furthermore, to evaluate the therapeutic potential of developed functional gummy against high fat diet-induced NAFLD in Wistar albino rats by assessing treatment associated changes in body weight and key serum hepatic biomarkers, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

2. Materials and Methods

2.1. Sample collection

Fresh mature coconuts (*Cocos nucifera*), sweet oranges (*Citrus sinensis*), and limes (*Citrus aurantifolia*) were bought from agricultural markets in Faisalabad, Pakistan. Roasted coffee beans (*Coffea arabica*), black peppercorns (*Piper nigrum*), food-grade bovine gelatin, and stevia leaf powder (*Stevia rebaudiana*) were obtained from a commercial market to University of Agriculture, Faisalabad (UAF), Pakistan. All the raw materials were packed in perforated food-grade polyethylene bags and taken directly to the Functional and Nutraceutical Food Research Unit (FNFR), UAF.

2.2. Preparation of raw materials

The raw materials were prepared according to previously reported methods with slight modifications. The outer layer of coconut (husk) and brown testa were separating off to obtain inner white solid endosperm (coconut meat). The coconut meat was blanched in boiled distilled water for 3

min, drained, and crushed to flakes using a sterilized mechanical grater. Whole roasted coffee beans were selected, sorting, washing, rinsing, roasting, cooling, dried at 40 °C, ground to fine powder, sieved through a 200-mesh (75 µm) sieve, and stored in tightly sealed amber bottles at 4 °C according to Wu et al. (2022). Mature black pepper were cleansed, air dried in hot air at 50 °C for 6 hours, crushed in the laboratory using a pulverizing machine, sieved through 250 µm sieve, and then stored in light-proof polyethylene pouches at 4 °C as following the modified methods of Liu et al. (2018) and Urooj et al. (2026c). Stevia leaf powder was prepared according to Dilrukshi et al. (2024) by washing fresh leaves, air dried in shaded area (27 ± 2 °C; 60% RH) for 5 hours, dried in oven at 55 °C for 24 hours, crushed, and then sieved for a particle size range of 88-180 µm. Orange juice was prepared by washing, rinsing, peeling, deseeding, manually squeezing, and filtering fresh oranges through sterile muslin cloth and a fine-mesh sieve, with the clarified juice stored at 4 °C until gummy preparation. Similarly, lime extract was prepared according to Das et al. (2022) by washing, rinsing, peeling, cutting, mechanically extracting, and filtering *Citrus limon* juice through sterile muslin cloth to remove insoluble materials, followed by storage in screw capped glass bottles at 4 °C until further use.

2.3. Gelatin-based functional cocofee gummies making process

The cocofee gummies as illustrated in Figure 2, preparations were performed with minor modifications from the preparation procedure of functional gummy candies as described by Roudbari et al. (2024). Gelatin (25 g) was dissolved in 250 mL of fresh orange juice by boiling at 100 °C while continuously stirring until complete dissolution was attained. Powdered coffee (500 mg), black pepper powder (100 mg), stevia (2 mg), coconut husk (50 mg), and lime extract were mixed with the gelatin solution and well homogenized to ensure uniform dispersion of ingredients as outlined in Table 1. Mixture was poured in sterile silicon molds and left to cool at room temperature until an initial gelification took place. The gummies were then stored in a refrigerator at 4°C.

2.4. Nutritional composition

The nutritional composition of gummies was determined based on the respective AOAC methodologies (AOAC, 2019). The moisture content was determined gravimetrically by drying gummies in a hot air oven at 105±5°C for 24

hours and measuring the weight difference between dry and wet gummies. The fat contents were quantified using a Soxhlet apparatus (LEX 3030 F, LabTech, Korea) with 6 n-hexane cycles. The crude fiber content was measured by treating the gummies with H₂SO₄ and NaOH until acid and alkaline free samples were prepared using Labconco Fibertech (Kansas City, Missouri, USA). The protein content of gummies was estimated by acid digestion followed by applying Kjeldahl methodology using Kjeltec (Technich GmbH, Behr Labor, Germany) for determination of nitrogen content. The nitrogen content was multiplied with a conversion factor of 6.25 for estimation of protein contents. For the measurement of ash contents, the samples after acid and alkali treatments were charred in a muffle furnace (MF-1/02, PCSIR, Pakistan) at 600 ± 8°C for 5 hours. Total carbohydrate content was estimated by difference methods.

2.5. Phytochemical composition

2.5.1. Extraction of phytochemicals from gummies

For phytochemical analysis, gummy samples were extracted according to the method described by Asif et al. (2025a) with slight modifications. 6 g of sample was dissolved in 20 mL of methanol and agitated continuously for 45 min at room temperature, then centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant was carefully collected, filtered through a 0.45 µm polytetrafluoroethylene (PTFE) membrane filter, and stored at -80 °C until further analysis.

2.5.2. Determination of total phenolic content (TPC)

Total phenolic content (TPC) of gummy extracts was determined using the Folin-Ciocalteu (FC) colorimetric method following the procedure proposed by Naqvi et al. (2022) with slight modifications. 1 mL of methanolic gummy extract was added to 5 mL distilled water and 0.8 mL FC reagent in a 10 mL volumetric flask. After leaving it for 8 min at room temperature, continuously stirred, 1.2 mL of 20% (w/v) sodium carbonate (Na₂CO₃) was added. The resulting solution was then diluted to 10 mL by adding distilled water and incubated for 2 h at room temperature in darkness. Absorbance reading was taken at 760 nm with a UV-Visible spectrophotometer taking the reagent blank as control. Calibration was performed using gallic acid and expressed as mg GAE per 100 g of sample.

2.5.3. Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) was determined according to Asif et al. (2025b) as adopted in the study. 1 mL of gummy

methanol extract was taken and combined with 4 mL of distilled water. Then, 300 µL of 25% sodium nitrite (NaNO₂) was added to it. The reaction mixture was kept for incubation for 5 min at room temperature. Then, 300 µL of 10% aluminium chloride (AlCl₃) was added to it. Then, 2 mL of 4% sodium hydroxide (NaOH) and 2.4 mL of distilled water was added to the reaction mixture. The reaction mixture was left for standing for 10 min at room temperature. The absorbance of the reaction mixture was recorded on a UV-Visible spectrophotometer at 510 nm. Quantification was done using a standard calibration curve of epicatechin, and the values were expressed in mg epicatechin equivalent per 100 g of gummy sample (mg ECE/100 g).

2.5.4. Determination of antioxidant potential (DPPH Assay)

Antioxidant activity of gummy extract was evaluated using DPPH radical scavenging assay based on Roudbari et al. (2024). In DPPH assay, 40 µL of gummy extract (dilution 1:10) was added to 2960 µL of a 6 × 10⁻⁵ M DPPH methanolic solution. The resulting mixture was kept in dark for 30 min at room temperature. The absorbance of sample was then measured at 515 nm with the help of a UV-Vis spectrophotometer with methanol as blank. Trolox antioxidant was used for the preparation of a calibration curve, and antioxidant activity was reported in terms of mM TE/100 g of gummy.

$$\text{DPPH scavenging (mM TE/100 g)} = \frac{C \times DF \times V}{W} \times 100$$

where: C = Trolox equivalent concentration obtained from the calibration curve (mM); DF = dilution factor; V = extracted volume (L); W = weight of gummy sample (g)

2.6. Mineral composition

The mineral composition of gummies was analyzed by adopting methodology proposed by Székelyhidi et al. (2024), with minor adjustments. First, 0.2 g of each gummy sample was weighed in a microwave digestion vessel. Digestion of samples was conducted with 5 mL of concentrated nitric acid (HNO₃) and 2 mL of 30% (w/v) hydrogen peroxide (H₂O₂) through CEM MARS Xpress 6 One Touch microwave digestion system (CEM Corporation, USA) at 210 °C and 200 PSI. Upon completion of digestion, solutions were left to cool to ambient temperature and were diluted to a total volume of 20 mL with deionized water. Concentrations of macro- and microelements were measured using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Agilent 5110, Agilent

Table 2. Treatment plan for disease induced fatty liver disease rats

Gro up	Status	Experiment al Induction	Daily Treatment
G ₀	Negative Control	Non-induced	Normal
G ₁	Positive Control	NAFLD Induced	Normal
G ₂	Therapeutic Group	NAFLD Induced	5 g gummy per day
G ₃	Therapeutic Group	NAFLD Induced	10 g gummy per day

Technologies, USA) in simultaneous mode according to Tošić et al., 2015 Calibration standards were made through serial dilutions of a 1000 mg/L multi-element stock solution in 1% (w/w) HNO₃, while reagent blanks were prepared under the same conditions. ICP-OES was carried out using these parameters: RF power, 1.2-1.3 kW; plasma gas (Ar) flow rate, 15 L/min; auxiliary gas (Ar) flow rate, 1.5 L/min; viewing height, 5-12 mm; copy and reading time, 3 s. Concentrations of elements were measured in terms of their analytical wavelengths: potassium (766.491 nm), calcium (317.933 nm), magnesium (279.553 nm), iron (238.204 nm), manganese (257.610 nm), and boron (249.772 nm). The calibration curves having coefficients of determination R² = 0.999 were used for quantitative analysis of minerals.

2.7. Color analysis

The color analysis for functional gummies was done following the technique used by Boninsegna et al. (2024). Color analysis was done using the HunterLab colorimeter. The measurement of color was done at 3 points and was presented in the CIE L*a*b* color system; where L* stands for lightness, a* stands for the scale from red (+) to green (-) and b* stands for yellow (+) to blue (-). The change in color (ΔE)), chroma (C*) and whitening index (WI) were determined using the formula

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$
$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$
$$WI = \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

2.8. Sensory evaluation

Sensory assessment of cocofee gummies on the basis of their sensory parameters was conducted as per the procedure detailed by Fayyaz et al. (2026) and Asif et al. (2023) with minor alterations. Ten semi-trained panelists (5 males and 5 females ranging in age from 22-45 years) from National Institute of Food Science and Technology (NIFSAT), University of Agriculture Faisalabad, Pakistan, evaluated the products. Before starting the sensory evaluation process, the panelists were briefed about the sensory parameters and procedures for the evaluation. Appearance, texture (chewability), flavor, aroma, and overall acceptability of the gummy samples were assessed by the panelists using a 9-point hedonic scale, whereby 1 = dislike extremely and 9 = like extremely as the minimum score acceptable. Random 3-digit numbers were assigned to the samples which were given to the panelists in a randomized order in similar environmental conditions.

2.9. Ethical approval and induction of disease

Twelve male albino Wistar rats (n = 3) (age: 8-10, weeks: 185 ± 20 g) were acquired from the Central Animal Facility, University of Agriculture, Faisalabad (UAF), Pakistan. The rats were individually housed in polypropylene cages containing sterile polycarbonate bedding, with all environmental factors kept strictly regulated (temperature: 25 ± 2 °C; relative humidity: 50 ± 10%; light-dark cycle: 12/12 h; lights on at 07:00). All procedures were performed as per guidelines and were ethically approved by the Institutional Biosafety and Bioethics Committee of UAF (Approval No. 694/ORIC). After the acclimation period, rats belonging to G₁, G₂ and G₃ were provided experimental HFD on free choice basis for 4 weeks to induce fatty liver disease. Rats from normal group (G₀) were fed normal diet. Induction of NAFLD was confirmed on day 0.

2.10. Preparation of high-fat diet

The experimentally formulated HFD followed the standard formula provided in Table 3. All raw materials were blended together to provide an accurate percentage of w/w of 100% weight in all material and preparation of diet was done in the pellet form once in a week with slight modifications according to the methods of Urooj et al. (2026d).

2.11. Body weight

Body weight of each mouse was measured at day 0, day 15, and day 30 with the use of calibrated digital balance as per the suggestion of Urooj et al (2026c), and then computed using the following formula:

Table 3. High-fat diet formulation recipe

Ingredients	Quantity (% w/w)
Corn Starch	60.00
Casein	4.30
Mineral Mixture (AIN-93G-MX)	1.70
Corn Oil	6.90
Vanaspati Ghee	8.60
Vitamin Mixture (AIN-93-VX)	0.86
Carboxymethyl Cellulose (CMC)	4.30
Wheat Bran	4.40
Refined Cane Sugar (Sucrose)	8.50
Cholic Acid	0.45

$$\text{Body weight gain (\%)} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100\%$$

2.12. Determination of liver markers

The blood samples were collected on three pre-defined days: day 0, day 15, and day 30 after overnight 12-hour fasting, as illustrated in Fig. 3. The samples were centrifuged at 3000 rpm for 10 minutes to obtain the serum which was kept at -20°C until biochemical tests were done. Levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were quantified using kinetic IFCC without pyridoxal phosphate method (Catalog No. ALT-1120, BioSystems S.A., Spain) as described by Begum et al. (2025). AST was quantified using kinetic UV IFCC method (Catalog No. AST-1130, BioSystems S.A., Spain). Levels of alkaline phosphatase (ALP) were quantified using recommended DGKC colorimetric kinetic p-nitrophenylphosphate (p-NPP) (Catalog No. ALP-1040, BioSystems S.A., Spain).

2.13. Statistical analysis

All assays and animal experiment readings were taken in triplicates and reported in terms of mean $\pm$ standard deviation (SD). Statistical analysis was done by using Statistix 8.1 software, where Two-Way Factorial ANOVA was used to evaluate the efficacy (Montgomery, 2017). The mean difference between the groups and time intervals were determined using Tukey's HSD test at $p < 0.05$.

3. Results and Discussions

3.1. Nutritional composition

In nutritional composition of cocofee gummies Nitrogen-free extract (NFE) was the predominant component ($57.84 \pm 0.98\%$), followed by moisture ($27.28 \pm 0.12\%$), crude fat ($5.90 \pm 0.95\%$), crude protein ($3.60 \pm 0.05\%$), crude fiber ($2.78 \pm 0.01\%$), and ash ($2.60 \pm 0.18\%$), as presented in Figure 4. Given the high value of NFE, the gummies demonstrated to consist of highly digestible carbohydrates that can provide energy, while the presence of moisture explains the chewy nature of the gummies. Crude fat, protein, and fiber result from the use of coconut husks, coffee, and black pepper in the preparation process, while the ash content results from the minerals present in the mixture of coconut husks, coffee, and orange juice. The observed proximate composition is consistent with previous findings on coffee-containing products and other plant ingredients. Stanek et al. (2021) found protein content between 3-5% in coffee products, a figure that is in accordance with the current study results. Lin et al. (2024) recorded ash content between 3.5-5.5% in roasted coffee beans, while Freitas et al. (2024) found roasted coffee containing 10-15% protein after roasting. According to Le et al. (2024), roasting causes a slight reduction in the amount of crude fiber because of the breakdown of fiber components through heat treatment. It is evident from the



Figure 1. Raw materials for the formulation of cocofee gummies powder. (a) Coffee beans, (b) Coconut husk, (c) Black pepper, (d) Stevia, (e) Gelatin



Figure 2. Pictorial representation of coffee gummies



Figure 3. Collection of blood sample for serum biochemical parameters

results above that the addition of coffee, coconut husk, black pepper, and orange juice leads to the formation of nutritionally improved gummies containing carbohydrates, dietary fiber, proteins, and minerals.

3.2. Phytochemical analysis

The phytochemical study revealed the presence of a relatively high amount of bioactive phytochemicals having significant antioxidant activity in functional cocofee gummies. The TPC content was 24.1 mg GAE/100 g of the sample, while TFC was 4.9 mg ECE/100 g. Antioxidant activity measured via DPPH test was 22.06 mM TE/100 g of gummy sample, as shown in Figure 5. High phenolic and flavonoid content can be explained by the addition of such ingredients as roasted coffee, black pepper, and orange juice because all these ingredients contain natural antioxidants. Roasted coffee is rich in CGAs, which are mainly responsible for the antioxidant activity, while black pepper and citrus fruits provide additional flavonoids and polyphenols. The obtained phytochemical content is comparable to that in coffee and coffee-based products reported previously in scientific literature. El-Sayed and

Youssef (2019) state that chlorogenic acids are the main phenolic compounds responsible for the high antioxidant activity of coffee. The same way, Masek et al. (2020) found the phenolic content in brewed coffee to vary between 100-300 mg GAE/L depending on the type of coffee used, brewing method, and extraction conditions. Górecki and Hallmann (2020) recorded flavonoid contents in roasted coffee beans in a range of 1-3 mg catechin equivalents/g, while Tsai and Jioe (2021) noted that light roasting retains higher contents of phenolic and flavonoid compounds compared to dark roasting. Similar results were reported by Muzykiewicz-Szymańska et al. (2021), who found that

Table 4. Effect of cocofee gummies on body weight (g) of rats

Groups	Day 0	Day 15	Day 30
G ₀	183 ± 0.753 ^c	180 ± 0.042 ^a	178 ± 0.042 ^a
G ₁	190 ± 0.292 ^b	191 ± 0.003 ^b	192 ± 0.954 ^b
G ₂	192 ± 0.854 ^a	190 ± 0.933 ^c	188 ± 0.833 ^c
G ₃	188 ± 0.744 ^c	185 ± 0.892 ^d	181 ± 0.022 ^d

Table 5. Effect of cocofee gummies on ALT, ALP and AST against NAFLD

Groups	Day 0	Day 15	Day 30
ALT (U/L)			
G ₀	54.0 ± 0.93 ^c	52.0 ± 0.89 ^c	51.0 ± 0.05 ^d
G ₁	69.0 ± 0.85 ^{ab}	70.0 ± 0.04 ^a	71.0 ± 0.05 ^a
G ₂	71.0 ± 0.98 ^a	66.0 ± 0.95 ^b	61.0 ± 0.49 ^b
G ₃	68.0 ± 0.84 ^b	67.0 ± 0.74 ^b	54.0 ± 0.74 ^c
ALP (U/L)			
G ₀	119.0 ± 0.74 ^b	114.0 ± 0.73 ^b	104.0 ± 0.42 ^b
G ₁	118.0 ± 0.85 ^c	116.0 ± 0.32 ^a	108.0 ± 0.73 ^a
G ₂	120.0 ± 0.64 ^a	112.0 ± 0.85 ^c	103.0 ± 0.75 ^c
G ₃	117.0 ± 0.32 ^d	111.0 ± 0.59 ^d	100.0 ± 0.58 ^d
AST (U/L)			
G ₀	80.0 ± 0.72 ^a	71.0 ± 0.98 ^c	68.0 ± 0.84 ^a
G ₁	78.0 ± 0.00 ^b	73.0 ± 0.03 ^a	63.0 ± 0.92 ^c
G ₂	75.0 ± 0.07 ^d	70.0 ± 0.32 ^d	67.0 ± 0.74 ^b
G ₃	77.0 ± 0.58 ^c	72.0 ± 0.49 ^b	63.0 ± 0.64 ^c

roasted coffee shows strong antioxidant activity as DPPH radicals scavenger due to a combination of chlorogenic acids, melanoidins, and other polyphenolic compounds. Therefore, the use of roasted coffee with black pepper and orange juice increased the bioactive compound content of the gummies and made them a functional confectionery product.

3.3. Mineral composition

Composition of minerals in cocofee gummies is depicted in Figure 6. In terms of macroelements, potassium had the highest concentration of 334.00 ± 8.71 mg/100g followed by calcium (24.89 ± 1.51 mg/100 g) and magnesium (11.42 ± 0.68 mg/100 g). As for microelements, iron had the highest concentration of 0.49 ± 0.01 mg/100 g followed by boron (0.43 ± 0.03 mg/100 g) and manganese (0.12 ± 0.01 mg/100 g). High levels of potassium content in cocofee gummies are mainly due to addition of fresh orange juice and coconut containing substances which are good sources of potassium. Potassium being an electrolyte can help prevent metabolic syndrome and NAFLD. Similarly, calcium plays a vital role in intracellular signaling, lipid metabolism, whereas magnesium serves as a cofactor for more than 300 enzymatic reactions. Magnesium deficiency has frequently been associated with chronic inflammation and hepatic steatosis (Kaviya, 2023). Concentrations of trace minerals further enhance the nutritional value of the formulated gummies. Iron is indispensable for oxygen transport and numerous

oxidation-reduction reactions, while manganese functions as an essential cofactor of mitochondrial manganese superoxide dismutase (MnSOD), a key antioxidant enzyme protecting hepatocytes against oxidative damage. Boron has also gained considerable attention due to its anti-inflammatory properties. The present findings are consistent with previous studies reporting appreciable mineral concentrations in fruit-based functional confectionery products. Roudbari et al. (2024) demonstrated that antioxidant rich functional gummy candies formulated with natural ingredients retained considerable amounts of essential minerals, particularly potassium and magnesium, owing to the use of fruit matrices.

3.4. Color analysis

Color characteristics of functional cocofee gummies are presented in Figure 7. The cocofee gummies exhibited L*, a*, and b* values of 28.9, 5.8, and 11.01, respectively. The low L* value indicated a dark appearance of the gummies, while the positive a* and b* values indicated slight redness and yellowness in the formulation. The dark brown color of the cocofee gummies could be associated with presence of coffee and the cooking process during preparation. Coffee is a source of melanoidins along with other brown-colored compounds that form upon roasting and lead to the dark color of products based on coffee. The dark color of the gummies might also result from the Maillard reaction and caramelization at 80°C. Such findings were reported by Iriondo-DeHond et al. (2021) as the higher cooking temperature resulted in negative effects on color properties leading to darkening. Cocofee gummies had C* value of 25.83 ± 1.7 , implying moderate color intensity. The C* is responsible for the vividness of the product color. The relatively low value of C* might be associated with the use of the coating of coconut husk that decreased the brightness of the color. Such results were confirmed by Kingwascharapong et al. (2020). The Whitening Index (WI) of cocofee gummies was 12.14 ± 1.9 , implying that the product possessed a relatively low level of whitening. This was not surprising since coffee products are known to possess a brown color due to the formation of melanoidins from roasted coffee components. Unlike the whitening products, coffee products do not whiten since roasting causes the formation of melanoidins which results in the dark color of the products. The dark color of the gummies may have been brought about by the presence of coconut husks and black pepper in the product. Color difference

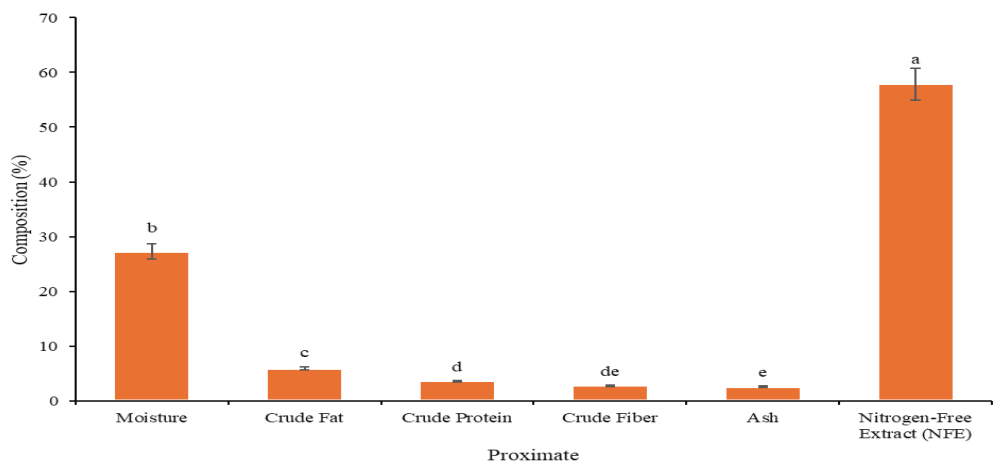


Figure 4. Proximate composition (%) of the developed functional cocofee gummy

(ΔE) of cocofee gummies was 23, implying that there was a noticeable color difference in the product. ΔE represents the amount of color difference visible to the human eye. The ΔE observed could be a result of ingredients, roasting of coffee and thermal processing of gummy products.

3.5. Sensory acceptability

The sensory acceptability of functional cocofee gummies indicated that consumers had high acceptability for all analyzed sensory parameters such as appearance, texture, taste, smell, and acceptability. Among the sensory characteristics, appearance received the highest hedonic score (8.8 ± 0.3), followed by texture (7.9 ± 0.6), overall acceptability (7.2 ± 0.4), taste (6.6 ± 0.62), and aroma (5.2 ± 0.4) as presented in Figure 8. This high appearance score is due to the dark brown color contributed by roasted coffee and the coconut covering, whereas a favorable texture could be attributed to optimal gelatin level and moisture level,

giving rise to a soft and chewy texture. The moderate scores for taste and aroma are likely due to the inherent bitterness of roasted coffee and the volatiles present, along with the pungent flavor of black pepper. However, the acceptability score indicated good acceptability of the prepared gummies among consumers. This result is supported by previous literature on functional gummies. In their study, Shende et al. (2020) found that appearance, taste, and texture are the key factors responsible for consumer acceptance of gummy confections. Likewise, Santos et al. (2022) showed that the cooking temperature and moisture level used during the processing of gummies have a significant impact on their texture and consumer acceptance. Roudbari et al. (2024) also found that gelatin-based gummies possess a smooth, elastic, and chewable texture. Moreover, as noted by Zakidou et al. (2021) reported that roasted coffee aroma comprises complex fruity, floral, nutty, spicy, and caramelized volatile compounds that influence consumer preference. These findings suggest that incorporation of roasted coffee, coconut husk, black pepper, and orange juice produced a sensory acceptable functional gummy with desirable appearance, texture, and overall acceptability, supporting its potential as a novel health promoting confectionery product.

3.6. Efficacy trials

3.6.1. Effect of cocofee gummies on body weight

Results related to the effect of cocofee gummies on body weight are shown in Table 3. Results revealed that the disease control group (G_1) showed a slight increase in body weight (192.0 ± 0.95 g) compared with the normal control group (G_0 ; 178.0 ± 0.04 g) ($p < 0.01$). Treatment with cocofee gummies resulted in a significant, dose-dependent reduction in body weight, with the 10 g cocofee gummy group (G_3) showing the lowest final body weight ($181.0 \pm$

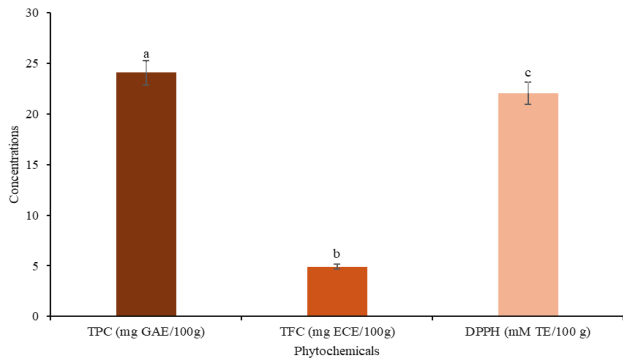


Figure 5. Antioxidants and phytochemical properties of the developed cocofee gummy

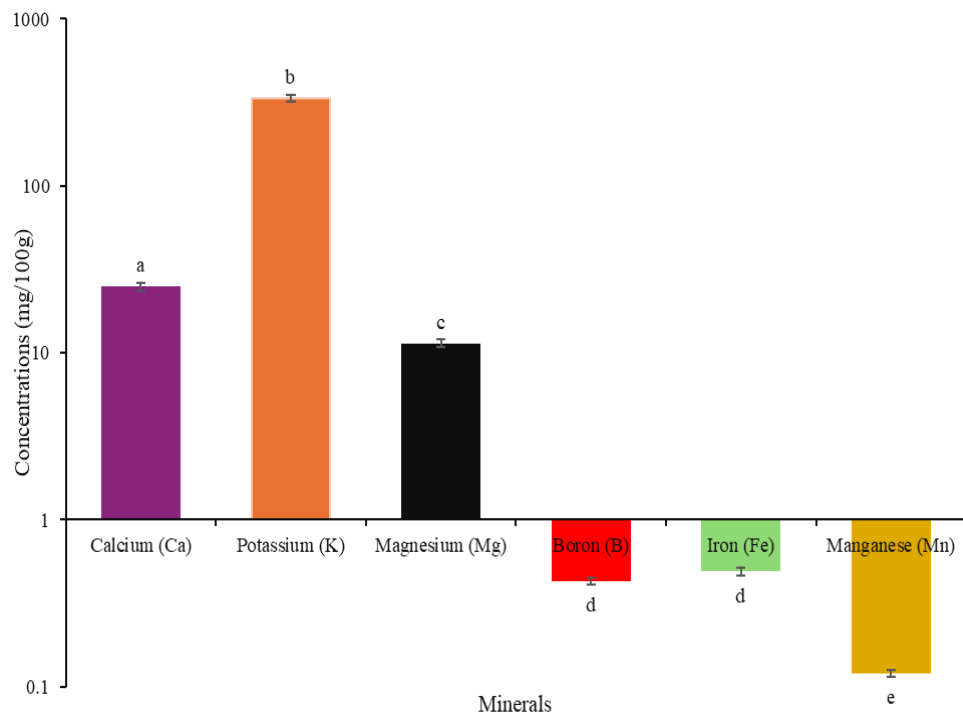


Figure 6. Mineral composition of the developed cocofee cocofee gummy

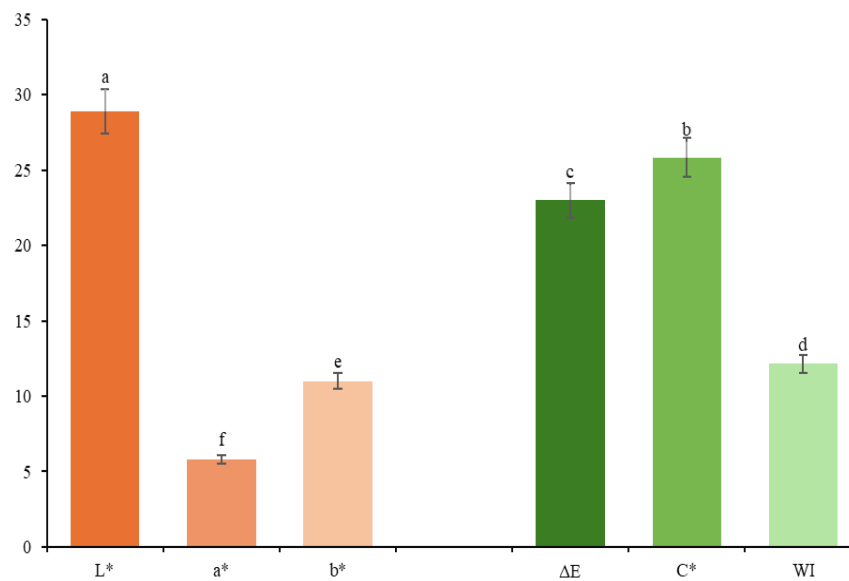


Figure 7. Color characteristics of the developed functional cocofee gummy

0.02 g), followed by the 5 g cocofee gummy group (G_2 ; 188.0 ± 0.83 g), indicating significant improvement compared with the disease control group ($p < 0.05$). The reduction in body weight attributed to the presence of

coffee-derived bioactive compounds, particularly caffeine, chlorogenic acid, and dietary fiber, which have been reported to improve lipid metabolism, increase energy

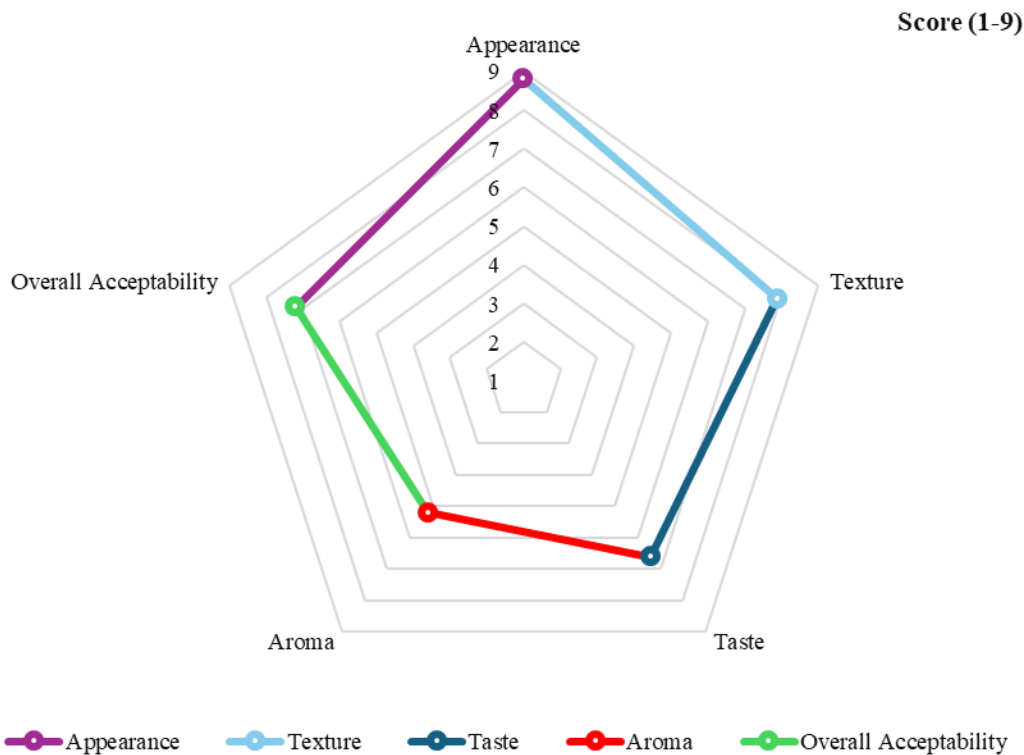


Figure 8. Sensory evaluation of the developed functional cocofee gummy

expenditure, and reduce fat accumulation (Wang et al., 2023).

3.6.2. Effect of cocofee gummies on alanine transaminase (ALT)

As shown in Table 5, cocofee gummy supplementation significantly ($p < 0.01$), influenced serum ALT concentrations throughout the experimental period. Normal control group (G_0) showed a slight reduction in ALT levels from 54.0 ± 0.93 U/L on day 0 to 51.0 ± 0.05 U/L on day 30. In contrast, the disease control group (G_1) showed a gradual increase in ALT concentration from 69.0 ± 0.85 U/L to 71.0 ± 0.05 U/L, indicating persistent hepatocellular injury in untreated NAFLD rats. Treatment with cocofee gummies resulted in a significant reduction in ALT concentration for all treatment groups. Animals administered with 5 g cocofee gummies (G_2) demonstrated a progressive reduction in ALT levels, ranging from 71.0 ± 0.98 U/L to 61.0 ± 0.49 U/L, while the highest reduction in ALT was seen in the case of 10 g cocofee gummies (G_3) from 68.0 ± 0.84 U/L to 54.0 ± 0.74 U/L by the end of the study period. The reduction of ALT in the treatment groups

may be explained by the effects of hepatoprotection due to bioactive compounds contained in coffee, such as chlorogenic acid, caffeine, and polyphenols, that are known to decrease inflammation, oxidative stress, and lipids within the liver (Calabrò et al., 2020).

3.6.3. Effect of cocofee gummies on alkaline phosphatase (ALP)

Findings about the effect of cocofee gummies on the level of serum alkaline phosphatase (ALP) have been shown in Table 5. Significant influence ($p < 0.05$) of cocofee gummies on the levels of serum ALP has been observed in the duration of experiment. In G_0 , the reduction in ALP level has been observed from 119.0 ± 0.74 U/L on day 0 to 104.0 ± 0.42 U/L on day 30. Likewise, in G_1 , reduction in ALP level has been comparatively low from 118.0 ± 0.85 U/L to 108.0 ± 0.73 U/L, showing the continuous impairment of liver function in untreated NAFLD rats. The use of cocofee gummies caused higher reduction in the level of serum ALP. Rats that were treated with 5 g cocofee gummies (G_2) had reduction in ALP from 120.0 ± 0.64 U/L to 103.0 ± 0.75 U/L. However, maximum reduction has been noted in

10 g cocofee gummies group (G_3) in which reduction in ALP from 117.0 ± 0.32 U/L to 100.0 ± 0.58 U/L. From the above results, we can say that cocofee gummies enhanced the health of the liver because of decreased ALP levels, with the highest dosage having the best impact against NAFLD-associated liver lipids. The consumption of cocofee gummies resulted in reduced levels of ALP due to the dual effects of cocoa flavonoids and coffee chlorogenic acid that inhibit oxidative stress and lipid peroxidation in the liver (Li et al., 2018). These compounds also enhance gut barrier integrity to prevent endotoxin release into the liver.

3.6.4. Effect of cocofee gummies on aspartate aminotransferase (AST)

The findings concerning the impact of cocofee gummies on serum aspartate aminotransferase (AST) were provided in Table 5. It was shown that cocofee gummy consumption significantly influenced serum AST concentrations during the experimental period. The normal control group (G_0) showed a reduction in AST levels from 80.0 ± 0.72 U/L on day 0 to 68.0 ± 0.84 U/L on day 30. Similarly, the disease control group (G_1) showed a reduction from 78.0 ± 0.00 U/L to 63.0 ± 0.92 U/L. Treatment with cocofee gummies also resulted in a lowering of the serum AST levels in both test groups. The 5 g cocofee gummies (G_2) treated rats experienced AST lowering from 75.0 ± 0.07 U/L to 67.0 ± 0.74 U/L while 10 g cocofee gummies treated (G_3) experienced lowering of AST from 77.0 ± 0.58 U/L to 63.0 ± 0.64 U/L with more effect observed in 10 g cocofee gummy group. These results corroborate previous in vivo studies assessing the role of bioactive polyphenols in hepatic steatosis. Begum et al. (2025) reported that the high flavanol cocoa based formula and the administration of coffee with high polyphenols have been shown to lower serum transaminase enzymes (AST & ALT) through the process of scavenging reactive oxygen species (ROS), stimulating endogenous antioxidant enzymes (SOD & CAT) and lipogenesis inhibition in high fat diet animal models (Dimeji et al., 2024). It is clear from the lowered AST level of 10 g cocofee gummies that the combination of coffee and cocoa polyphenols prevents hepatocyte membrane destruction and the subsequent leaking of AST enzymes into the blood.

Conclusion

The innovative formulation, characterization, and in vivo therapeutic assessment of a new functional botanical "Cocofee" gummy was achieved. Using the synergy of

coffee, coconut, black pepper, stevia, and citrus bases, the novel chewing delivery system contains a rich pool of dietary polyphenols, natural antioxidants, and macro- and microelements. Due to an optimized gelatin-based matrix, functional bioactive were successfully retained resulting in high physical stability, pleasant visual properties, and good sensory acceptability of the product. In vivo studies have shown that oral administration of cocofee gummies can attenuate HFD-induced changes in Wistar albino rats. Supplementation with gummies led to a dose and time-dependent reduction of body weight gain and a significant reduction in serum hepatic markers (ALT, AST, and ALP). Therefore, Cocofee gummies can be considered a convenient and efficient nutraceutical product that possesses both consumer appeal and specific metabolic effects.

Authors Contribution

Tooba Arif: Conceptualization, Methodology, Writing - original draft, Mahnoor Saleh: Validation, Formal analysis, Writing - original draft, Ramisa Maryam: Data curation, Methodology, Ayesha Saddiqa: Investigation, Formal analysis, Javaria Ashfaq: Validation, Methodology, Maheen Sabir: Formal analysis, Data curation, Baila Ahmad: Validation, Writing - review & editing, Esha Arif: Investigation, Data curation, Muhammad Asif: Conceptualization, Methodology, Supervision, Writing - review & editing, Anum Urooj: Conceptualization, Methodology, Supervision, Writing - review & editing.

Conflict of Interest

Authors declared that there were no conflicts of interest.

Funding

This research has received no funding towards the conduct, authorship, or publication of this manuscript.

References

- Aiello, F., Caputo, P., Rossi, C. O., Restuccia, D., & Spizzirri, U. G. (2024). Formulation of antioxidant gummies based on gelatin enriched with citrus fruit peels extract. *Foods*, 13(2), Article 320.
- Amini-Salehi, E., Letafatkar, N., Norouzi, N., Joukar, F., Habibi, A., Javid, M., Sattari, N., Khorasani, M., Farahmand, A., Tavakoli, S., Masoumzadeh, B., Abbaspour, E., Karimzad, S., Ghadiri, A., Maddineni, G., Khosousi, M. J., Faraji, N., Keivanlou, M.-H., Mahapatro, A., ... Nayak, S. S. (2024). Global prevalence of nonalcoholic fatty liver disease: An updated review meta-analysis comprising a population of 78 million from 38 countries. *Archives of Medical Research*, 55, Article 103043.
- Analinasari, A., Berliana, D., & Shintawati, S. J. (2024). Defects of coffee beans with different postharvest processes and roasting temperatures on volatile compounds of coffee beans from coffee small-scale

- industries of West Lampung, Indonesia. *Trends in Sciences*, 21, Article 7695.
- AOAC International. (2019). *Official methods of analysis of AOAC International* (21st ed.). AOAC International.
- Arif, M. R., Hafeez, R., Shabbir, H. A., Fatima, K., Yousaf, S., Usman, A., Fatima, A., Urooj, A., & Asif, M. (2026). Formulations and optimization of snacks enriched with mango seed kernel and egg shell powders to modulate satiety for obesity management. *Food and Nutrition Insights*, 4(1), 37–52.
- Arif, T., Saddiqa, A., Butt, M. S., Asghar, A., Iqbal, M., Urooj, A., Butt, H. D., & Ahmad, M. (2026a). Formulation, nutritional, and phytochemical characterization of functional tomato condiment fortified with *Mentha piperita* (peppermint) and *Piper nigrum* (black pepper). *Indus Journal of Bioscience Research*, 3(5), Article 1287.
- Arif, T., Urooj, A., & Shafqat, A. (2026b). Development, characterization, and therapeutic evaluation of functional cocofee gummies for type 2 diabetes management in Wistar albino rats. *Policy Research Journal*, 4(6), 77–78.
- Asif, M., Abbas, M., Fatima, K., Ahmad, N., Ahmed, S., Meghwar, P., Hosseini, E., Nasir, A., & Hassan, S. A. (2025a). Innovative corn extrudates incorporating barley-oat flour blends: Nutritional, functional, and sensory evaluation. *Applied Food Research*, Article 100812.
- Asif, M., Khan, M. K. I., Khan, M. I., Maan, A. A., Helmick, H., & Kokini, J. L. (2023). Effects of citrus pomace on mechanical, sensory, phenolic, antioxidant, and gastrointestinal index properties of corn extrudates. *Food Bioscience*, 55, Article 103012.
- Asif, M., Maan, A. A., Nazir, A., Khan, M. I. M., & Khan, M. K. I. (2025b). Effect of chickpea on the physicochemical, nutritional, antioxidant, and organoleptic characterization of corn extrudates. *Journal of the Science of Food and Agriculture*, 105(3), 2059–2067.
- Bayram, H. M., Majoo, F. M., & Ozturkcan, A. (2021). Polyphenols in the prevention and treatment of non-alcoholic fatty liver disease: An update of preclinical and clinical studies. *Clinical Nutrition ESPEN*, 44, 1–14.
- Begum, Q., Begum, M., Tabassum, A., & Mahboob, T. (2025). Effect of green tea, coffee, and cocoa powder administration on antioxidant enzymes of liver in experimental rat model. *Indus Journal of Bioscience Research*, 3(6), 252–260.
- Bosso, H., Barbalho, S. M., De Alvares Goulart, R., & Otoboni, A. M. (2023). Green coffee: Economic relevance and a systematic review of the effects on human health. *Critical Reviews in Food Science and Nutrition*, 63, 394–410.
- Butt, H. D., Gill, P., Kubra, K. T., Ahmad, B., Urooj, A., & Arif, M. R. (2026). Development and characterization of nutrient-dense oat-based energy crackers for combating malnutrition. *Policy Research Journal*, 4(4), 530–546.
- Calabrò, A., Procopio, A. C., Primerano, F., Larussa, T., Luzza, F., Di Renzo, L., De Lorenzo, A., Gualtieri, P., & Abenavoli, L. (2020). Beneficial effects of coffee in non-alcoholic fatty liver disease: A narrative review. *Hepatoma Research*, 6, Article 69.
- Campbell, P., Symonds, A., & Barritt, A. S., IV. (2021). Therapy for nonalcoholic fatty liver disease: Current options and future directions. *Clinical Therapeutics*, 43(3), 500–517.
- Casas-Grajales, S., Reyes-Gordillo, K., Cerda-García-Rojas, C. M., Tsutsumi, V., Lakshman, M. R., & Muriel, P. (2019). Rebaudioside A administration prevents experimental liver fibrosis: An in vivo and in vitro study of the mechanisms of action involved. *Journal of Applied Toxicology*, 39(8), 1118–1131.
- Chaudhry, A., Zahoor, Z., Batool, H., Fatima, A., Fatima, A., Ahmad, B., & Urooj, A. (2026). Quantifying the influence of *Hibiscus sabdariffa* (roselle) on compositional stability and sensorial attributes of locally food formulations for weaning age. *The Research of Medical Science Review*, 4(3), 1639–1650.
- Das, S., Deka, A., & Saikia, K. K. (2022). Comparative analysis of bioactive and physiochemical properties of Citrus limon Burm and Citrus aurantifolia juice. *International Journal of Scientific Development and Research*, 7(12), 984–989.
- Del Bo', C., Perna, S., Allehdan, S., Rafique, A., Saad, S., AlGhareeb, F., Rondanelli, M., Tayyem, R. F., Marino, M., Martini, D., & Riso, P. (2023). Does the Mediterranean diet have any effect on lipid profile, central obesity and liver enzymes in non-alcoholic fatty liver disease (NAFLD) subjects? A systematic review and meta-analysis of randomized control trials. *Nutrients*, 15(10), Article 2250.
- Dilrukshi, H. M. W., Ulpatakumbura, B. S. K., Gunarathna, K. M. R. U., Iqbal, M. C. M., Lai, O. M., & Marikkar, J. M. N. (2024). Evaluation of Stevia leaf (*Stevia rebaudiana* Bertoni) powder as sugar-substitute in cashew nut fortified cookies. *Ceylon Journal of Science*, 53(4), 599–608.
- Dimeji, I. Y., Gujja, G. M., Adeoye, S. W., Jabba, H. L., Jibrin, S., & Baba, Z. M. (2024). Flavonoid-rich cocoa extract alleviates oxidative stress, inflammation and liver dysfunction induced by high-fat diets in Wistar rats. *Research Journal of Phytochemistry*, 18(1), 20–33.
- Domingues, D. S., Ramalho, J. C., & Partelli, F. L. (2023). Coffee: From plant to cup. *Plants*, 13, 2346–2352.

- El-Sayed, S. M., & Youssef, A. M. (2019). Potential application of herbs and spices and their effects in functional dairy products. *Heliyon*, 5(6), Article e01989.
- Fayyaz, F., Fatima, A., Bakht, N., Anwer, M., Khan, A., Hussain, M., Saddique, K., & Asif, M. (2026). Influence of sorghum flour substitution on cooking, textural, and sensory properties of pasta. *Journal of Physical Education, Health and Social Sciences*, 4, 893–904.
- Fazal, A., Butt, H. D., Hira, Z., Mazhar, U., Ashraf, A., Mushtaq, H., & Urooj, A. (2026). Formulation and functional evaluation of protein enriched herbal antidiabetic bar for glycemic control. *Policy Research Journal*, 4(5), 10–11.
- Freitas, V. V., Borges, L. R., Castro, G. A. D., Almeida, L. F., Crepalde, L. T., dos Barros Kobi, H., Vidigal, M. C. T. R., dos Santos, M. H., Fernandes, S. A., & Maitan-Alfenas, G. P. (2024). Influence of roasting levels on chemical composition and sensory quality of Arabica and Robusta coffee: A comparative study. *Food Bioscience*, 59, Article 104171.
- Górecki, M., & Hallmann, E. (2020). The antioxidant content of coffee and its in vitro activity as an effect of its production method and roasting and brewing time. *Antioxidants*, 9(4), Article 308.
- Herawati, D., Armawan, M. S., Nurhaliza, N., Mu'arij, F. A., Hunaefi, D., & Noviasari, S. (2024). Impact of bean origin and brewing methods on bioactive compounds, bioactivities, nutrition, and sensory perception in coffee brews: An Indonesian coffee gastronomy study. *International Journal of Gastronomy and Food Science*, 35, 100892–100901.
- Iqbal, S., Urooj, A., Afzal, M., Bashir, A., Rasheed, M. M., Khan, M., Asif, M., Arshad, N., & Fatima, A. (2026). Nutritional, phytochemical, and sensory characterization of gluten-free cookies developed from rice, chickpea, and banana peel composite flour. *Policy Research Journal*, 4(3), 502–516.
- Iriondo-DeHond, A., Rodríguez Casas, A., & del Castillo, M. D. (2021). Interest of coffee melanoidins as sustainable healthier food ingredients. *Frontiers in Nutrition*, 8, Article 730343.
- Kaviya, T. (2023). Development of nutraceutical jelly gummies from amla (*Phyllanthus emblica* L.) using natural sugar sources. *International Journal of Food Science and Nutrition*, 10, 279–301.
- Kingwascharapong, P., Arisa, K., Karnjanapratum, S., Tanaka, F., & Tanaka, F. (2020). Effect of gelatin-based coating containing frog skin oil on the quality of persimmon and its characteristics. *Scientia Horticulturae*, 260, Article 108864.
- Le, B. X. N., Van Thach, P., Phan, Q. K., Pham, G. B., Pham, Q. H., & Do, A. D. (2024). Coffee husk by-product as novel ingredients for cascara kombucha production. *Journal of Microbiology and Biotechnology*, 34(3), 673–680.
- Li, S., Tan, H. Y., Wang, N., Cheung, F., Hong, M., & Feng, Y. (2018). The potential and action mechanism of polyphenols in the treatment of liver diseases. *Oxidative Medicine and Cellular Longevity*, 2018, Article 8394818.
- Lin, Y.-W., Lu, W.-C., Liao, C.-H., & Huang, P.-H. (2024). Innovative coffee filter cups enhance pour-over coffee quality while providing bioactive benefits: Challenges and opportunities. *Journal of Agriculture and Food Research*, 15, Article 101024.
- Liu, H., Zheng, J., Liu, P., & Zeng, F. (2018). Pulverizing processes affect the chemical quality and thermal property of black, white, and green pepper (*Piper nigrum* L.). *Journal of Food Science and Technology*, 55(6), 2130–2142.
- Maqsood, S., Asif, M., Alvi, T., Shakoore, S., & Zahir, A. (2025). Probiotics and functional foods in metabolic disease: Emerging solutions for obesity and diabetes. *Journal of Diabetes & Metabolic Disorders*, 24, Article 185.
- Masek, A., Latos-Brozio, M., Kałużna-Czaplińska, J., Rosiak, A., & Chrzęścijańska, E. (2020). Antioxidant properties of green coffee extract. *Forests*, 11(5), Article 557.
- Montgomery, D. C. (2017). Design and analysis of experiments (9th ed.). John Wiley & Sons.
- Munteanu, C., & Schwartz, B. (2023). The effect of bioactive aliment compounds and micronutrients on non-alcoholic fatty liver disease. *Antioxidants*, 12(4), Article 903.
- Muzykiewicz-Szymańska, A., Nowak, A., Wira, D., & Klimowicz, A. (2021). The effect of brewing process parameters on antioxidant activity and caffeine content in infusions of roasted and unroasted Arabica coffee beans originated from different countries. *Molecules*, 26(12), Article 3681.
- Naqvi, S. N. U. A., Anwar, A., Almas, I., Khalid, I., Muzammal, S. F., Urooj, A., & Arif, A. (2025). Exploring the storage effect on the physicochemical and microbiological properties of peach-plum juice. *RADS Journal of Biosciences*, 4(1), 12–19.
- Naqvi, S. N. U. A., Khadim, M. A., Muzammal, S. F., Gul, S. A., Ahmad, B. A., Urooj, A., & Asif, M. (2022). Effect of lysozyme and bromelain on physicochemical, textural and sensorial properties of mozzarella cheese. *Journal of Tianjin University Science and Technology*, 57(3), 45–61.
- Nascimento, M. O., Celestino, S. M. C., Veiga, A. D., De Jesus, B. D. A., & De Oliveira, L. D. (2024). Quality of arabica coffee grown in Brazilian Savannah and impact

- of potassium sources. *Food Research International*, 188, 114500–114521.
- Pathak, M. P., Pathak, K., Saikia, R., Gogoi, U., Patowary, P., Chattopadhyay, P., & Das, A. (2023). Therapeutic potential of bioactive phytoconstituents found in fruits in the treatment of non-alcoholic fatty liver disease: A comprehensive review. *Heliyon*, 9(4), Article e15347.
- Peteliuk, V., Rybchuk, L., Bayliak, M., Storey, K. B., & Lushchak, O. (2021). Natural sweetener *Stevia rebaudiana*: Functionalities, health benefits and potential risks. *EXCLI Journal*, 20, 1412–1430.
- Roudbari, M., Barzegar, M., Sahari, M. A., & Gavlighi, H. A. (2024). Formulation of functional gummy candies containing natural antioxidants and stevia. *Heliyon*, 10(11), Article e31581.
- Santos, D., Pintado, M., & Lopes da Silva, J. A. (2022). Potential nutritional and functional improvement of extruded breakfast cereals based on incorporation of fruit and vegetable by-products: A review. *Trends in Food Science & Technology*, 125, 136–153.
- Shabbir, A., Asif, H., Shakoor, S., Hussain, M., Fatima, A., Fatima, I., Urooj, A., & Asif, M. (2026). Development and characterization of flat bread incorporated with *Moringa* powder for nutritional, biofunctional, and sensory evaluation. *Policy Research Journal*, 4(4), 233–246.
- Shende, D., Kour, M., & Datta, A. K. (2020). Evaluation of sensory and physico-chemical properties of Langra variety mango leather. *Journal of Food Measurement and Characterization*, 14(6), 3227–3237.
- Stanek, N., Zarębska, M., Biłos, Ł., Barabosz, K., Nowakowska-Bogdan, E., Semeniuk, I., Błaszkiwicz, J., Kulesza, R., Matejuk, R., & Szkutnik, K. (2021). Influence of coffee brewing methods on the chromatographic and spectroscopic profiles, antioxidant and sensory properties. *Scientific Reports*, 11, Article 21377.
- Székelyhidi, R., Giczi, Z., Pallag, R., Lakatos, E., & Sik, B. (2024). Physicochemical and textural properties of gummy candies prepared with fruit vinegar. *Applied Food Research*, 4, Article 100473.
- Thomas, P.-A. (2023). Functional gummies: The (still) rising star of the nutraceutical space... and the production challenges brands can face on the ascent. *Agro FOOD Industry Hi Tech*, 34(2).
- Tsai, C.-F., & Jioe, I. P. (2021). The analysis of chlorogenic acid and caffeine content and its correlation with coffee bean color under different roasting degree and sources of coffee (*Coffea arabica* Typica). *Processes*, 9(11), Article 2040.
- Urooj, A., & Asif, M. (2026a). Formulation and characterization of polyphenol-rich functional porridge: A preliminary pilot randomized trial in patients with *Helicobacter pylori*-associated peptic ulcer disease. *Journal of Food Innovation, Nutrition, and Environmental Sciences*, 3(3), 304–326.
- Urooj, A., Mumtaz, F., Shabbir, A., Sultan, M., Tirmazi, S. M. B., Sarwar, M. S., & Asif, M. (2026b). Exploring the ameliorative effects of *Anethum graveolens* and *Foeniculum vulgare* seeds on dyslipidemia: A comparative efficacy in high fat diet-induced model. *Policy Research Journal*, 4(2), 506–527.
- Urooj, A., Shabbir, A., Hafeez, R., Rasheed, M. M., Asif, M., Aslam, I., Fatima, T., Shakoor, A., Zaman, K., & Faryad, A. (2026c). Physicochemical, phytochemical, and sensory evaluation of cookies fortified with jujube powder. *Policy Research Journal*, 4(3), 273–280.
- Urooj, A., Shabbir, A., Shahbaz, A., Ahmed, S., Ul Hassan, H. N., Asif, M., Usman, A., Tofique, M., Abbas, S., & Safdar, A. (2026d). Synergistic effects of *Cinnamomum zeylanicum* and inositol on metabolic and hormonal parameters in women with polycystic ovary syndrome. *Journal of Physical Education, Health and Social Sciences*, 4(1), 637–648.
- Vojvodić Cebin, A., Bunić, M., Mandura Jarić, A., Šeremet, D., & Komes, D. (2024). Physicochemical and sensory stability evaluation of gummy candies fortified with mountain germander extract and prebiotics. *Polymers*, 16, 259–278.
- Wang, L. L., Zhang, P.-H., & Yan, H.-H. (2023). Functional foods and dietary supplements in the management of non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Frontiers in Nutrition*, 10, Article 1014010.
- Wang, Q., Hu, G.-L., Qiu, M.-H., Cao, J., & Xiong, W.-Y. (2024). Coffee, tea, and cocoa in obesity prevention: Mechanisms of action and future prospects. *Current Opinion in Food Science*, 13, 100741–100782.
- Wu, H., Lu, P., Liu, Z., Sharifi-Rad, J., & Suleria, H. A. R. (2022). Impact of roasting on the phenolic and volatile compounds in coffee beans. *Food Science & Nutrition*, 10, 2408–2425.
- Yeager, S. E., Batali, M. E., Guinard, J.-X., & Ristenpart, W. D. (2023). Acids in coffee: A review of sensory measurements and meta-analysis of chemical composition. *Critical Reviews in Food Science and Nutrition*, 63, 1010–1036.
- Zakidou, P., Plati, F., Matsakidou, A., Varka, E.-M., Blekas, G., & Paraskevopoulou, A. (2021). Single origin coffee aroma: From optimized flavor protocols and coffee customization to instrumental volatile characterization and chemometrics. *Molecules*, 26(15), Article 4609.

Disclaimer/Publisher's Note: The views, opinions, and data presented in all publications are exclusively those of the respective author(s) and contributor(s) and do not reflect the stance of Pagepal or its editorial team. Pagepal and the editor(s) disclaim any responsibility for harm to individuals or damage to property resulting from the use of any ideas, techniques, instructions, or products mentioned in the content.