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Functional Food Rich in Bioactive Components for Management of Obesity and Related Metabolic Changes in Obese Women

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ABSTRACT

Background: Obesity is a global health problem that requires a safe and efficient approach. The objective of the research was to study how to manage obesity with functional food and dietary advice. Female obese subjects were given a daily amount of functional food for 2 months along with dietary advice. The functional food consisted of a mixture of powdered orange pectin, carrot, eggplant, oat, wheat bran, green tea, green coffee bean, *Nigella sativa* seeds, flax seed, roasted lemon, cumin seeds, fennel seeds, turmeric, ginger, cinnamon, and chili pepper. Anthropometric and biochemical parameters were assessed before and after dietary intervention. Bioactive components in the functional food were analyzed.

Results: Results showed obese subjects to demonstrate dyslipidemia and elevated insulin resistance, plasma malondialdehyde, glucose, and interleukin-6, together with reduced liver and kidney function compared to normal controls. Intervention with functional food with dietary advice produced significant improvement in the determined biochemical parameters except for triglycerides, high-density lipoprotein-cholesterol, nitric oxide, and alanine transaminase, which only showed insignificant improvements. The functional food also demonstrated a significant reduction in body mass index and waist/hip ratio. Analysis of the functional food showed high phenolic and flavonoid contents and phytosterols with an ω -3/ ω -6 ratio of 1/10. Dietary fibers were calculated to be 30.94%.

Conclusion: The intervention with functional food together with dietary advice has antiobesity effect with potential reduction of comorbidities associated with obesity, which might be due to the presence of phenolic compounds, phytosterols, ω -3 fatty acids, and dietary fibers.

1 | Introduction

Obesity is termed globesity due to its high prevalence worldwide. The pathogenic changes in obesity and its complications are considered a real public health problem that requires deep

investigations. Elevated inflammatory cytokines and reactive oxygen species are demonstrated in obese subjects. Obese subjects are prone to develop metabolic syndrome with high glucose tolerance, dyslipidemia, proinflammatory conditions, and insulin resistance in addition to a predictable involvement of

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CVDs, cardiovascular diseases; ELISA, enzyme-linked immunosorbent assay; GAE, gallic acid equivalent; HC, hip circumference; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IL-6, interleukin-6; IR, insulin resistance; LDL-C, low-density lipoprotein-cholesterol; MAFLD, metabolic dysfunction-associated fatty liver disease; MDA, malondialdehyde; NO, nitric oxide; QE, quercetin equivalent; TC, total cholesterol; TGs, triglycerides; WC, waist circumference

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hepatic, cardiac, and renal pathology [1]. The serious medical complications of obesity are referred to as insulin resistance syndrome. Besides, it is worth mentioning that iron deficiency anemia might be developed in obese subjects, which may be ascribed to elevated cytokines [2].

Numerous studies have described the importance of medicinal plants and natural products as remedies for human diseases. Bioactive constituents of plant food are among such natural products. Functional food is defined as a food product rich in bioactive constituents that have health benefits beyond basic nutrition, with the ability to reduce the risk of diseases [3–5]. Therefore, functional foods rich in biologically active ingredients like phenolic compounds, omega-3 fatty acids, phytosterols, carotenoids, tocopherols, fibers, and many other functional ingredients that could afford weight reduction, antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, and hypolipidemic effects might be beneficial in ameliorating the pathological condition present during obesity. The main concern in using functional foods is their safety compared to drugs administered for weight reduction with untolerated side effects. Plant food mixture from flax seed [6]; roasted lemon, ginger, cumin [7–9]; turmeric [10, 11]; orange pectin [12]; *Nigella sativa* seeds [13]; fennel [14, 15]; eggplant [16]; green coffee bean [17, 18]; carrot [19]; green tea [9, 18]; wheat bran [20]; cinnamon [21]; chili pepper [9]; and oat [22] is expected to contain bioactive ingredients with promising biological activities toward obesity.

The main goal of the present work was to study the potential remedial effect of obesity and its associated pathological changes in obese females by intervention with functional food composed of a blend of the aforementioned plant foods in dried powder form. The bioactive constituents within such a plant food blend are expected to have a synergistic effect toward obesity when combined together.

2 | Subjects, Materials, and Methods

2.1 | Ethical Approval and Informed Consent

The procedures in the present study adhered to the ethical standards of the Helsinki Declaration (1964) and its subsequent revisions. The study was approved by the ethical committee of the National Research Centre, Egypt, with an approval number of 19/175. All participants provided informed consent before the start of the study.

2.2 | Materials and Methods

2.2.1 | Materials

2.2.1.1 | Plant Materials. Flax seed (*Linum usitatissimum*), oat (*Avena sativa*), black cumin (*Nigella sativa*), green coffee bean (*Coffea arabica*), fennel (*Foeniculum vulgare*), turmeric root (*Curcuma longa*), ginger root (*Zingiber officinale*), cumin (*Cuminum cyminum*), carrot (*Daucus carota*), eggplant (*Solanum melongena*), wheat bran, green tea (*Camellia sinensis*), dried lemon (*Citrus limon*), cinnamon (*Cinnamomum verum*), chili pepper (*Capsicum annum*), and orange (*Citrus sinensis*) were purchased from local markets, Cairo, Egypt.

2.2.1.2 | Chemicals. Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Petroleum ether and ether of analytical grade were obtained from Fluka Chemika (Buchs, Switzerland). Aluminum chloride (anhydrous sublimed, $\geq 99.8\%$), gallic acid, quercetin, Folin–Ciocalteu reagent, and standards for fatty acids, hydrocarbons, and phytosterols were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other used chemicals were of analytical grade.

2.2.2 | Methods

2.2.2.1 | Preparation of Plant Materials. Fresh carrot roots and eggplant were washed separately by tap water and cut into small pieces, and the latter was sprinkled with lemon juice, and both were dried by solar energy. Parts rich in pectin from orange fruit (the white parts under the outer peel and the parts left after squeezing) were separated and dried in an air-circulated oven at 40°C until complete dryness. All plant materials were crushed separately and kept in a deep freeze until used.

2.2.2.2 | Preparation of the Functional Food. The crushed, dried powders of all the prepared plant materials were mixed together and reduced into fine powder to produce the functional food. The high percentages were supplemented from orange parts, carrot, eggplant, and oat (each of 15%), followed by wheat bran (7%), green tea (7%), green coffee bean (5.5%), *Nigella sativa* seeds (5%), flax seed (5%), and roasted lemon (5%), and the lowest were provided from cumin seeds (1%), fennel seeds (1%), turmeric (1%), ginger (1%), cinnamon (1%), and chili pepper (0.5%).

2.2.2.3 | Chemical Analysis

2.2.2.3.1 | Proximate Composition. Proximate composition (moisture, protein, fat, ash, and carbohydrates) of the functional food was determined according to AOAC [23]. For the determination of moisture content, 2 g of functional food powder was dried at $130 \pm 3^{\circ}\text{C}$ to obtain a constant weight; the moisture was obtained by difference. For assessing the ash, briefly, 3–5 g of functional food powder was placed in a preweighed and preheated porcelain crucible and incinerated at $550 \pm 5^{\circ}\text{C}$ in a furnace and weighed. The fat content was determined by extracting the functional food powder with petroleum ether in a Soxhlet apparatus. The solvent was evaporated using a rotary evaporator under vacuum and then dried at 105°C in an oven until a constant weight was obtained, which represented the fat content. For protein estimation, approximately 500 mg of functional food powder was digested using Kjeldahl (Gerhardt, Germany) (digestion, distillation, and titration apparatus), and then, protein content was calculated by multiplying nitrogen content by 6.25. The carbohydrate content was calculated by subtracting the sum of protein, fat, moisture, and ash content from 100.

2.2.2.3.2 | Determination of Total Phenolic and Total Flavonoid Content in the Functional Food. During the study, three batches of functional foods were prepared at different times, and therefore, phenolic and flavonoid contents were assessed in the three batches. For the determination of total phenolic content, 200 mg from the functional food was extracted with 2 mL of methanol (80%) containing 1% hydrochloric acid at

room temperature in a shaker for 2 h and then centrifuged at 3000 rpm for 10 min. The upper layer was collected in a clean tube, and re-extraction of the residue was carried out using the same previous procedure. The extracts were combined and used for the determination of total phenolic colorimetry using Folin–Ciocalteu reagent [24]. Briefly, 1 mL of the extract was mixed with 2.5 mL of 10% (w/v) Folin–Ciocalteu reagent. After 5 min, 2.0 mL of sodium carbonate (7.5%) was added and the mixture was incubated at room temperature for 30 min. Afterward, the absorbance of the sample was measured using a spectrophotometer (Jasco, V-730, Japan) at 765 nm against a blank without extract. Gallic acid was used as a standard. The standard was used in different concentrations. The calibration curve was plotted (the concentrations of standard against the absorption) (Figure 1). The concentration of the sample was obtained from the calibration curve. The result was expressed as gallic acid equivalent (GAE) in mg/g and mg/15 g dry sample. Total flavonoid content was determined by the aluminum chloride colorimetric method [25]. An aliquot of 1 mL of the previous extract was mixed with 0.2 mL of 10% (w/v) aluminum chloride in methanol, 0.2 mL (1 M) potassium acetate, and 5.6 mL of distilled water. The mixture was incubated for 30 min at room temperature, followed by the measurement of absorbance at 415 nm against the blank. Quercetin was used as a standard. The standard was used in different concentrations. The calibration curve was plotted (the concentrations of standard against the absorption) (Figure 1). The total flavonoid concentration of the sample was obtained from a calibration curve, and the result was expressed as mg quercetin equivalent (QE) per g of 15 g dry sample. Both total phenolic and flavonoids were assessed in triplicate (in the three batches of the functional food) and expressed as mean $\pm$ SD.

2.2.2.3.3 | Assessment of Fatty Acids Profile, Hydrocarbons, and Phytosterol Content of the Functional Food.

The unsaponifiable fraction of the prepared functional food was prepared according to AOAC [23]. Briefly, 2.5 mL of 2 M potassium hydroxide in ethanol was added to the test tube containing the fat portion of the prepared functional food for

saponification, followed by closing the test tube cap tightly, mixing well by vortex, and refluxing in a water bath at a temperature of 65°C–75°C for 1 h. After reflux, 2 mL of deionized water and 3 mL of HPLC-grade hexane were added to the mixture, vortexed for 1 min, and allowed to stand until the layers separated. The hexane fraction was transferred to another test tube, and the extraction of the aqueous phase was repeated with 3 mL hexane. All hexane fractions were collected in the same test tube, and the solvent was evaporated to dryness under moderate nitrogen flow to be subjected to GLC analysis of hydrocarbons and phytosterols. The unsaponifiable fraction was analyzed by GLC using an Agilent Technologies 6890N Network GC system instrument. GLC conditions: The oven temperature was initially set at 80°C and held for 1 min, then increased at a rate of 8°C min⁻¹ to a final temperature of 250°C and held for 25 min. The inlet temperature was maintained at 250°C (split ratio of 15:1). Column: Separation was achieved using a ZB-5 capillary column (5% phenyl–95% dimethylpolysiloxane, length: 30 m, diameter: 530 μ m, film thickness: 50 μ m). Detector: Detection was performed using an FID set at 300°C (flow: 3 mL/min, carrier gas: N₂ (30 mL/min), H₂ (30 mL/min), and air (300 mL/min)). Identification of hydrocarbons and phytosterols in the unsaponifiable matter quantitatively was carried out by comparison of their retention times matched with co-injected authentic reference compounds. Quantization was based on peak area integration.

Fatty acid methyl esters of the prepared functional food were prepared according to the AOAC method 996.06 [23]. Functional food ground sample (1 g) was mixed with 50 mg of pyrogallol. Then, hydrolysis was carried out with 10 mL of 8.3 M hydrochloric acid in a water bath at 70 $\pm$ 5°C for 40 min, followed by extraction into 25 mL of ether and 25 mL of petroleum ether, and methylated using 2 mL of 7% boron trifluoride at 100°C for 45 min. The extract was then dehydrated over anhydrous sodium sulfate and prepared for GLC analysis. Assessment of the methyl ester was carried out by injecting 2 μ L into a Hewlett-Packard (HP) system 6890 gas chromatograph equipped with FID. An HP-5 capillary column (30 m $\times$ 0.32 mm i.d.; 0.25 μ m film thickness) was used to separate the different methyl esters under specified

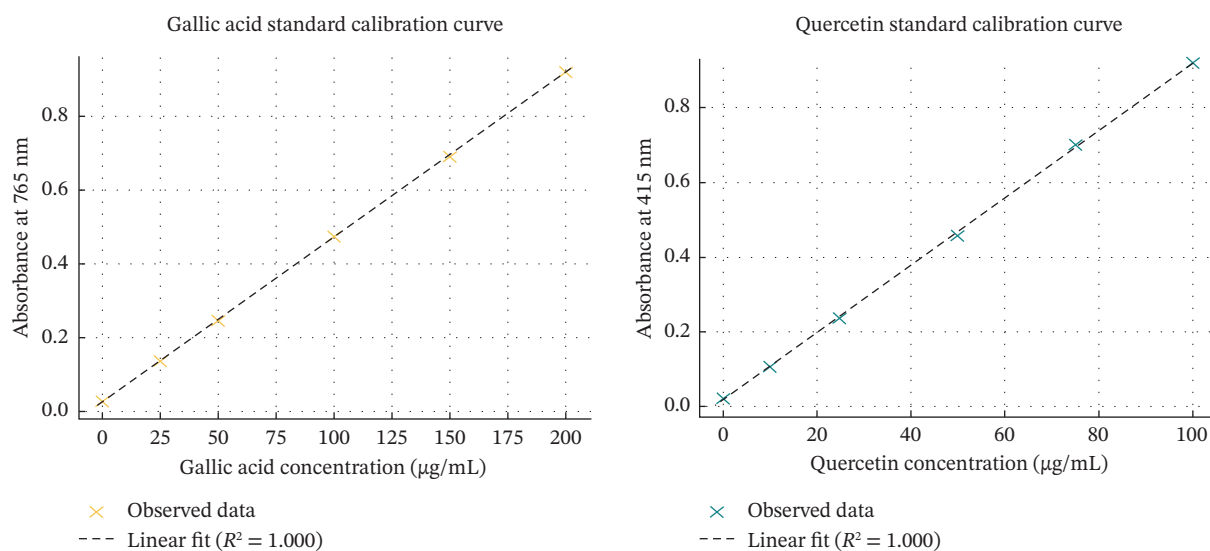


FIGURE 1 | Calibration curves of (gallic acid and quercetin).

conditions. Fatty acid methyl esters were quantitatively identified by direct comparison of their retention times matched with standards of the fatty acid methyl esters analyzed under the same conditions. Quantization was based on peak area integration.

2.2.2.3.4 | Dietary Fiber Contents of the Functional Food. Dietary fibers of each ingredient that are present in the functional food were obtained from USDA food composition tables [26] and were calculated on a dry basis in each ingredient and finally in the whole functional food.

2.2.2.4 | Organoleptic (Sensory) Evaluation of the Functional Food. A nine-point hedonic scale was employed for the sensory evaluation, incorporating both verbal and numerical categories to assess the degree of preference (Table 1). Ten individuals from Food Industries and Nutrition Institute, National Research Centre, Egypt, participated in the 9-point hedonic scale for the evaluation of the prepared functional food formula. All panelists evaluated the functional food formula through eight parameters, including color, aroma, flavor, bitterness, spiciness, texture/mouthfeel, aftertaste, and overall acceptability. The verbal hedonic scale is considered the most effective tool for expressing respondents' degrees of preference in sensory evaluation. For statistical analysis, the verbal hedonic scale is converted to a numerical hedonic scale [27].

2.2.2.5 | Design of the Clinical Study. The subjects under study were 44 female subjects from the National Research Centre, Cairo, Egypt, who willingly participated in the study. These subjects were 34 female obese patients with ages ranging from 29 to 62 years old, and 10 female healthy subjects of matched age worked as a control group with an ideal body mass index (BMI) ranging from 20 to 24.9. Group sizes reflect the maximum number of eligible, consenting participants available during the recruitment window. The reduced number of obese individuals was due to the fact we selected only those who were completely free from chronic diseases, and those who did not consume any medications. Concerning the small number of healthy controls, this was attributed to the prevalence of overweight and obesity, where it was difficult to find females with an ideal BMI. Obese patients were given the functional food (15 g/day) for two months. A sufficient quantity of functional food was given weekly to each obese subject to avoid bad storage conditions. The functional food was prescribed to be consumed by adding it to food. The time period of 2 months

was adopted according to previous nutritional intervention studies by functional food and nutraceuticals on obese subjects, which ranged from 6 to 8 weeks with acceptable results [28, 29]. All the subjects completed the study without dropping out. The obese subjects were assessed nutritionally through measuring anthropometric parameters and taking a questionnaire for 24-h dietary recall (for 3 days) with the frequency of consumed food items. The assessed anthropometric parameters were weight, length, waist circumference, hip circumference, with calculation of BMI, and waist/hip ratio. Patients were advised to reduce calories and carbohydrates from their diets; substitute saturated fats, purified flour and its products, and whole milk and full-fat milk products with polyunsaturated fats, whole grains and cereal products, and skimmed milk and low-fat milk products, respectively; and to take a daily specific amount of fresh vegetables and fresh fruits (low in total sugar). Anthropometric parameters were re-measured after dietary intervention.

Biochemical analysis of blood was carried out at the start and end of the study. Blood samples were obtained from fasted obese and control normal subjects. Blood hemoglobin was determined using Drabkin's solution according to Vankampen and Zijlstra [30]. The blood samples were collected in a heparinized tube for the separation of plasma. Plasma aspartate transaminase (AST) and alanine transaminase (ALT) activities were determined as an indicator of liver function according to Reitman and Frankel [31]. The amino group was enzymatically transferred by ALT or AST present in the sample from alanine or aspartate to the carbon atom of 2-oxoglutarate, yielding pyruvate or oxaloacetate, respectively, which react with 2,4-dinitro-phenylhydrazine to give respective hydrazine-measured colorimetric. Plasma levels of creatinine and urea were assessed as representative of kidney function using previous methods [32, 33]. Plasma level of creatinine was assessed using buffered kinetic Jaffé reaction without deproteinization, while urea was determined by an enzymatic method using urease-UV fixed rate. Fasting plasma glucose was determined calorimetrically [34]. Glucose was exposed to enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacted under the catalysis of peroxidase with phenol and 4-aminoantipyrine to form a red violet quinoneimine dye that was measured at 546 nm. Plasma total cholesterol (TC) [35] was determined after enzymatic hydrolysis of cholesterol esters by cholesterol esterase. TC was then oxidized by cholesterol oxidase to produce hydrogen peroxide, which, when combined with phenol and 4-amino antipyrine in the presence of peroxidase-produced quinoneimine dye, which was measured quantitatively at 500–550 nm. Triglycerides (TGs) [36] were determined using the enzymatic colorimetric method. TGs were hydrolyzed by lipoprotein lipase to produce glycerol, which was then phosphorylated to glycerol-3-phosphate by adenosine triphosphate in a reaction catalyzed by glycerol kinase. Glycerol-3-phosphate was oxidized by phosphate oxidase to produce hydrogen peroxide. Then, hydrogen peroxide in the presence of peroxidase induced the oxidative coupling of 4-chlorophenol and 4-aminoantipyrine to produce red color quinoneimine dye, which was measured at 546 nm. High-density lipoprotein-cholesterol (HDL-C) was assessed by the aforementioned method of cholesterol after precipitation of both low-density lipoprotein and very low-density lipoprotein by phosphotungstate and magnesium ion [37]. Low-density lipoprotein-

TABLE 1 | Verbal and numerical hedonic scale.

Verbal hedonic scale	Numerical hedonic scale
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

cholesterol (LDL-C) was calculated from the following equation: $LDL-C = TC - HDL-C (TGs/5)$. TC/HDL-C ratio was computed as a predictor of cardiovascular disease (CVD) risk. The chemical reagents for assessing the aforementioned biochemical parameters were obtained from the Egyptian Company for Biotechnology-Spectrum Diagnostic. Plasma insulin was assessed by the enzyme-linked immunosorbent assay (ELISA) technique using the Sunlong kit with Catalogue No. SL0933Hu. Homeostasis model of insulin resistance (HOMA-IR) was calculated according to Matthews et al. [38]. Plasma malondialdehyde (MDA) was assessed as an indicator of lipid peroxidation and oxidative stress using a Sunlong ELISA kit (Catalogue No. SL1135Hu). Plasma interleukin-6 (IL-6), an inflammatory biomarker, was estimated by adopting the ELISA technique (Sunlong kit Catalogue No. SL1001Hu). All ELISA methods used in the present research were quantitative sandwich enzyme immunoassay. A monoclonal antibody specific for human porcine insulin, MDA, or IL-6 has been precoated onto a microplate. Standard, samples, and biotin-linked detection antibodies specific for insulin, MDA, or IL-6 were pipetted into wells; after incubation, the insulin, MDA, and IL-6 were bound by the immobilized antibodies and detected by the corresponding detection antibodies. After washing away any unbound substances, streptavidin-HRP was added. Substrate solution was added to the wells, and color developed in proportion to the quantity of insulin, MDA, or IL-6 bound in the initial step. The color development was stopped, and the intensity of the color was measured at 450 nm using ELISA plate reader (ASYS HITECH, EXPERT PLUS, Austria). The biochemical parameters of patients were compared before and after the intervention study, and both were compared with those of the healthy normal control.

2.2.2.6 | Statistical Analysis. A formal a priori sample size calculation based on a specific expected effect size was not feasible due to the lack of reliable preliminary data and the pragmatic nature of recruitment. All data were expressed as mean $\pm$ SD. Normality tests were done using the Shapiro–Wilk test, and since assumptions of normality could not be met, the data were analyzed using nonparametric tests. The food intake and the anthropometric parameters of obese subjects before and after intervention were statistically analyzed by Wilcoxon’s signed rank test. The data for biochemical parameters were analyzed by Wilcoxon’s signed rank test to compare the paired “before” and “after” effect, followed by Mann–Whitney’s test to compare the healthy control group to the “before group,” as well as the healthy control group to the after group. In all cases, $p < 0.05$ was used as the criterion of statistical significance and specific p values were reported along with 95% CI ranges for effect size. The results of the proximate composition, total phenolic, and flavonoids were expressed as mean $\pm$ SD.

3 | Results

3.1 | Chemical Analysis of the Functional Food

Table 2 summarizes the chemical composition of the functional food. Proximate composition of the functional food revealed that protein were present as 10.77%, fat was 9.1%, and ash was 5.09%. Carbohydrates represented by available (digestible) + nonavailable carbohydrates (dietary fibers) was present as 66.7%.

TABLE 2 | Chemical composition of the functional food (mean $\pm$ SD), each of three replicates.

Ingredients of dry sample	Dietary supplement
Moisture (g/100 g)	8.34 $\pm$ 0.111
Protein (g/100 g)	10.77 $\pm$ 0.221
Fat (g/100 g)	9.10 $\pm$ 0.366
Ash (g/100 g)	5.09 $\pm$ 0.168
Available and nonavailable carbohydrates (g/100 g)*	66.7 $\pm$ 0.713
Total phenolic compounds ((mg GAE)/g)	68.96 $\pm$ 0.451
Total flavonoids ((mg QE)/g)	48.77 $\pm$ 0.475

*Calculated by differences. Nonavailable carbohydrates (total dietary fibers) were calculated to be = 30.94%.

Dietary fibers were calculated to be 30.94%. The functional food prepared in the present study showed high content of phenolic compounds (68.96 mg GAE/g) and total flavonoids (48.77 mg QE/g), which account for 1 g and 0.731 mg/15 g, respectively.

Table 3 shows the fatty acid profiles of the functional food. Palmitic acid (14.203%) was the major saturated fatty acid, followed by lauric acid (1.38%). Linoleic acid (ω -6) was the highest polyunsaturated fatty acid (37.983%), followed by oleic acid (ω -9) (36.883%), and then, linolenic acid (ω -3) was the least percentage (4.104%). The functional food showed a high percentage of unsaturated fatty acids (79.827%) compared to the saturated (18.647%). The ratio ω -3/ ω -6 was shown to be 1/10.

The analyzed hydrocarbons and phytosterols of the functional food are shown in Table 4. The results revealed a high percentage of hydrocarbons (70.999%) and heneicosane (C21) was the major

TABLE 3 | Fatty acids in the functional food as a percentage of total fatty acids.

Fatty acids	Fatty acid percentage of total fatty acids
Lauric acid (C12: 0)	1.380
Myristic acid (C14: 0)	1.324
Palmitic acid (C16: 0)	14.203
Stearic acid (C18: 0)	1.069
Oleic acid (C18: 1)	36.883
Linoleic acid (C18: 2)	37.983
Linolenic acid (C18: 3)	4.104
Arachidonic acid (C20: 0)	0.671
Eicosenoic acid (C20: 1)	0.857
Total identified saturated fatty acids	18.647
Total identified monounsaturated fatty acids	37.74
Total identified polyunsaturated fatty acids	42.087
Total identified unsaturated fatty acids	79.827
ω -3/ ω -6	1/10

TABLE 4 | Hydrocarbons and phytosterols in the functional food (as percentage of total hydrocarbons and phytosterols).

Hydrocarbon and phytosterols	Dietary supplement
Hydrocarbon	
Tridecane (C13)	0.804
Tetradecane (C14)	0.699
Pentadecane (C15)	1.068
Heptadecane (C17)	0.576
Octadecane (C18)	0.453
Eicosane (C20)	9.089
Heneicosane (C21)	21.685
Docosane (C22)	2.656
Tricosane (C23)	4.229
Tetracosane (C24)	6.582
Pentacosane(C25)	4.366
Hexacosane (C26)	7.695
Heptacosane (C27)	6.959
Squalene (C30)	4.138
Total hydrocarbon	70.999
Phytosterols	
Campesterol	5.963
Stigmasterol	9.819
β -sitosterol	4.445
Total phytosterols	20.227

one (21.685%) followed by eicosane (C20) (9.089%). Phytosterols were present as 20.227%. The main phytosterol was stigmasterol (9.819%) followed by campesterol (5.963%), while β -sitosterol was the lowest phytosterol (4.445%) in the functional food.

3.2 | Organoleptic (Sensory) Evaluation of the Functional Food

Sensory evaluation of the functional food formula revealed overall panelists acceptability scores ranging from 7 to 8 on a 9-point hedonic scale (7.55 ± 0.369), indicating that the product was “liked moderately” and “liked very much” by most panelists. The aroma (7.75 ± 0.354), color (7.7 ± 0.369), and flavor (7.35 ± 0.579) of the functional food formula were the most appreciated attributes, while bitterness (5.55 ± 0.438) and aftertaste (6.1 ± 0.316) received slightly lower scores, likely due to the presence of ingredients such as green tea and spices. The spiciness (6.2 ± 0.258) was considered acceptable, with the warmth from ginger and chili. Even with these minor reductions, the overall acceptability (7.55 ± 0.369) suggests strong panelists’ acceptance, which revealed the strong potential marketing for a functional food product enriched with natural bioactive ingredients. Figure 2 and Table 5 show the mean hedonic scores for each sensory attribute of the functional food formula.

3.3 | Baseline Characteristics and Anthropometric Parameters of Obese Patients Before and After Intervention

Baseline characteristics are demonstrated in Table 6. Anthropometric parameters are presented in Table 7 in comparison to

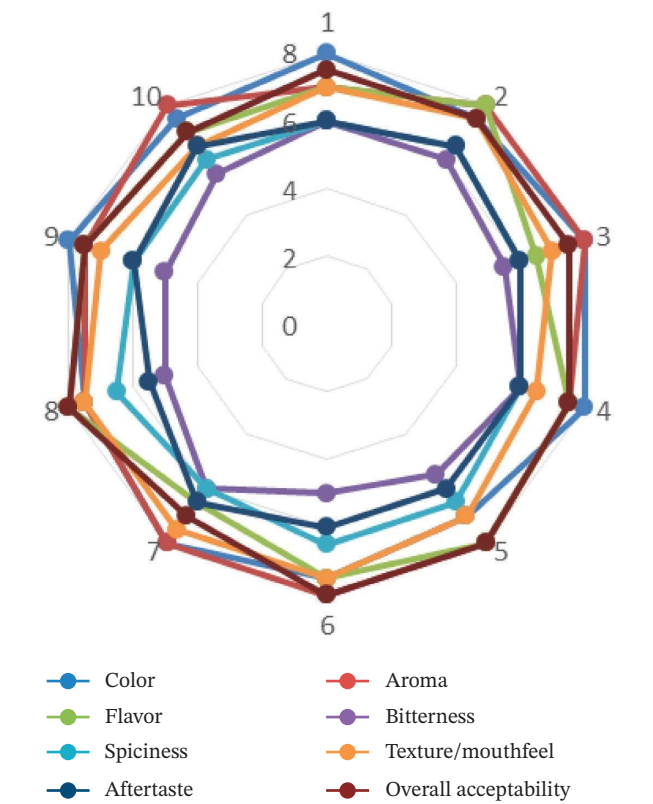


FIGURE 2 | Sensory attributes of the formulated functional food formula based on a 9-point hedonic scale for color, aroma, flavor, bitterness, spiciness, texture, aftertaste, and overall acceptability ($n = 10$).

normal values [39–41]. The body weight, BMI, WC, HC, and WC/HC of the studied subjects were significantly reduced after dietary intervention.

3.4 | Biochemical Parameters of Obese Subjects Before and After Dietary Intervention

Table 8 shows the different biochemical parameters of obese patients before and after dietary intervention in comparison to healthy controls. Plasma lipid profile (TC, LDL-C, and T-C/HDL-C), MDA, IL-6, glucose, insulin, and IR showed significant elevation in obese patients compared with normal healthy subjects. Plasma AST activity and creatinine were significantly higher in obese patients than in normal subjects. Blood hemoglobin exhibited a significant reduction in obese compared to normal healthy subjects. Plasma TG, HDL-C, urea, and ALT activity in obese individuals showed no changes compared to normal controls. Plasma TC, TG, LDL-C, HDL-C, and TC/HDL-C of obese subjects were improved significantly after dietary intervention. Plasma activity of AST, levels of creatinine, and urea of obese subjects were reduced significantly after the dietary intervention. The patients showed significantly lower levels of plasma MDA and IL-6 after dietary intervention compared to before supplementation. Significant decreases in plasma levels of glucose, insulin, and IR were noticed in obese subjects after dietary intervention when compared to before intervention. Blood hemoglobin increased significantly in obese patients after 2 months of functional food supplementation compared to before the intervention.

TABLE 5 | Sensory evaluation scores of the formulated functional food formula based on a 9-point hedonic scale.

	Color	Aroma	Flavor	Bitterness	Spiciness	Texture/mouthfeel	Aftertaste	Overall acceptability
Mean	7.7	7.75	7.35	5.55	6.2	7.1	6.1	7.55
±SD	±0.349	±0.354	±0.579	±0.438	±0.258	±0.394	±0.316	±0.369

Note: (n = 10).

TABLE 6 | Baseline characteristics of obese and control healthy subjects (mean ± SD).

Parameters	Obese female subjects	Control healthy subjects
Age (years)	42.08 ± 8.06	41.7 ± 8.00
BMI (kg/m ²)	35.92 ± 4.31	24.3 ± 5.2
Medications	-ve	-ve
Smoking	-ve	-ve

3.5 | Food Intake of Obese Subjects

Table 9 demonstrated the mean food intake/day of obese subjects before and after intervention with functional food in comparison to the recommended dietary allowance (RDA). Calories were shown to be reduced by 15% after 2 months of intervention. There were significant declines in fat calcium, iron, sodium, and zinc intake after 2 months of the study, while vitamin C intake showed a significant increase.

4 | Discussion

Obesity as a global health problem requires efficient treatment due to its serious complications. The antiobesity synthetic drugs have severe side effects; therefore, searching for natural agents for obesity management represented by functional food was the goal of the present work.

Abdominal adiposity represented by WC was reported to enhance oxidative stress in obese individuals and to be positively correlated with IL-6, IR, dyslipidemia, and inflammation [42]. In obese subjects, IL-6 was positively correlated with HOMA-IR, BMI, TGs, LDL-C, and TC and negatively correlated with HDL-C [40]. There is an association of the elevated IL-6 with IR and the development of Type 2 diabetes, which was reported to be due to its inhibitory effect on glucose transporter-4 and insulin receptor

substrate-1 expression [43]. The elevated plasma glucose, IR, TC, and LDL-C in the obese subjects with WC of 105.85 cm and BMI of 35.92 kg/m² in the present work agreed with the above-mentioned studies. Also, in the current study, elevated MDA, IL-6, and TC/HDL-C reflected a state of elevated oxidative stress, inflammation, and elevated CVD risk in the obese subjects, respectively.

Obesity might be associated with anemia in adults [2]; however, Qin et al. [44] showed that obese women were less likely to be anemic, which disagreed with the present study. Renal dysfunction was demonstrated to be related to obesity due to a change in renal hemodynamics [45]. Concerning liver function, IR had a significant association with the risk of elevated ALT and AST in obese adults [46], and this might be due to the increased incidence of metabolic dysfunction-associated fatty liver disease (MAFLD) during obesity. Liver and kidney functions in obese subjects in the present study were affected, but ALT and AST activity and creatinine were still within the normal range.

Dietary intervention with the functional food and dietary advice could improve the anthropometric and the majority of biochemical parameters, where reduction of inflammation (IL-6) and oxidative stress (MDA) was noticed. Also, insulin resistance, hypercholesterolemia, and elevated LDL-C were improved with concomitant reduction of TC/HDL-C, the risk factor of CVDs, together with improvement of both liver and kidney functions. These health benefits of the functional food might be attributed to the presence of phenolic compounds, including flavonoids, α -linolenic acid, phytosterols, and dietary fibers determined in the present study. Phytosterols were reported as a novel nutraceutical for the management of obesity and metabolic syndrome. They are structurally similar to cholesterol and have anti-inflammatory, antioxidant, immune-modulatory, and cholesterol-lowering effects [47]. Alpha-linolenic acid has been shown to reduce CVD risk factors [48]. Phenolic compounds have the ability to reduce food intake, lipogenesis, inflammation,

TABLE 7 | Anthropometric parameters (mean ± SD) of obese subjects before and after dietary intervention.

Parameters	Before intervention	After intervention	p value	Normal values for females
Age (year)	42.08 ± 8.06	42.08 ± 8.06	1	
Weight (kg)	96.63 ± 16.85	90.83* ± 16.06	< 0.001	
Height (meter)	1.64 ± 0.32	1.64 ± 0.32	1	
BMI (kg/m ²)	35.92 ± 4.31	33.77* ± 4.09	< 0.001	18.5–24.9 [#]
WC (cm)	105.85 ± 10.61	101.08* ± 10.61	< 0.001	80 or less ¹
HC (cm)	124.31 ± 6.79	119.62* ± 5.96	< 0.001	
WC/HC	0.85 ± 0.05	0.84* ± 0.06	< 0.001	0.8 or less ²

Abbreviations: HC, hip circumference; WC, waist circumference.

*Significant changes using Wilcoxon's signed rank test ($p < 0.05$), $n = 34$.

[#][39].

¹[40].

²[41].

TABLE 8 | Different biochemical parameters (mean $\pm$ SD) of obese patients before and after 2 months of dietary intervention with the functional food in comparison to healthy controls.

Parameters	Functional food supplementation		Healthy control subjects	p value (Mann–Whitney) healthy control/before	95% CI for effect size healthy control/before		p value (Mann–Whitney) healthy control/after	95% CI for effect size healthy control/after		
	p value (Wilcoxon) before/after				Lower	Upper		Lower	Upper	
	Before	After								
Hemoglobin (g/dL)	14.25 ± 1.41	15.48 ± 0.89	15.16 ± 0.63	< 0.001*	< 0.049*	–0.691	–0.026	0.501	–0.260	0.506
Glucose (mg/dL)	102.6 ± 23.82	86.3 ± 13.35	79.9 ± 3.92	< 0.001*	< 0.001*	0.871	0.974	0.565	–0.280	0.490
Insulin (mU/L)	14.10 ± 4.22	7.32 ± 1.80	6.98 ± 1.21	< 0.001*	< 0.001*	0.883	0.976	0.989	–0.395	0.385
IR	3.03 ± 1.15	1.84 ± 0.55	1.37 ± 0.202	< 0.001*	< 0.001*	1	1	0.006*	0.241	0.789
TC (mg/dL)	204.3 ± 24.8	167.8 ± 17.7	167.0 ± 15.0	< 0.001*	< 0.001*	0.694	0.933	0.565	–0.280	0.490
TG (mg/dL)	90.4 ± 29.08	74.6 ± 12.29	83.8 ± 8.02	< 0.001*	< 0.001*	–0.397	0.382	0.002*	–0.831	–0.353
HDL-C (mg/dL)	47.2 ± 10.62	54.4 ± 9.07	58.9 ± 13.83	< 0.001*	< 0.001*	–0.676	0.002	0.614	–0.478	0.294
LDL-C (mg/dL)	139.0 ± 24.25	98.5 ± 23.20	91.4 ± 14.15	< 0.001*	< 0.001*	0.947	0.990	0.149	–0.095	0.622
TC/HDL-C ratio	4.51 ± 1.16	3.18 ± 0.64	2.96 ± 0.56	< 0.001*	< 0.001*	0.722	0.940	0.293	–0.182	0.564
MDA (ng/mL)	176.98 ± 18.75	146.92 ± 14.79	139.38 ± 10.6	< 0.001*	< 0.001*	0.877	0.975	0.042*	0.044	0.700
IL-6 (ng/mL)	47.85 ± 11.98	34.88 ± 5.67	36.00 ± 2.92	< 0.001*	< 0.001*	0.389	0.844	0.133	–0.630	0.082
AST (U/L)	24.54 ± 7.53	15.85 ± 6.26	12.88 ± 1.75	< 0.001*	< 0.001*	0.928	0.986	0.118	–0.073	0.635
ALT (U/L)	11.69 ± 4.33	9.15 ± 5.24	8.13 ± 2.79	> 0.05	0.084	–0.036	0.657	0.655	–0.467	0.307
Creatinine (mg/dL)	0.98 ± 0.12	0.86 ± 0.14	0.68 ± 0.08	< 0.001*	< 0.001*	1	1	< 0.001*	0.539	0.891
Urea (mg/dL)	35.03 ± 12.33	26.9 ± 5.35	29.0 ± 4.66	< 0.006*	0.262	–0.167	0.575	0.119	–0.637	0.069

*Means show statistically significant difference by applying the Wilcoxon signed rank test (before/after), followed by Mann–Whitney's test (healthy control/before) and (healthy control/after) ($p < 0.05$), $n = 34$ for obese and $n = 10$ for healthy control.

TABLE 9 | Mean food intake/day of obese subjects (mean $\pm$ SD) before and after intervention with functional food.

Parameters	Food intake before intervention	% RDA	Food intake after intervention	% RDA	p value
Calories (kcal.)	1302.3 $\pm$ 498.6	—	1108.7* $\pm$ 429.2	—	0.02
Protein (g)	58.2 $\pm$ 25.93	88.15	49.1 $\pm$ 16.27	74.38	0.060
Carbohydrates (g)	177.9 $\pm$ 89.18	136.86	154.3 $\pm$ 78.29	118.69	0.070
Fibers (g)	4.94 $\pm$ 2.66	19.76	5.98 $\pm$ 5.17	23.92	0.945
Fat (g)	38.8 $\pm$ 12.64	—	32.1* $\pm$ 9.21	—	0.025
Vitamin B ₁ (mg)	0.63 $\pm$ 0.41	70	0.57 $\pm$ 0.38	63.33	0.365
Vitamin B ₂ (mg)	0.75 $\pm$ 0.33	83.33	0.69 $\pm$ 0.55	76.67	0.065
Vitamin C (mg)	25.3 $\pm$ 13.46	42.08	78.4* $\pm$ 70.11	130.7	< 0.001
Calcium (mg)	498 $\pm$ 229.1	62.26	410* $\pm$ 172.8	51.22	0.011
Copper (mg)	0.65 $\pm$ 0.3	92.86	0.61 $\pm$ 0.31	87.14	0.441
Iron (mg)	10.8 $\pm$ 5.6	132.72	9.4* $\pm$ 4.87	116.17	0.014
Magnesium (mg)	112.4 $\pm$ 49.95	42.42	125.7 $\pm$ 51.5	47.44	0.353
Phosphorus (mg)	895 $\pm$ 213	154.35	803 $\pm$ 221	138.36	0.06
Potassium (mg)	1682 $\pm$ 769	35.79	1498 $\pm$ 609	31.86	0.087
Sodium (mg)	2741 $\pm$ 769	182.76	1593* $\pm$ 609	106.2	< 0.001
Zinc (mg)	16.3 $\pm$ 11.99	239.7	6.7* $\pm$ 3.05	98.24	< 0.001

Abbreviation: RDA, recommended dietary allowance.

*Means are statistically significant when comparing food intake before and after intervention by the functional food by applying Wilcoxon's signed rank test ($p < 0.05$), $n = 34$.

and oxidative stress and promote beta oxidation of fatty acids [49]. Dietary fibers enhance satiety, reduce food intake and retard cholesterol absorption, and decrease serum cholesterol [50]. Reduction of oxidative stress, as remarked by the reduction in MDA after treatment with the functional food, could be attributed to the presence of antioxidant bioactive constituents. Such antioxidants could have potential therapeutic implications on the metabolic changes associated with obesity. Oxidative stress is accused in the progression of obesity, leading to chronic inflammation and dysfunction of metabolic processes [51, 52]. Antioxidants have been reported to possess anti-inflammatory action and have the ability to regulate cellular processes related to adipose tissue function and energy metabolism along with improving insulin sensitivity, which is considered a key factor in maintaining healthy body weight [51–53].

The individual ingredients of the functional foods prepared in the present work showed beneficial effects toward obesity as reported previously. Ginger, cumin, and dried lemon were shown to reduce body weight and improve lipid profile, serum glucose, and liver function in obese rats due to the presence of phenolic compounds and flavones [16]. The activity of roasted lemon was also attributed to its contents of volatile constituents and α -linolenic acid [54]. Ginger effects were related to remodeling of adipocytes and enhancing fatty acid oxidation [8]. The biological activity of ginger might be attributed to its contents from phenolic compounds like gingerols, shogaols, and flavonoids in addition to terpenoids [55]. Also, the phenolic contents of green tea like catechin and epigallocatechin gallate were shown to prevent abdominal obesity [18, 56]. Polyphenol, especially chlorogenic acid in coffee, has beneficial effects as an antiobesity. Polyphenol antioxidative actions help scavenge ROS and downregulate nuclear factor- κ B to produce favorable anti-inflammatory effects [18]. Phenolic compounds and volatile

constituents in *Nigella sativa* were reported to reduce oxidative stress and inflammation and improve lipid profile and liver and kidney functions in rats [13, 57]. Eggplant that is rich in phenolic and alkaloid compounds was reported to have antioxidant, antihyperlipidemic, and antidiabetic activities through an effect on lipoprotein lipase activity and the inhibition of pancreatic lipase. Eggplant can control diabetes via its antioxidant effect and the reduction of α -glucosidase and α -amylase [16]. Phenolic pigments like curcumin in turmeric stimulate energy expenditure, inhibit adipogenesis, improve lipid metabolism, and have anti-inflammatory effects due to an impact on gut microbiota [11], thereby contributing to antiobesity action.

Cinnamon, which is another component of the functional food, was reported to significantly reduce body weight and BMI but not WC, which might be ascribed to cinnamaldehyde as an active ingredient that delays stomach emptying, improves gastrointestinal motility, promotes adrenaline secretion and energy expenditure, and enhances the release of serotonin from intestinal enterochromaffin cells [21, 58]. It also promotes fat oxidation in adipose tissue and reduces serum leptin and intestinal glucose absorption [59–61]. Cinnamon also contains polyphenols that suppress adipose tissue-induced inflammation [62].

Fennel, which is present in the functional food, was reported to improve liver and kidney function, and lipid profile and reduce oxidative stress, appetite, and body weight gain in obese rats [15]. The isoflavones in fennel work like phytoestrogen, which could affect a serotonergic system by inhibiting serotonin reuptake, resulting in elevation of serotonin in synaptic clefts that promotes satiety and reduction of body weight gain [63].

Capsicum annuum was incorporated at the least amount in the functional food to avoid its burning taste with concomitant utilization of its bioactivity. The antiobesity effect of *Capsicum*

annuum was related to antiadipogenesis and antioxidant activities due to the presence of capsaicin and related capsaicinoids, trigonelline, cinnamic derivatives, C4-substituted pyridine, vitamins C and E, carotenoids, and phenolic compounds. Also, *Capsicum annum* enhances fat oxidation, catecholamine secretion, and energy expenditure and reduces fat accumulation [9].

Oat, an ingredient of the functional food used in the current study, was reported to control hyperglycemia and to reduce blood lipids, body weight, and HOMA-IR due to the presence of β -glucan. The mechanism might be related to increased intestinal viscosity that reduces the absorption of carbohydrates, fats, and food in general in addition to its impact on gut microbiota that can release bioactive compounds, which reduce the risk of obesity [22]. Wheat bran can reduce visceral fat and body weight [20] due to its contents of arabinoxylan, an insoluble fiber which can also improve fasting plasma glucose in diabetics [64]. Orange pectin, which is a soluble dietary fiber, was shown to improve lipid profile and to reduce cardiovascular risk in rats with nonalcoholic fatty liver [12]. Flaxseed-containing bread was shown to reduce BMI and improve lipid profile, blood sugar, and oxidative stress in diabetic patients, which was ascribed to the presence of dietary fibers (gum), proteins, and lignans (phenolic compounds) [6]. Carrot consumption was shown to reduce BMI, an effect that was ascribed to its effect on intermediary nucleotide polymorphisms rs4445711, which referred to genetic implication of its antiobesity activity [19]. Such carrot activity might be related to the presence of fibers and carotenoids like β -carotene, α -carotene, and β -cryptoxanthin. β -cryptoxanthin was reported to reduce BMI and visceral fat, while serum β -carotene was inversely correlated with inflammatory biomarkers and IR [65, 66].

According to the aforementioned discussion, the different ingredients incorporated in the functional food could afford synergistic antiobesity effect together with improving plasma lipid profile, glucose, and IR. The ingredients might also participate in improving oxidative stress, inflammation, and liver and kidney function. Hemoglobin might be elevated as a result of reducing obesity and the inflammatory cytokine (IL-6).

The significant reduction in calories and fat intakes without a doubt participated in BMI reduction. Functional food supplementation may have a hand in caloric reduction, which could be attributed to an appetite suppressant effect. The insignificant increase in dietary fiber intake might be due to the elevated contents of fibers in the functional food in addition to the advice given to the patients to replace purified cereals by whole grains and to increase the vegetable intake. The significantly increased intake of vitamin C might participate in reducing MDA and subsequently oxidative stress and inflammation. Although the increase in magnesium intake was not statistically significant, it may improve glucose metabolism and insulin sensitivity [67]. Reduced intake of sodium after intervention might have health benefits for obese subjects by managing blood pressure.

The bioactive constituents analyzed in the present study in the functional food with the reduction of caloric intake could have a hand in the management of the MAFLD common in obese subjects. Such management of MAFLD could be reflected in the improvement of liver function, IR and plasma MDA, IL-6, and lipid profile. No standard dietary strategies are present concerning MAFLD management; however, maintaining

a Mediterranean diet with physical activity and caloric restriction was previously recommended [68].

Study limitations: MDA was assessed as a biomarker of oxidative stress during the present study; however, more biomarkers of oxidative stress might be analyzed to add to the study. Dietary advice was used along with the functional food; this coin-tervention makes it impossible to attribute effects to the functional food alone; however, it is mandatory for the obese to control their diet even with the administration of a remedy or the consumption of functional food. Limitations include a modest sample size and an imbalance between groups (34 obese, 10 normal weight). These were due to recruitment constraints. Although the sample size may reduce power to detect small effects, we used appropriate statistical methods for unequal groups and reported effect sizes and confidence intervals. One of the limitations is the lack of physical activity in obese subjects.

5 | Conclusion and Recommendation

Dietary intervention with functional food and dietary advice could improve the anthropometric and the majority of biochemical parameters in obese females. The health benefits of the functional food might be attributed to the presence of polyphenols, flavonoids, linolenic acid, phytosterols, and dietary fibers. The nutritional implication of the present study is that consumption of the present studied functional food together with dietary advice could prevent the progression to metabolic syndrome in obese subjects and help in the reduction of body weight. It is suggested that the functional food could produce more promising health benefits if it is consumed a longer than 2 months safely. This functional food could be produced on an industrial scale and marketed so as to be available for all; shelf-life and microbial safety must be assessed in such a case.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data are present within the manuscript.

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