Formulation and evaluation of novel nutraceuticals rich in protein, vitamins, minerals, natural flavors, and steviol glycosides for improving quality of life

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Research Article

Keywords: Nutraceuticals, Whey protein concentrate and skim milk powder, Vitamins and minerals, Natural flavors, Steviol glycosides, Quality of life (QoL)

Posted Date: June 5th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2992200/v2

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Abstract

For a wide range of people, including malnourished adults with non-communicable diseases (NCDs), micronutrient deficiency, underweight and overweight people, pregnant and lactating women, and adolescents, the current research aimed to present innovative nutraceutical formulations devoted to improving the quality of life (QoL) while promoting health. The novel nutraceutical formulations were structured on a scientific basis with evidence-based health benefits, with daily values (DVs) according to the Food and Drug Administration (FDA), containing a high-quality whey protein concentrate (WPC 70%), skim milk powder (SMP 34%), vitamins (folic acid, B\textsubscript{12}, C), minerals (iron, zinc), natural flavors (vanilla, cinnamon, coffee, chocolate), and stevia leaf extract sweetener. The novel formulations were subjected to the physicochemical, microbiological, and sensory evaluation. The results showed significant differences (P < 0.05) among formulations with natural flavors added in different amounts to provide 10 g of protein (20% DV), elemental iron and zinc, vitamin C, folic acid, and vitamin B\textsubscript{12} (25% DV), with elemental calcium (20% DV) emerged from milk derivatives. The serving sizes of the four formulas were different according to the difference in the amount of natural flavor while keeping the same amounts of the other ingredients. Following the chocolate-flavored formulation as the first in order of overall acceptability, were coffee, cinnamon, and vanilla-flavored formulation, according to the sensory evaluation.

Highlights

- Nutraceuticals are foods with pharmaceutical effects improving the quality of life.
- Four novel nutraceutical formulations were structured on a scientific basis.
- High-quality whey protein concentrate and skim milk powder were used.
- Steviol glycosides, natural flavors, vitamins, and minerals were added.
- The physicochemical, microbiological, and sensory evaluations were investigated.

1. Introduction

Malnutrition and infection have complicated relationships. Malnutrition is the main cause of immunodeficiency in the globe, increasing the risk of non-communicable diseases (NCDs) and the severity of viral infections. Malnutrition is prevalent and at an elevated risk for occurring in more than 70% of hospitalized patients. Therefore, nutritional therapy specialists who use functional foods to enhance quality of life (QoL) and lessen the severity of diseases like diabetes, obesity, heart disease, arthritis, hepatitis, and depression should regularly include the prevention and treatment of malnutrition by supplementing the patients’ diets with healthy nutraceuticals. In order to ensure that therapeutically useful meals with micro- and macronutrients are given in high-quality, safe, nutritionally dense formulae without contaminants, nutritional therapy is also involved in food processing technologies (Almoselhy, 2021a). A food or component of a food that offers medicinal or health advantages, including the prevention or treatment of disease, was the first definition of the term "nutraceutical" given by Stephen L. DeFelice in 1989. Currently, it is redefined as a food product or secondary metabolite capable of providing
health benefits, to treat or prevent diseases in the medical context (Santini & Novellino, 2018; Santos et al., 2022).

Modern lifestyle, genetics, and nutritional overload through high-fat diet (HFD) attributed prevalence and diabetes outcomes with various complications primarily due to obesity in which energy-dense diets frequently affect metabolic health. Adaptation of altered diet systems such as the western diet, Mediterranean diet, low glycemic index diet, relatively low carbohydrate consumption, and vegetarian diet trends to optimize calorie requirements for weight reduction and disease management (Prasad et al., 2022). Wellness, quality of life (QoL), and well-being refer to the positive, personal state that is contrary to illness (Meiselman, 2016). Health-related quality of life (HRQoL) is an important aspect of physical, social, and psychological well-being. Advanced diet quality was prospectively related with better quality of life and functional capability (Gopinath et al., 2014). By 2050, the proportion of people in Europe who are 65 and older is predicted to reach an all-time high and make up more than 30% of the population. The quality of life across the lifetime was the focus of the 6th European Conference on Sensory and Consumer Research, where a key issue was the function of food in healthy ageing (Giacalone et al., 2016). Understanding consumer food choices and creating ways to encourage healthy eating habits depend on seeming well-being (Jaeger et al., 2022). Maintaining a healthy diet during pregnancy may be crucial in creating the groundwork for how children will react to food consumption in their early years (Delahunt et al., 2022).

The usage of nutritional supplements among athletes has significantly expanded, both to improve performance and to satisfy dietary needs. Therefore, adulterated products emerged on the market, with serious side effects such as strokes, acute liver injury, kidney failure, and even death. To identify the adulteration of crucial products, numerous analytical assessments were devised (Hashem et al., 2020; Filho et al., 2022; Martins et al., 2022). 85% of the total milk used to make cheese is discarded as whey, which is rich in valuable components, especially lactose sugar and soluble proteins like beta-lactoglobulin (β-Lg) (40–50%), alpha-lactalbumin (α-La) (12–15%), bovine serum albumin (BSA) (5%), and immunoglobulins (8%) with high contents of essential amino acids and high digestibility with high nutrient content (Gantumur et al., 2023). As a reliable and reasonably priced carrier and stabilizer of bioactive compounds, whey protein concentrate (WPC) has been employed for encapsulation. In comparison to maltodextrin or Arabic gum, it provides higher protection for bioactive compounds (Ji et al., 2022). Whey protein before breakfast may improve postprandial glucose excursions in centrally obese individuals after fasting low-moderate intensity exercise without affecting hunger or subsequent calorie intake (Allerton et al., 2021). Whey protein concentrate (WPC 70% protein) was used over the other forms (isolate or hydrolysate) as it is well-documented the superior characteristics of whey protein concentrate with more bioactive components than the other forms. WPCs are currently used as fat mimetics in reduced-fat formulations (Lammert et al., 2014).

Vanillin, the molecule responsible for the flavor of vanilla, is extracted from vanilla pods, making vanilla, the "Queen" of spices, one of the most widely used flavors in the food and cosmetics sectors worldwide (Wilde et al., 2019). The antioxidative, anti-apoptotic, anti-inflammatory, neuroprotective, and anticancer
properties of vanillin are well documented. Antioxidant, antiproliferative, depressive, and anti-glycating effects are all present in vanilla. Doxo-induced toxicity in H9c2 cardiac cells is lessened by vanilla. Cardiomyocytes could be shielded by vanillin against doxo-induced cell damage. Vanillin was applied therapeutically to mitigate the cardiotoxicity caused by anthracyclines and enhance the long-term efficacy of antineoplastic therapy (Sirangelo et al., 2020).

Cinnamon, due to its wide range of bioactive phenolic compounds, including catechin, protocatechuic acid, quercetin, epicatechin, p-coumaric acid, p-hydroxybenzoic acid, syringic acid, rosmarinic acid, caffeic acid, ferulic acid, and chlorogenic acid, has been used in traditional medicine for its protective role. These compounds have therapeutic effects against inflammation, oxidative stress, diabetes, obesity, hypertension, hypercholeste (Akilen et al., 2013; Das et al., 2022). Due to its functional compound, which also lowers the risk of cancer, hyperlipidemia, and hyperglycemia and has anti-bacterial and anti-tyrosinase activities, cinnamon has been shown to have antimicrobial properties in clinical studies. It is also used as a natural anti-browning additive in the active packaging materials (Senevirathne et al., 2022; Siew et al., 2022).

Coffee contains more than 1000 chemical compounds, from which the biological effects of coffee are due to caffeine, chlorogenic acid, trigonelline, cafestol, kahweol, and ferulic acid (Surma & Oparel, 2021). Caffeinated coffee intake increased to a moderate level (1–7 cups per week) in an elderly Mediterranean cohort at elevated cardiovascular risk, but not at higher levels. linked with a decrease in visceral adipose tissue (VAT), trunk fat, and overall body fat. Decaffeinated coffee was not related to adiposity indicators. In an elderly population with obesity, moderate caffeine consumption may be a weight-management approach (Henn et al., 2023).

Cocoa powder; a rich source of flavonoids has a beneficial role in lipid metabolism due to its polyphenols, reducing the risk of coronary heart disease (CHD), reducing blood pressure (BP), and increasing plasma antioxidant capacity (Khan et al., 2012). Numerous cognitive outcomes are positively impacted by cocoa consumption. These positive benefits appear to be accompanied by a rise in cerebral blood flow or cerebral blood oxygenation following cocoa consumption. Young people who consumed cocoa flavanols on a regular basis showed improved cognitive function and higher levels of neurotrophins (Martín et al., 2020). Flavanols, which are a type of flavonoid, are abundant in cocoa. The antioxidant and anti-inflammatory properties of these substances are what give cocoa its health-promoting properties. Cocoa polyphenols interact in both directions with the gut microbiota once they enter the intestine. These substances have the ability to alter the gut microbiota's makeup through prebiotic mechanisms. They inhibit the development of pathogenic bacteria like Clostridium perfringens while promoting the growth of beneficial gut bacteria like Lactobacillus and Bifidobacterium. Contrarily, bioactive cocoa metabolites can improve gut health by acting as anti-inflammatory agents, boosting immunity, and lowering the chance of a number of diseases (Sorrenti et al., 2020).

Stevia leaf extract as a zero-calorie sweetener contains steviol glycosides, chlorogenic acids, caffeoylquinic acids, and dicaffeoylquinic acids, with great health benefits, antioxidant, antibacterial,
antiviral, and anti-inflammatory effects (Ai et al., 2022; Moongngarm et al., 2022). Stevia preparation display health-promoting effects, especially the antidiabetic action. Steviol glycosides are the compounds responsible for the sweet taste of stevia (Gardana & Simonetti 2018; Molina-Calle et al., 2016). Steviol glycosides have anti-inflammatory, oral health-promoting, anti-hypertensive, and cancer-prevention properties. Additionally, they assist in controlling blood sugar levels by influencing glucose uptake, enhancing insulin production, or raising the concentration of glucose transporters (Kurek & Krejpcio, 2019).

In light of the growing need for high-quality and secure nutraceutical formulations, the current study set out to develop and assess four innovative dietary supplements in order to establish their validity with the most desirable qualities and consumer acceptance. It was determined using physicochemical, microbiological, sensory analysis, and nutrition data.

2. Materials and Methods

2.1. Materials

2.1.1. Raw Materials

All ingredients used in the processing of the novel nutraceutical formulations were purchased from the local market in Egypt, such as whey protein concentrate (WPC 70% protein), skim milk powder (SMP 34% protein), natural flavors of vanilla, cinnamon, instant coffee, and raw dark cocoa, zero calorie sweetener of stevia leaves extract (steviol glycosides), anti-caking agent as silicon dioxide (silica - E551), ferrous fumarate (33% elemental iron), zinc gluconate (13% elemental zinc), ascorbic acid (vitamin C), sodium folate (98% folic acid - vitamin B₉), and cyanocobalamin (10% vitamin B₁₂).

2.1.2. Chemicals and Reagents

All solvents and chemicals used in the study were of analytical and HPLC grade.

2.2. Methods

2.2.1. Formulation of the nutraceutical formulations

The novel nutraceutical formulations were designed according to the method described in the Egyptian Standards (ES:2730/2007) for “Protein-Rich Supplementary Foods” (Committee Name: Food for Special Dietary Uses) referenced mainly from the Indian Standard (IS: 8220/1976) for “Specification for Protein-Rich Concentrated Nutrient Supplementary Foods” with modifications recommended by the Special Food
All ingredients as shown in Table (1), were mixed and homogenized using a mechanical high-speed homogenizer HSH (Ingenieurbüro CAT, M. Zipperer GmbH, Drive motor Unidrive X 1000 D, Germany) at 1000 rpm for 2 min., then packaged in sealed metallized polypropylene (MPP) sachets and kept in a cool dry place away from direct sunlight till analysis and sensory evaluation.

**Analytical Methods**

**Physical characteristics and proximate composition of the nutraceutical formulations**

The novel nutraceutical formulations were evaluated for physical characteristics and proximate composition. Appearance, texture, and flavor were determined along with moisture, ash, protein, fat, fiber, and carbohydrate content (calculated by difference) according to the AOAC Official Method 965.33 (2016) and the total energy (Calories) per 100 g was calculated.

**Determination of steviol glycosides in the nutraceutical formulations**

Steviol glycosides content was determined according to the method described by Vaněk et al. (2001) using high-performance liquid chromatography (HPLC).

**Determination of vitamins in the nutraceutical formulations**

Folic acid (vitamin B$_9$) and vitamin B$_{12}$ were determined according to the method described by Salvati et al. (2016) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Whereas, vitamin C was determined according to the AOAC Official Method 967.21 (2012) using high-performance liquid chromatography (HPLC).

**Determination of minerals and heavy metals in the nutraceutical formulations**

Minerals (sodium, calcium, iron, and zinc) and heavy metals (lead, cadmium, and arsenic) were determined in the novel nutraceutical formulations according to the method described by Sepe et al. (2003). Sodium and calcium were determined using inductively coupled plasma optical emission
spectrometry (ICP-OES). Whereas, iron, zinc, lead, cadmium, and arsenic were determined using inductively coupled plasma mass spectrometry (ICP-MS).

**Determination of preservatives in the nutraceutical formulations**

Preservatives (benzoates and sorbates) were determined in the novel nutraceutical formulations according to the method described by Saad et al. (2005) using high-performance liquid chromatography (HPLC).

**Chemical migration testing for the packing material (metallized polypropylene MPP)**

Testing for chemical migration is done on packing materials that come into touch with food to see if chemicals are transferred from packing materials to food. The chemical migration test directs the manufacturers to examine the security of packaging materials, which should not endanger people or alter the characteristics of food. According to the method described by Zhang et al., (2022), a chemical migration test was performed on packing material made from metallized polypropylene (MPP).

**Microbiological analysis of the nutraceutical formulations**

According to the procedure outlined in ISO 4833-1:2013 (2013), the total combined yeasts and molds count (TYMC) and the aerobic plate count (APC) were performed. Whereas, the detection of *Salmonella* was carried out in accordance with ISO 6579-1:2017 (2017a), the detection of *Bacillus cereus* was carried out in accordance with ISO 7932: 2004 (2004), the detection of *Escherichia coli* was carried out in accordance with ISO 16649-2: 2001 (2001), and the detection of *Listeria species* was carried out in accordance with ISO 11290-1:2017 (2017b).

**Determination of aflatoxin and deoxynivalenol in the nutraceutical formulations**

Analysis of aflatoxin (B1, B2, G1, G2) and deoxynivalenol was carried out according to the method described by Sulyok et al. (2020) using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

**Sensory evaluation of the nutraceutical formulations**

30 trained sensory panelists in the field of food science (15 women and 15 men, ages 25 to 70) were chosen on the basis of voluntariness with the understanding that the samples under evaluation were
The only participants in this research had to be regular users of this kind of product. Participants who had a milk, flavor, or sweetener allergy were not permitted to join. A nine-point Hedonic scale, which indicates the degree of participants' general liking or disliking for quality characteristics such as appearance, aroma, taste, texture, and acceptability, was used to ask participants to rate the product. According to the evaluator, each attribute's score ranged from 1 to 9 (1: extremely dislike; 9: extremely like). The average intensity score for each attribute was determined and tallied when the evaluation process was finished. The samples were given to the panelists in the form of powdered nutraceutical formulations in sealed sachets labelled with the four flavors (vanilla, cinnamon, coffee, and chocolate), to be prepared with cold drinking water in disposable plastic cups with spoons. To ensure that the items given to the panelists were of the greatest acceptable quality and safety, the samples were kept for 6 months (the period of chemical and microbiological examination) before the sensory evaluation was conducted.

**Nutrition facts of the nutraceutical formulations**

Nutrition facts of the nutraceutical formulations were determined from the analytical methods such as proximate composition, minerals, and vitamins compliant with the recommendations of the Food and Drug Administration (FDA, 2013).

**Statistical analysis**

All measurements were performed in three replicates, whereas the sensory evaluation was carried out in 30 replicates. Data were shown as mean ± standard deviation (SD). Analysis of variance (ANOVA) was conducted with the SPSS software at P<0.05.

**3. Results And Discussion**

**Physical characteristics and proximate composition of the nutraceutical formulations**

The proximate composition (moisture, ash, protein, fat, fiber, carbohydrate, and energy) of the novel nutraceutical formulations is shown in Table (2). It is noticed significant differences (P<0.05) among different formulations due to the differences in composition because of the different amounts of the natural flavors added, as the nutraceutical formulations were designed according to the specific regulations which recommend labeling the serving unit which differs among the formulations as follows; vanilla-flavored formulation (21 g); cinnamon-flavored formulation (23 g); coffee-flavored formulation (23 g); chocolate-flavored formulation (25 g), and we evaluated the proximate composition on a 100 g basis of each formulation which makes differences between the formulations despite being containing the same ingredients other than the natural flavors. Also, the added natural flavors resulted in differences in proximate compositions as some of them have excess plant protein content as in the chocolate-flavored...
formulation which contains raw dark cocoa powder with plant protein content, fat, carbohydrate, fiber, minerals, and vitamins. Also, the cinnamon-flavored formulation contains carbohydrates and fiber, whereas, the coffee-flavored formulation powder contains carbohydrates exceeding that of the vanilla-flavored formulation.

Steviol glycosides, vitamins, minerals, heavy metals, and preservatives in the nutraceutical formulations

Steviol glycosides, vitamins, minerals, heavy metals, and preservatives in the dietary supplements were determined and tabulated in Table (3). No significant difference was detected among all samples considering the content of steviol glycoside which was the same in all samples coinciding with the preparation content added in the same amounts per 100 g of formulation. Significance differences (P<0.05) among formulas were observed in vitamins and minerals contents due to the difference in composition as the additives were added in different ratios according to the serving unit which was 21, 23, 23, 25 g for the vanilla, cinnamon, coffee, and chocolate-flavored formulation, respectively (not per 100 g). Cinnamon-flavored and chocolate-flavored formulations had more contents of iron and zinc due to the richness of cinnamon and cocoa powders in the 2 elements. No heavy metals (lead, cadmium, and arsenic) were detected in any of the samples. Also, no preservatives (benzoates and sorbates) were detected in any of the samples. The results indicate the safety of products for human consumption.

Chemical migration analysis for the packing material

The concentrations of the measured chemical elements were compared with their migration limits in accordance with the Commission Regulation (EU) No. 10/2011 on plastic materials and articles intended to come into contact with food in order to quantify the chemical migration and risk of packing materials. Table (4) displays the results of the chemical migration testing, all of which fell below the limit of detection (LOD), which is set at 0.01 ppm for all elements based on the instruments used. This proves the safety of the packing material made from metallized polypropylene (MPP), which is used in product packaging (Abd El-Baset & Almoselhy, 2023). MPP is a superior food packaging material that protects the superior quality and safety of food products because the aluminum layer used in metallized packing is directly applied onto the polymer surface of the packing material through the vacuum deposition technique without the use of adhesives, which could potentially cause toxic chemicals from the adhesive layer to migrate into a food matrix in laminated multilayer films of packing materials (Zhang et al., 2022).

Microbiological analysis of the nutraceutical formulations
Table (5) shows the results of the microbiological analysis of the nutraceutical formulations. Microbial contamination and the presence of spores were not detected. The maximum values observed for the aerobic plate count (APC) and total combined yeasts and molds count (TYMC) were 0.63 and 0.22 CFU/g, respectively. No *Bacillus cereus* and *E. coli* were detected in any sample. *Salmonella spp.* and *Listeria spp.* were absent in all samples. Also, there were significant differences (P<0.05) in APC and TYMC between different samples under a different type of flavor which reflects the effect of natural flavor on the microbiological activity of the product, where the APC values were 0.30, 0.40, 0.53, and 0.63 CFU/g in cinnamon, coffee, vanilla, and chocolate-flavored formulations, respectively. The TYMC values were 0.13, 0.15, 0.17, and 0.22 CFU/g in cinnamon, coffee, vanilla, and chocolate-flavored formulations, respectively, indicating the superiority of cinnamon as an antimicrobial followed by coffee, vanilla, and chocolate flavor. The detected values of the microbiological activity in all samples were lower than the permitted range stipulated by the Commission Regulation (EC) No 2073/2005.

**Aflatoxin and deoxynivalenol analyses in the nutraceutical formulations**

Determination of aflatoxin and deoxynivalenol is considered a routine analysis in food samples with the emergence of mycotoxins in dairy products as a result of the contamination of the milk by the contaminated feeding stuff consumed by the cows or by the direct fungal contamination of the dairy product which may result in the formation of mycotoxins (*Becker-Algeri et al., 2016; Flores-Flores & González-Peñas, 2018; Van Egmond, 1983*). From the results shown in Table (6), for aflatoxin (B1, B2, G1, G2) and deoxynivalenol, the obtained values did not reach the limit of quantitation (LOQ) by the analytical instrument which has been assigned as 0.2 µg/kg for aflatoxins and 50 µg/kg for deoxynivalenol. These values (LOQ) were considerably less than the ranges permitted by the official regulations. Thereby, all the tested samples of the nutraceutical formulations with different types of natural flavor were safe for consumption as the aflatoxins and deoxynivalenol values did not reach the benchmark values assigned by Commission Regulation (EU) No 165/2010 for aflatoxins and Commission Recommendation (2006/576/EC) for deoxynivalenol. Also, there were no significant differences between different samples under different types of flavor, and hence, there was no effect of flavor on the contents of aflatoxins and deoxynivalenol which indicated the absence of any contamination during the processing of products.

**Sensory evaluation of the nutraceutical formulations**

Sensory evaluation of the nutraceutical formulations is an important step to knowing the consumer’s perception of the final product. The sensory attributes of appearance, aroma, taste, texture, and overall liking were evaluated (P<0.05) as being in the range of 1 to 9. As shown in Table (7), there were significant differences (P<0.05) in all sensory attributes. In terms of appearance, taste, and texture, the chocolate-flavored formulation was the best followed by coffee, cinnamon, and vanilla with the last
position despite the vanilla-flavored formulation being the best in the aroma followed by a cinnamon, coffee, and chocolate-flavored formulation which reflects the apparent effect of changing natural flavors. The chocolate-flavored formula was the best acceptable according to the sensory evaluation scores, followed by coffee, cinnamon, and vanilla-flavored formulations.

**Nutrition facts of the nutraceutical formulations**

Nutrition facts (Figure 1.) are considered a mandatory act for labeling dietary supplements with essential nutrients including fat, carbohydrate, dietary fiber, protein, minerals, vitamins, and calories per serving for health purposes. 2,000 calories a day is used for general nutrition advice (FDA, 2013). Considering the nutrition facts information provided on the outer package, the labeled food product is better than the unlabeled. The labeling of food products as containing "natural" ingredients creates a potent marketing tool, as consumers pay more for natural products that express naturalness and being a better and more safe product (Hartmann et al., 2018). To endorse healthier eating behaviors, regulatory authorities around the world encourage food labeling with mandatory basic nutrition information on the front of the pack (FOP) with the calorie and nutrition information already provided on the back or the side of the package. It was shown that the most effective nutrition label increased the promotion, marketing, and sales of foods (Dubois et al., 2021). The Five-Color Nutrition Label based was effective in promoting healthier food products (Ducrot et al., 2016).

Figure 1. shows the nutrition facts of the four nutraceutical formulations where significant differences are shown among all products considering the energy per serving. The chocolate-flavored formulation had the highest energy per serving at 90 calories followed by coffee (86 calories), cinnamon (84 calories), and vanilla with the least energy per serving (79 calories). The differences were attributed to the different compositions of the added flavor as the raw dark cocoa contains excess dietary fiber, carbohydrate, fat, and plant protein which causes the excess increase in values compared with vanillin which had the least calories. Also, cinnamon had dietary fiber and carbohydrates, and instant coffee had carbohydrates.

It is noticeable to say that all formulations were low-calorie without any vegetable fat added from refined edible oils (as many products have a considerable vegetable fat content as a source of energy) to avoid the processing contaminants emerging in the refined edible oils (Almoselhy, 2021b; Almoselhy et al., 2021) and the products were free from any added sugars or animal fats except for the very low-fat content in the skim milk powder (SMP 34% protein) and whey protein concentrate (WPC 70% protein) and the total sugars contents appearing in the nutrition facts were calculated from the milk sugar (lactose) which is present in SMP and WPC. The aforementioned values in nutrition facts tables will be helpful to the consumers to guide them in selecting the most suitable formulations for their health benefits.

**Novelty impact statement and health benefits of the innovative nutraceutical formulations**
The novelty impact statement of the current study can be summed up as a new concept for the formulation of nutraceutical dietary supplements on a scientific basis in accordance with the mandatory registration regulations of the National Nutrition Institute (NNI) - Ministry of Health (MOH) - Egypt, with daily values in accordance with the FDA's recommendations (FDA, 2013). The formulations were free from artificial colors, flavors, fillers, and preservatives to avoid any potential negative effects including allergic reactions, weight gain, gas, flatulence, and bloating. To adhere to the assertions that added sugars should be reduced in a healthy diet, no added sugar was present. The benefits of the present formulations are derived from the use of the improved high-speed homogenization technique for product processing (Almoselhy, 2022, Azab et al., 2022) and the appropriate ingredient choice, starting with the high-quality protein source of whey protein concentrate (WPC 70% protein) and skim milk powder (SMP 34% protein) apart from the cheap plant protein such as soy protein, especially with the raised concerns in many studies that the phytoestrogens (isoflavones) in soy may feminize men including lowering testosterone levels and raising estrogen levels in men despite the results of a recent study with no significant effects of soy protein or isoflavone intake indicating that regardless of dose and study duration, neither soy protein nor isoflavone exposure affects total testosterone (TT), testosterone (FT), estradiol (E₂) or estrone (E₁) levels in men (Reed et al., 2021). In addition to being a good source of calcium and milk protein, skim milk powder (SMP 34%) was included as a functional ingredient utilized as a natural nutraceutical filler to improve the texture and taste and ensuring shelf life stability. Vitamins (folic acid, B₁₂, C), minerals (iron, zinc), the healthy sweetener (steviol glycosides), and natural flavors (vanilla, cinnamon, coffee, chocolate) were added in effective concentrations and all the formulations were processed and packaged in sealed metallized polypropylene (MPP).

**Conclusions**

The current research paper’s goal was to present novel nutraceutical dietary supplements on a scientific basis with validated health benefits and examine their physicochemical, microbiological, and sensory characteristics to ensure their quality and safety for consumption by adults in order to fight malnutrition and support a healthy lifestyle. It was determined that all formulations were of the highest quality and safety, with the greatest customer acceptance being recorded in the following order: chocolate > coffee > cinnamon > vanilla-flavored formulations.

**Declarations**

**Ethics approval and consent to participate**

Sensory evaluation performed in this study was carried out in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.
Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' contributions

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

Acknowledgements

Not applicable.

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References


Tables

**Table 1. Nutritional composition of formulations per 100 gm (ingredients expressed in grams)**

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Vanilla</th>
<th>Cinnamon</th>
<th>Coffee</th>
<th>Chocolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey protein concentrate (WPC 70%)</td>
<td>38.38±1391</td>
<td>35.04±4427</td>
<td>35.04±4718</td>
<td>35.24±1328</td>
</tr>
<tr>
<td>Skim milk powder (SMP 34%)</td>
<td>61.09±094</td>
<td>55.76±769</td>
<td>55.76±769</td>
<td>51.28±88</td>
</tr>
<tr>
<td>Natural flavor</td>
<td>0.01</td>
<td>8.69±696</td>
<td>8.69±696</td>
<td>16</td>
</tr>
<tr>
<td>Steviol glycosides</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Silica (E551)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Sodium folate (98% Folic acid – B9)</td>
<td>0.000490</td>
<td>0.000448</td>
<td>0.000448</td>
<td>0.000412</td>
</tr>
<tr>
<td>Cyanocobalamin (10% Vitamin B12)</td>
<td>0.000071</td>
<td>0.000065</td>
<td>0.000065</td>
<td>0.000060</td>
</tr>
<tr>
<td>Ascorbic acid (Vitamin C)</td>
<td>0.071429</td>
<td>0.065217</td>
<td>0.065217</td>
<td>0.06</td>
</tr>
<tr>
<td>Ferrous fumarate (33% Fe)</td>
<td>0.065238</td>
<td>0.059565</td>
<td>0.059565</td>
<td>0.0548</td>
</tr>
<tr>
<td>Zinc gluconate (13% Zn)</td>
<td>0.137381</td>
<td>0.125435</td>
<td>0.125435</td>
<td>0.1154</td>
</tr>
</tbody>
</table>

**Table 2. Physical characteristics and proximate composition of the nutraceutical formulations**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vanilla</th>
<th>Cinnamon</th>
<th>Coffee</th>
<th>Chocolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Creamy white</td>
<td>Brownish</td>
<td>Brownish</td>
<td>Brownish</td>
</tr>
<tr>
<td>Texture</td>
<td>Fine powder</td>
<td>Fine powder</td>
<td>Fine powder</td>
<td>Fine powder</td>
</tr>
<tr>
<td>Flavor</td>
<td>Vanilla</td>
<td>Cinnamon</td>
<td>Coffee</td>
<td>Chocolate</td>
</tr>
<tr>
<td>Moisture %</td>
<td>4.28±0.20d</td>
<td>4.50±0.12b</td>
<td>4.43±0.09c</td>
<td>4.66±0.12a</td>
</tr>
<tr>
<td>Ash %</td>
<td>4.45±0.03d</td>
<td>4.87±0.00b</td>
<td>4.54±0.06c</td>
<td>4.97±0.03a</td>
</tr>
<tr>
<td>Protein %</td>
<td>48.05±0.02a</td>
<td>44.17±0.03b</td>
<td>44.04±0.03c</td>
<td>42.64±0.08d</td>
</tr>
<tr>
<td>Fat %</td>
<td>2.85±0.01b</td>
<td>2.61±0.03c</td>
<td>2.61±0.02c</td>
<td>4.00±0.06a</td>
</tr>
<tr>
<td>Fiber %</td>
<td>0.00±0.00c</td>
<td>4.60±0.04b</td>
<td>0.00±0.00c</td>
<td>5.28±0.06a</td>
</tr>
<tr>
<td>Carbohydrate %</td>
<td>40.37±0.13b</td>
<td>39.25±0.27c</td>
<td>44.38±0.17a</td>
<td>38.45±0.43d</td>
</tr>
<tr>
<td>Energy (calories)</td>
<td>379.33±0.38a</td>
<td>366.37±0.63d</td>
<td>377.17±0.44b</td>
<td>370.92±0.85c</td>
</tr>
</tbody>
</table>
Values are mean ± standard deviation (n=3). a-d Means in the same row with different superscripts are significantly different (P<0.05).

**Table 3. Determination of steviol glycosides, vitamins, minerals, heavy metals, and preservatives in the nutraceutical formulations**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
<th>Vanilla</th>
<th>Cinnamon</th>
<th>Coffee</th>
<th>Chocolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetener</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steviol glycosides (mg/kg)</td>
<td></td>
<td>2004±2^a</td>
<td>2000±3^a</td>
<td>2001±1^a</td>
<td>2003±1^a</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid (Vitamin B₉) µg/kg</td>
<td></td>
<td>4763±13^a</td>
<td>4350±24^c</td>
<td>4356±10^b</td>
<td>4084±4^d</td>
</tr>
<tr>
<td>Cyanocobalamin (Vitamin B₁₂) µg/kg</td>
<td></td>
<td>74±5^a</td>
<td>65±6^b</td>
<td>65±4^b</td>
<td>62±2^c</td>
</tr>
<tr>
<td>Ascorbic acid (Vitamin C) mg/kg</td>
<td></td>
<td>715±5^a</td>
<td>656±7^b</td>
<td>652±2^c</td>
<td>601±1^d</td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mg/kg)</td>
<td></td>
<td>1192±5^b</td>
<td>1088±9^c</td>
<td>1087±8^c</td>
<td>1292±12^a</td>
</tr>
<tr>
<td>Calcium (mg/kg)</td>
<td></td>
<td>9533±17^a</td>
<td>8863±13^b</td>
<td>8696±11^c</td>
<td>8156±16^d</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td></td>
<td>217±5^b</td>
<td>780±4^a</td>
<td>196±4^d</td>
<td>201±3^c</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td></td>
<td>180±8^c</td>
<td>380±6^a</td>
<td>164±6^d</td>
<td>305±7^b</td>
</tr>
<tr>
<td>Heavy metals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead (mg/kg)</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cadmium (mg/kg)</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Arsenic (mg/kg)</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Preservatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzoates (as Benzoic acid) mg/kg</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sorbates (as Sorbic acid) mg/kg</td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: Not detected.

Values are mean ± standard deviation (n=3). a-d Means in the same row with different superscripts are significantly different (P<0.05).

**Table 4. Chemical migration testing for the packing material (metalized polypropylene - MPP)**

<table>
<thead>
<tr>
<th>Chemical element (ppm)</th>
<th>Formula</th>
<th>After soaking in 10% ethyl alcohol</th>
<th>After soaking in 3 acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper (Cu)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Barium (Ba)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Lithium (Li)</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
</tbody>
</table>

LOD – Limit of detection (0.01 ppm)

**Table 5. Microbiological analysis of the nutraceutical formulations**
ND: Not detected

Values are mean ± standard deviation (n=3). a-d Means in the same row with different superscripts are significantly different (P<0.05).

**Table 6. Determination of aflatoxin and deoxynivalenol in the nutraceutical formulations**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Vanilla</th>
<th>Cinnamon</th>
<th>Coffee</th>
<th>Chocolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B1</td>
<td>&lt;LOQ (0.2 µg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxin B2</td>
<td>&lt;LOQ (0.2 µg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxin G1</td>
<td>&lt;LOQ (0.2 µg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxin G2</td>
<td>&lt;LOQ (0.2 µg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>&lt;LOQ (50 µg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LOQ – Limit of quantitation

**Table 7. Sensory evaluation of the nutraceutical formulations**

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>Formula</th>
<th>Vanilla</th>
<th>Cinnamon</th>
<th>Coffee</th>
<th>Chocolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td></td>
<td>8.23±0.21±0.03</td>
<td>8.73±0.03</td>
<td>8.82±0.03</td>
<td>8.92±0.07</td>
</tr>
<tr>
<td>Aroma</td>
<td></td>
<td>8.72±0.03</td>
<td>8.43±0.05</td>
<td>8.20±0.05</td>
<td>8.06±0.04</td>
</tr>
<tr>
<td>Taste</td>
<td></td>
<td>8.25±0.05</td>
<td>8.28±0.08</td>
<td>8.42±0.06</td>
<td>8.76±0.13</td>
</tr>
<tr>
<td>Texture</td>
<td></td>
<td>8.24±0.06</td>
<td>8.33±0.06</td>
<td>8.54±0.10</td>
<td>8.90±0.10</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td></td>
<td>8.36±0.09</td>
<td>8.44±0.14</td>
<td>8.50±0.06</td>
<td>8.66±0.09</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (n=30). a-d Means in the same row with different superscripts are significantly different (P<0.05).

**Figures**
Figure 1

Nutrition Facts

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.