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New aspects on nutraceutical formulations of whey protein concentrate sweetened with steviol glycosides for patients with non-communicable diseases

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ABSTRACT

The current paper aimed to highlight new aspects of nutraceuticals with proven health benefits for patients with non-communicable diseases (NCDs). Four nutraceutical formulations were structured scientifically according to the Indian Standard (IS: 8220/1976) for "Specification for Protein-Rich Concentrated Nutrient Supplementary Foods". High-quality milk derivatives with considerable protein content (10g protein/serving) rich in essential amino acids with valuable branched chain amino acids (BCAAs) reaching about 18% of total amino acids content and calcium content 325 mg/serving were included in whey protein concentrate (WPC) and skim milk powder (SMP). Zero-calorie artificial sweetener steviol glycosides (SGs) from a natural source (stevia leaf extract) were added with vitamins, minerals, and natural flavors. One of the new aspects is the valuable nutrient-nutrient interaction, as in the synergistic effect between WPC and SGs. Also, the presence of natural flavors (vanilla-cinnamon-coffee-raw cocoa) has great effects with their contents of bioactive components and antioxidants. Valuable applications of these nutraceuticals were discussed here to show their medicinal benefits with regular consumption by patients with NCDs and those with special food needs, such as in malnourished people, pregnant and lactating women, adolescents, and athletes to improve their physical activity, mentality, and overall performance summed up in the term of improving quality of life (QoL). Research design was carried out by applying evidence-based approaches. Well-structured scientific publications were examined thoroughly to collect updated information about using these nutraceuticals as adjuvant therapy in the treatment protocols for patients with NCDs.

KEYWORDS

Nutraceuticals; Branched-chain amino acids; Whey protein concentrate; Steviol glycosides; Quality of life

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Introduction

Malnutrition and the role of nutraceuticals in improving quality of life (QoL) in patients with non-communicable diseases (NCDs)

Malnutrition increased with the incidental climate change, resulting in decreased crop productivity, negatively affecting food security in almost all countries [1]. Infection and malnutrition have complex interactions. Worldwide, immunodeficiency is mostly brought on by malnutrition, which raises the risk of NCDs and the severity of viral infections. More than 70% of hospitalized patients are at a higher risk of developing malnutrition. Therefore, nutritional therapy specialists should regularly include the prevention and treatment of malnutrition by enhancing the patients' diets with healthy nutraceuticals or functional foods to improve QoL and lessen the severity of NCDs like diabetes, obesity, heart disease, arthritis, hepatitis, and depression. Nutritional treatment is also involved in food processing technologies to ensure that therapeutically effective meals with micro- and macronutrients are supplied in high-quality, secure, nutritionally packed formulations without contaminants [2].

Nutraceuticals in Iranian traditional medicine

The foundation of Iranian traditional medicine is food treatment. Avicenna and Abu Reyhan Biruni were two well-known Iranian scientists who healed patients by merely altering their diets. As a result, classic medical texts written in Persian and other Muslim languages list and describe a substantial variety of herbs, medicinal plants, and food flavorings. It is interesting to note that choosing a certain variety of plant, fruit, or spice from a specific region was frequently strongly advised, according to research on ancient Iranian medical and pharmacology writings. This reveals the ancient doctor's interest in biodiversity and how climate affects food's nutritional value and, consequently, its curative capabilities [3]. The usage of herbs in a health-promoting lifestyle is approved by the scientific community with an increment of 68% during the Covid-19 pandemic by rural women [4].

Historical background and significance of steviol glycosides (SGs) sweetener

Stevia was first identified by Moises Santiago de Bertoni as *Eupatorium rebaudianum* in Paraguay in 1899. It was given the new name *Stevia rebaudiana* in 1905 and belonged to the same family as chrysanthemums and sunflowers (*Asteraceae*). The plant's sweetening components were identified by French

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chemists in 1931 and given the name steviol glycosides. China began cultivating *S. rebaudiana* in 1984 and is currently the market leader in the stevia sector. The FDA and GRAS officially approved its usage in foods and beverages in the USA in 2008. The use of SGs as a sweetener in foods and beverages was authorized by the European Food Safety Authority (EFSA) in 2011. Today, stevia is consumed by 5 billion people worldwide. The market is filled with a variety of artificial sweeteners, including saccharine, aspartame, cyclamate, sodium benzoate, sucralose, neotame, and acesulfame. These are all low-calorie sweeteners used in the food and pharmaceutical industries. In contrast to SGs, their adverse effects on human health have limited the public use of them. The Food and Drug Administration (FDA) and the European Union (EU) both approved the SGs in 2008 and 2011, respectively. *S. rebaudiana* Bertoni is sweet, low in calories, and rich in nutrients. It includes a variety of sterols and anti-oxidants, including phenols, flavonoids, tannins, kaempferol, quercetin, riboflavin, alpha-carotene, and chlorogenic acid, all of which have a key role in controlling blood pressure, cholesterol, and blood sugar. Additionally, it has anti-microbial, anti-oxidant, anti-inflammatory, and anti-diabetic properties [5].

The EFSA assessed the safety of SGs as a food additive in 2010, and an acceptable daily intake (ADI) of 4 mg/kg body weight (bw) was determined [6]. Substituting the added sugar in diets with SGs endorsed weight loss and a decrease in waist circumference in persons suffering from overweight [7]. SGs modulate adipogenic and lipogenic gene expression, decreasing the accumulation of lipids in mature adipocytes and enhancing glucose uptake with mitigation of insulin resistance. SGs are potent ingredients in functional foods for health promotion [8].

Nutraceutical properties of the natural flavors in formulations

Nutraceutical properties of vanilla

The "Queen" of spices, vanilla, is one of the most commonly used flavors in the food and cosmetics industries globally, with vanillin, the chemical that gives vanilla its flavor, extracted from vanilla pods [9]. Vanillin has been shown to have antioxidant, anti-apoptotic, anti-inflammatory, neuroprotective, and anticancer effects. Vanilla has antioxidant, antiproliferative, depressive, and anti-glycating properties. Vanilla reduces the toxicity caused by doxo in H9c2 cardiac cells. Vanillin may protect cardiomyocytes against doxo-induced cell toxicity. Vanillin was employed therapeutically to reduce the cardiotoxicity of anthracyclines and improve the long-term effectiveness of antineoplastic therapy [10].

Nutraceutical properties of cinnamon

Cinnamon has been used in traditional medicine for its protective properties 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. These substances exhibit anti-inflammatory, anti-oxidative, and therapeutic actions against diabetes, obesity, hypertension, and hypercholesterolemia [11,12]. The aromatic phenolic eugenol and cinnamaldehyde in cinnamon have inhibitory effects on *A. hydrophila*, *S. aureus*, *L. monocytogenes*, *E. coli*, and *S. enteritidis* [13]. Cinnamon has been demonstrated to have anti-bacterial qualities in clinical tests due to its functional component, which also reduces the risk of cancer, hyperlipidemia, and hyperglycemia and has anti-bacterial and anti-tyrosinase activity. The active packaging materials also use it as a natural anti-browning additive [14,15].

Nutraceutical properties of coffee

The biological benefits of coffee are attributed to caffeine, chlorogenic acid, trigonelline, cafestol, kahweol, and ferulic acid, among its more than 1000 chemical constituents [16]. In an older Mediterranean cohort with heightened cardiovascular risk, caffeine consumption increased to a moderate level (1–7 cups per week) but not greater levels. Associated with reduced body fat, trunk fat, and visceral adipose tissue (VAT). There was no connection between decaffeinated coffee and adiposity markers. Moderate caffeine use may be a weight-management strategy in an elderly population with obesity [17].

Nutraceutical properties of cocoa

According to Khan et al., cocoa powder, a rich source of flavonoids, plays a favorable function in lipid metabolism by lowering blood pressure, boosting plasma antioxidant capacity, and reducing the risk of coronary heart disease (CHD) [18]. Consuming cocoa has a favorable effect on many cognitive outcomes. Following cocoa consumption, there appears to be an increase in cerebral blood flow or cerebral blood oxygenation, which may be related to these favorable effects. The cognitive performance and neurotrophin levels of young people who regularly ingested cocoa flavanols improved [19]. In cocoa, there are lots of flavanols, a type of flavonoid. These compounds' anti-inflammatory and antioxidant capabilities are what give cocoa its health-promoting effects. Once they reach the intestine, cocoa polyphenols interact with the gut flora in bidirectional interactions. Through prebiotic mechanisms, these chemicals have the power to change the composition of the gut flora. They encourage the growth of Lactobacillus and Bifidobacterium, two healthy gut bacteria while inhibiting the growth of harmful bacteria like *Clostridium perfringens*. By acting as anti-inflammatory agents, enhancing immunity, and reducing the risk of several diseases, bioactive cocoa metabolites, on the other hand, can enhance gut health [20].

Nutraceutical and functional properties of whey protein concentrate (WPC)

Whey protein (WP), a powdered protein supplement, consists of a group of globular proteins, including immunoglobulins, bovine serum albumin (BSA), and α-lactoalbumin and β-lactoglobulin. WP is regarded as having great biological value because of its high essential amino acid content and excellent digestibility, promoting more effective gastrointestinal absorption. WPC has a maximum protein concentration of 80%, a low-fat content, and typically more beneficial components and lactose-based carbohydrates [21]. WPC was frequently used as a functional additive to enhance the functional characteristics of food products [22].

Branched Chain Amino Acids (BCAAs)

Approximately 18% of all amino acids and 63% of all hydrophobic amino acids are BCAAs, the main building blocks of most proteins. Leucine, isoleucine, and valine often have molar ratios of 2.2:1.0:1.6, demonstrating the interconnected nature of their metabolisms. Humans and animals are unable to synthesize BCAAs; as a result, they must receive them through food digestion. The intake and excretion of BCAAs maintain a delicate equilibrium under physiological circumstances. The

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body's BCAA supply comprises tissue (liver and skeletal muscle protein) and circulatory pools. The amount of BCAAs in the bloodstream is generally steady since they are mostly obtained by diet and tissue protein breakdown; they are primarily used up by oxidative breakdown and tissue protein synthesis, and their urine excretion is negligible. In addition to serving as nutritional substrates for protein synthesis, BCAAs also play significant roles in regulating energy balance, immunological response, and food metabolism through a number of signalling pathways. Abnormalities in BCAAs metabolism have been linked to a number of illnesses, a higher risk of diabetes, cancer, heart failure, and numerous fibrotic disorders [23].

In several clinical circumstances where proteolysis is elevated, branched-chain amino acid (BCAA) supplementation is utilized to enhance protein synthesis. Additionally, hepatic encephalopathy risk has been linked to reduced plasma BCAA levels in liver cirrhosis patients. According to the evidence, using BCAA supplements results in a little increase in muscle mass and body mass index, a rise in physical strength, and no change in fat mass. Additionally, BCAA supplementation reduces hepatic encephalopathy symptoms and is recommended as a second-line treatment [24].

The current paper aimed to highlight new aspects on nutraceutical formulations of WPC for patients with NCDs. The research design was carried out by applying evidence-based approaches. Well-structured scientific publications were examined thoroughly to collect updated information about using these nutraceuticals as adjuvant therapy in the treatment protocols for patients with NCDs to improve QoL.

Materials and Methods

Materials

Whey protein concentrate (WPC) of 70% protein content, skim milk powder of 34% protein content (SMP 34%), natural flavors in powders of vanilla, cinnamon, instant coffee, and raw dark cocoa, artificial sweetener of natural origin as SGs (stevia leaves extract), silicon dioxide of food grade (silica - E551) as an anti-caking agent added to powders, a mixture of added vitamins and minerals including ferrous fumarate (33% Fe), zinc gluconate (13% Zinc), ascorbic acid (vit. C), sodium folate (98% folic acid – vit. B_9), and cyanocobalamin (10% vit. B_{12}) were purchased from the local markets in Egypt [2].

Formulation, manufacturing, and packaging of nutraceutical formulations

The nutraceutical formulations were structured (Figure 1 and Table 1) as in previous work [2] according to the Indian Standard (IS: 8220/1976) for "Specification for Protein-Rich Concentrated Nutrient Supplementary Foods" [25], mixed by a mechanical high-speed homogenizer (HSH) at 1000 rpm for 2 min. [26,27], then sealed and packaged in metallized polypropylene (MPP) sachets, as the MPP is considered the best food packaging material, providing the safest protection and maintaining superior quality and safety [28]. The four formulations contain the same concentrations of WPC, SMP, vitamins, and minerals. However, they differ significantly in the contents of anti-caking agents, SGs, and natural flavors added in different effective amounts.

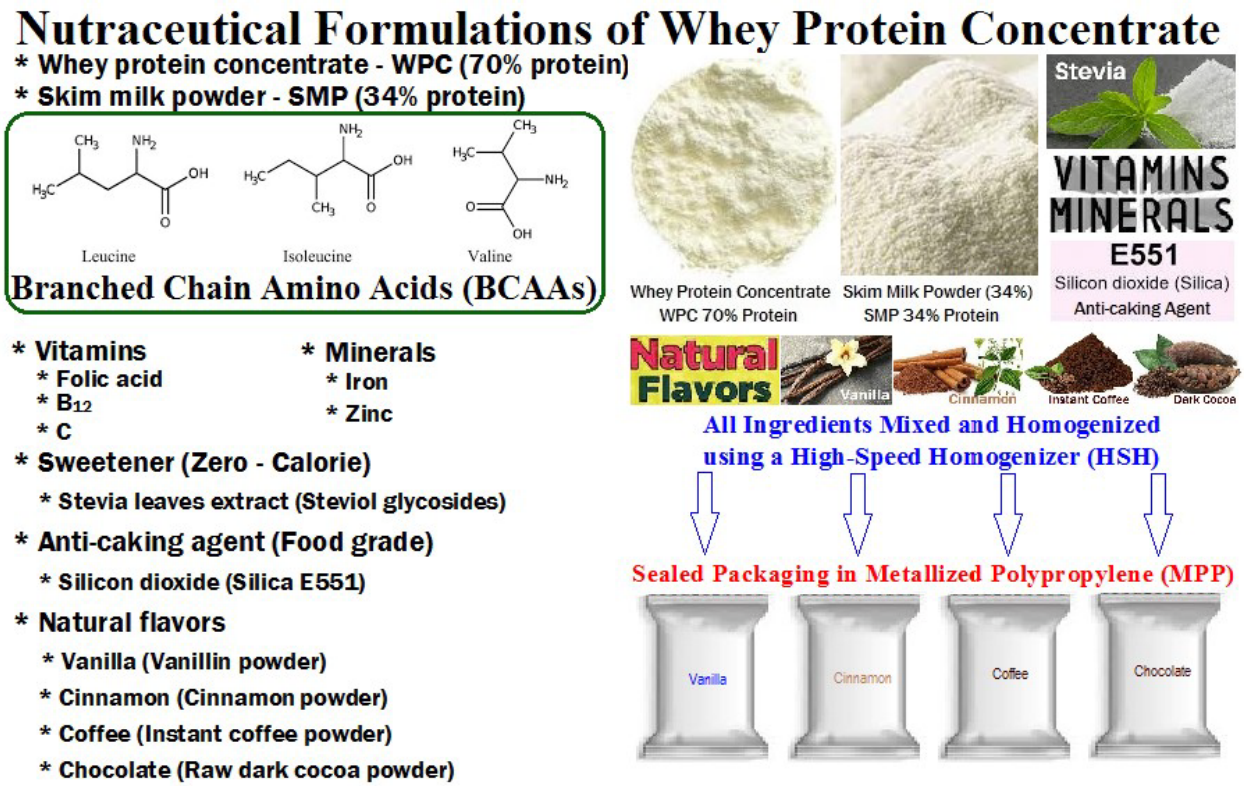

Figure 1. Experimental design of the nutraceutical formulations.

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Table 1. Nutritional composition (in grams) per serving of the nutraceutical formulations.

Ingredients (g)	Formula			
	Vanilla	Cinnamon	Coffee	Chocolate
	21 g	23 g	23 g	25 g
Whey protein concentrate (WPC 70%)	8.06	8.06	8.06	8.06
Skim milk powder (SMP 34%)	12.83	12.83	12.83	12.83
Natural flavor	0.0021	2.00	2.00	4.00
Steviol glycosides	0.042	0.046	0.046	0.050
Silica (E551)	0.0084	0.0092	0.0092	0.01
Vitamins and Minerals Mix.	0.0575	0.0575	0.0575	0.0575
Total weight/serving (g)	21	23	23	25

Proximate composition of the nutraceutical formulations

The proximate composition of the nutraceutical formulations was evaluated as moisture, ash, protein, fat, fiber, and carbohydrate content (calculated by difference), and the total energy (Calories) per serving was calculated along with vitamins, minerals, SGs per serving with DV referenced to recent updated Nutrition Facts Label by FDA [29,30].

Results and Discussion

Proximate composition of the nutraceutical formulations

The proximate composition of the nutraceutical formulations estimated per serving [2] with 10 g protein as 20% of the daily value (DV) with 25% of DV of calcium, iron, zinc, vit. C, folic acid, and vit. B_{12} according to the updated Nutrition Facts Label [31], which has been changed from the original Nutrition Facts Label [32] as shown in Table 2.

It is noticed the variation in serving sizes among the four nutraceutical formulations as they differ in the content of the natural flavors added in effective amounts as in vanilla natural flavor (0.0021g) with the least serving size (21g), cinnamon natural flavor (2g) with average serving size (23g), instant coffee natural flavor (2g) with average serving size (23g), and raw dark cocoa (4g) with the maximum serving size (25g). Also, SGs content varies among the different nutraceutical formulations due to the addition of SGs calculated per 100g and not per serving to be 0.2g/100g as the recommended content shall not exceed 2.5g/1000g or 0.25g/100g according to regulations of food additives.

Table 2. Proximate composition of the nutraceutical formulations per serving.

Parameter	Formula			
	Vanilla	Cinnamon	Coffee	Chocolate
	21g	23g	23g	25g
Moisture (g)	0.8988	1.035	1.0189	1.165
Ash (g)	0.9345	1.1201	1.0442	1.2425
Protein (g)	10.0905	10.1591	10.1292	10.66
Fat (g)	0.5985	0.6003	0.6003	1.00
Fiber (g)	0.00	1.058	0.00	1.32
Carbohydrate (g)	8.4777	9.0275	10.2074	9.6125
Steviol glycosides (mg)	42	46	46	50
Energy (Calories)	79	84	86	90
Calcium (mg)	325	325	325	325
Iron (mg)	4.5	4.5	4.5	4.5
Zinc (mg)	2.75	2.75	2.75	2.75
Vit. C (mg)	22.5	22.5	22.5	22.5
Folic acid (mcg)	100	100	100	100
Vit. B_{12} (mcg)	0.6	0.6	0.6	0.6

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Synergistic effect between WPC and SGs

With growth in the gastrocnemius and soleus muscle pads, a momentous increase in peroxisome-proliferator-activated receptor-γ coactivator-1 alpha (PGC-1α) expression, followed by a strikingly similar pattern in mitochondrial transcription factor A (TFAM) protein expression—two crucial mitochondrial biogenesis markers—and higher adenosine monophosphate-activated protein kinase or AMP-activated protein kinase (AMPK) phosphorylation, rats receiving WP with stevia leaves extract demonstrated higher maximum load-carrying capacities. Additionally, in resistance-trained rats, WP sweetened with stevia leaf extract promoted a significant reduction in retroperitoneal adipocyte diameter with an increase in the weight of brown adipose tissue pads. This is regarded as a promising method to be used in regular diet formulations containing WP and stevia for active and tolerant people with metabolic disorders, obesity, and diabetes to increase muscular mass and strength [33].

Three groups of diabetic rats were used to study the effects of WP supplemented with *S. rebaudiana* Bertoni: a group eating only whey protein (WP), a group eating only *S. rebaudiana* Bertoni, and a group eating the mixed diet (WP and *S. rebaudiana* Bertoni). Insulin secretion and its role in preventing streptozotocin-induced hyperglycemia were tracked. The radioimmunoassay (RIA) was used to test the plasma levels of insulin, and immunohistochemistry (IHC) was used to determine whether the cells were still viable. Results showed that diabetic rats given an experimental diet containing WP supplemented with *S. rebaudiana* had a better recovery from insulinemia, with insulin levels (0.13 ng/mL) similar to those of non-diabetic rats and exhibited a higher number of (66%) in immunohistochemistry (IHC) analysis of insulin-positive pancreatic B cells, while the other two diabetic rat groups eating only separate portions of *S. rebaudiana* Bertoni or WP exhibited 38 and 59% of positive cells, respectively. The results ABOVE show that whey WP supplemented with *S. rebaudiana* Bertoni has the ability to restore the viability of streptozotocin-damaged pancreatic B cells and, as a result, increases insulin secretion, indicating that this diet can be used as an adjunct or supplement in diabetic treatment protocols [34,35].

Emphasizing strengths of study

The current study successfully achieved its aim by highlighting new aspects on nutraceutical formulations of WPC with the documented well-known synergism between WPC and steviol glycosides. The study design is applicable and easily applied practically on a wide range on an industrial scale to present useful nutraceutical formulations of WPC sweetened with SGs and fortified with vitamins and minerals for improving QoL in patients with NCDs.

Limitations of study

The current paper presents only the nutraceutical formulations that have been officially structured according to the official standards and regulations to be available for consumers in a completely legal way. Although, there may be many other formulations structured in the laboratory or by manufacturers without official registration.

Future research

Future research should be carried out to formulate different innovative dietary supplements with different ingredients and concentrations of nutrients, including different forms of WP (concentrate, isolate, hydrolysate) with different vitamins, minerals, and other bioactive components in such a way that complies with the official regulations for registration of these valuable products to be available for consumers with a complete profile of physicochemical, microbiological, and sensory evaluation analyses.

Conclusions

The current paper successfully presented new aspects of officially registered nutraceutical formulations of WPC sweetened with SGs and supplemented with vitamins and minerals with natural flavors for improving QoL in patients with NCDs, malnutrition, micronutrient deficiency, special food needs in pregnancy, lactation, growth for improving the physical performance and maintaining good health. New aspects were introduced in an informative and concise way to highlight the useful synergism between WPC and SGs in augmenting the health benefits of nutraceutical formulations.

Author Contribution

Conceptualization, R.I.M.A.; methodology, R.I.M.A., A.U., P.G.A., M.A.M.; data curation, R.I.M.A., A.U., P.G.A., M.A.M.; writing—original draft preparation, R.I.M.A., A.U., P.G.A., M.A.M.; writing—review and editing, R.I.M.A., A.U., P.G.A., M.A.M.

Disclosure statement

No potential conflict of interest was reported by the authors.

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