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Antioxidant Status, Physicochemical and Immune-boosting Characteristics of New Formulated Functional Beverages

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ABSTRACT

Functional beverages have healthful benefits in reducing the risk of many diseases and enhancing immunity for the consumers. This study aimed to prepare healthy fresh blended beverages from extracts of Kiwi fruit, broccoli, ginseng and black seeds. Seven blending beverages were evaluated for changes in proximate, physio-chemical composition, antioxidant status, sensory properties, and their immunomodulatory parameters. This study revealed the possibility to produce seven functional blends of beverage characterized by their good sensory and nutritional properties. The obtained antioxidant status of seven blended beverages confirmed high potential antioxidant activity of their total phenolics, flavonoids and ascorbic acid contents. Furthermore, phagocytosis inhibition and lymphocytic population tests showed a highly significant increase in phagocytosis of macrophages and lymphocytic population for blended beverages especially blend 5 which consisting of 40:60% from Kiwi fruit: Broccoli juices. It could be concluded that functional blended beverages were effective as a source of minerals, antioxidants, and as immune boosting.

Keywords: Kiwi fruit; Broccoli; Ginseng; Black seeds; Immunomodulation

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INTRODUCTION

Our immune system is essential for our survival and adaptability. This is a defense mechanism of our body to protect us from viruses, bacteria, infection, and injury. The main Key players of the immune system are white blood cells, antibodies, lymphatic system, spleen, bone marrow, and thymus. Foods contain ingredients and bioactive compounds such as lipids, peptides, and antioxidants important for human nutrition. The idea of producing foods with boosted functionalities of inherent components has led the food industry to a surge of research activities and the development of “functional foods”. Therefore, the two major functional of the immune system are natural immunity (innate immunity) and it’s the first line of defense and acquired immunity (adaptive immunity) the second line of defense **(Martin, 2010).** But our immune system is getting weak day by day due to unhealthy behavior and eating habits, sedentary lifestyle, consumption of alcohol, smoking, and stress, all those factors decrease the body’s ability to fight infection and cause malnutrition and lead to reduction of vitamins concentration, zinc, copper and iron deficiency, metabolic damage and reduced intestinal absorption **(Farhadi et al., 2018).** So, nutritional status has a major influence on immune system function.

Immunomodulation means the alteration of immune response which may increase or decrease immune responsiveness. Enhancement in immune responsiveness is called immunostimulant. The potential role of natural bioactive components in preventing and treating chronic inflammatory disorders such as cancers, obesity, diabetes, rheumatoid arthritis, atherosclerosis, ischemic heart disease, and inflammatory bowel disease is now the subject of intense research **(Kim et al., 2018).**

Nutrients effectiveness on the human immune system was analyzed, as if the pathogen enters the human body, the immune system interferes with work and creates an immune response, which is enhanced by a diet rich in vitamins C & E and foods containing beta-carotene. One of the most important amino acids affecting the immune system's response against viruses and bacteria is arginine, which Stimulates T- cell response after surgeries and serious injuries. Vitamin B complex also affects the production of immune cells and lymph response, vitamin A enhances the work of T cells, vitamin D increases T cell production, while prebiotics promote the ability of develop and regulate the work of T cells. Iron and Zinc are among the most important minerals that influence the response of immune cells against wounds **(Karacabey and Ozdemir, 2012).**

Functional foods and nutritional supplements contain immune boosters like, polyphenols, flavonoids, polyunsaturated fatty acids, vitamins A, B complex, C, D, E and minerals including zinc, iron, selenium and copper lead to reduce viral risks and improving the efficiency of the immune system **(Alkhatib, 2020).**

Herbs, vegetables, and fruits have been investigated intensely with regards to protective effects they may provide to human tissues. Many of their activities were found to be related to oxygen radical scavenging **(Manfred et al., 2014)**. Also, they have a main role in boosting the immune system. The intake of functional foods, phytochemicals, probiotics, prebiotics, dietary fibres improve human health and immune response **(Caroline et al., 2019).**

Broccoli (*Brassica oleracea*) is a cruciferous vegetable rich in nutritional antioxidant such as vitamin C and E as well as phytochemicals (carotenoids, flavonoids, and glucosinolates) therefore, known for its disease-fighting properties like, cancer, cardiovascular disease, and diabetes **(Li et al., 2022)**. Emerging clinical evidence has shown that broccoli may offer a protective effect against severe symptoms of COVID-19 **(Bousquet et al., 2021),** highlighting its potential as a functional food with innate immune response and produce pro-inflammatory cytokines.

Kiwi fruit (*Actinidia deliciosa*) are commonly known as gold kiwi fruit, plentiful source of vitamin C, vitamin E, folate, potassium, and phytochemicals **(Richardson et al., 2018)**. Existing literature supports the fact that supplementation of kiwi fruit in diet can provide health benefits like improved immune, gastrointestinal, and cardiovascular functions **(Cundra et al., 2020)**.

Black seeds (*Nigella sativa*) increased the natural killer cell's functional activity and it’s a good source of carotenoids and Fe, K, D, zinc. Black seeds were identified as thymoquinone-rich source, which possesses potent anti-inflammatory effects on several inflammation-based models including experimental encephalomyelitis, colitis, peritonitis, oedema, and arthritis through suppression of the inflammatory mediators **(Fatima et al., 2020, Mukherjee et al., 2014)**.

Ginseng, a popular herb, is the root of plants in the genus Panax and well-known as a traditional medicine to immunomodulator. The main isolated components are polyphenolic compounds, polyacetylenes, triterpenoids, etc. roots, stems, and leaves are commonly used to boost the immune system by increasing the release of cytokines by macrophages and by activating the phagocytic property of macrophages. Administration of ginseng aqueous extracts has immunostimulatory effects on natural killer cells **(Sang et al., 2015, David et al., 2000).**

This study aimed to elaborate functional beverage blends and evaluate their effect on physicochemical properties, antioxidant status, and study their impact to increase the ability of immune system response.

MATERIALS AND METHODS

Raw Materials:

Four different raw materials were selected from the local market: Kiwi Fruit (*Actinidia deliciosa*) broccoli, (*Brassica oleracea*), Black seeds (*Nigella sativa L.)*, Ginseng (*Panax*) and Stevia sugar as a natural sweetener purchased from local market in Cairo, Egypt.

Chemicals and reagents

DPPH (2,2-Diphenyl-1-picrylhydrazyl) and Folin–Ciocalteu reagent were purchased from Sigma-Aldrich (USA), while, aluminium chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), sodium chloride (NaCl), nitroblue tetrazolium, phosphate buffered, ethanol, and methanol were obtained from El-Gomhoreya Co., Cairo, Egypt. All chemicals and solvents were of the analytical grade.

Preparation of extracts:

About eight kg from fresh kiwi and broccoli fruits purchased for obtained juice; fresh Kiwi was peeled off and squeezed to have fresh Juice by using Black & Decker home juicer, model: JE55, 60Hz, 450W, however for fresh Broccoli was cut into small cups, then mixed it and crushing by using home blender Philips, model: HR7772, 70Hz, 800W.

Powders of black cumin seeds and ginseng root were aqueous extracted by suspending 10 g for each one separately in 20 ml of distilled warm water 35 ºC then allowed to stand for 24 h. The extract was then decanted and filtered through a Whatman filter paper No one **(Ramesh et al.,2016)**.

Finally, different blends beverages were obtained, each exhibiting different ratios of kiwi and broccoli with same proportions of black seed and ginseng extracts, sweetened with stevia sugar as shown in (Table1). These blending beverages were stored in the refrigerator at 4°C until analysis.

Table 1. Compositional ratio of seven blends beverages

Blending beverages	Proportion of Ingredient (%)	
	kiwi fruit juice	Broccoli juice
1	80	20
2	70	30
3	60	40
4	50	50
5	40	60
6	30	70
7	20	80

All blends contained 0.1 % black seeds extract; 0.2 % ginseng extract and sweetened by stevia sugar.

Physiochemical analysis for prepared blending beverages:

Proximate chemical analysis including (moisture, ash, protein, fat, crude fiber and caloric value) was determined according to **(AOAC, 2019)**. Carbohydrates were calculated by difference using the following equation: % Total carbohydrates =100 – [% moisture + % ash + % protein + % fat + crude fiber]. While juice blends were evaluated as follows: Total Soluble Solid (TSS) were evaluated as Brix value using a hand Refractometer (Atago, Japan). Acidity was determined by titration to pH =8.1 with 0.1 M NaOH solution and expressed as gram of citric acid per 100 ml of juice according to the method of **(AOAC, 2019)**, Brix/acid ratio was calculated for each sample. The pH value was evaluated using Hanna pH meter HI 9021 m Germany.

Vitamin C of prepared juice blends was evaluated using 2,6 dichlorophenol-indophenol. The concentration of ascorbic acid calculated as mg of ascorbic acid per 100 ml of sample (**Ragana, 1979).**

Determination of total phenolic contents, total flavonoid content, and antioxidant activity for prepared blending beverages:

The total phenolic content was assessed by Folin-Ciocalteu method in accordance with **(Singleton and Rossi, 1965);** the results were presented as [mg of gallic acid equivalent (GAE) per 100 ml of sample]. The total flavonoid content was evaluated by $AlCl_3$ method in accordance with **(Zhishen et al., 1999)** and result were expressed as [mg catechin equivalent (CE) per 100 mL of sample]. The antioxidant activity was evaluated by 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical assay in accordance with **(Ansari et al., 2013, Mensor et al., 2001)** using a UV-VIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The DPPH radical scavenging ability of each sample is expressed as the half maximal inhibitory concentration on DPPH free radicals (IC_{50}).

Sensory attributes of prepared blending beverages:

The sensory characteristics of the prepared functional beverage blends were evaluated according to **(Watts et al., 1989)**. Twelve panellists from the staff members of Food Science Department, Faculty of Agriculture, Ain Shams University were chosen to evaluate the products. The panellists were asked to score appearance, colour, taste, odor, and over acceptability using a 9-point hedonic scale.

Immunomodulatory effect of prepared blending beverages:

Phagocytic response of macrophages:

The immunonodulatory effects of seven blending beverage were studied, *in vitro*. Phagocytic inhibition test depends on histamine, release from allergic reaction inhibit phagocytosis by the technique adapted by **(Soliman and Attia 2007, Budny et al., 1989).** was followed. Briefly, preparation of 0.1% nitroblue tetrazolium (NBT) solution labeled by flurescence material (if flurescence microscope will be using): 5 mg NBT was dissolved in 2.5 ml 0.9% NaCl protected from light by wrapping tube. Then the tube was placed in a conical plastic centrifuge tube, shacked until dissolved (30 to 60 unites). Then, 2.5 ml of 0.15 M phosphate buffered saline was added and tube labeled and stored at 2 to 8 ºC, this may be used up to 1 week from time of preparation.

Lymphocytic population test:

Lymphocytic population test achieved as follows: Approximately 5 ml of fresh defibrinated or heparinized blood specimen (10 units phenol-free heparin per ml blood) was mixed with an equivalent volume of Hank's solution. (Defibrinated blood must be processed within 2 hours, heparinized blood within 24 hours, and the specimen was not stored at refrigerator temperature during this period); one-part lymph Oflot (density gradient solution = 1.079 g/ml) was placed in the bottom of test tube and covered gently with a layer of an equal part of the diluted specimen, avoiding the layer mixing; centrifugation is done for about 20 minutes at 2800 rpm. The lymphocytes were removed, which appeared as a white ring on the border between the plasma and lymphoflot, this buffy coat was pipetted to another tube. The buffy coat was washed 3 times with phosphate buffer saline and every time centrifugation at 3000 rpm; then, 10% Hank's solution was prepared by adding 4.5 ml of Hank's solution and 0.5 ml of fetal calf serum (250 u was taken from this solution and Pipetted in the buffy coat; after that, 1.6 ml of Hank's 10% + 25 u of washed sheep RBCs were taken from the solution in equal amounts. Equal amount from the prepared solution (in step 5 and 6) were added to each other and incubated in a cool place (2-8 ºC). One drop taken from the solution after incubation, and 2 drops of Leishman's stain solution, were added, and incubated for 112 h at room temperature. One drop taken on slide and covered with cover slide and read under microscope. Finally, a total of 100 cells were counted as well as the rosette and non-rosette and normal expected range is 40-60% rosette according to **(Heny and Claman, 1989).**

Statistical Analysis

Results were obtained in triplicate and presented as means and standard error. Variance analysis (ANOVA) and Duncan's mean test were applied to identify possible differences between the obtained results ($P < 0.05$). The statistical procedures were performed using SAS version 4.10 software **(SAS, 1999).**

RESULTS AND DISCUSSION

Proximate analysis of prepared blending beverages:

Approximate analyses of the seven beverage blends were estimated and results are presented in Table (2), The obtained results revealed a slight difference in water content within all beverages ranged from 93.98 to 88.41%, kiwi fruit/ broccoli 20:80 (blend 7) recorded the highest amount of moisture content, while kiwi fruit/ broccoli 70:30 (blend 2) showed the lowest content amongst all.

The highest ash content was found in blend 3 with a value of 1.441% followed by blends 2, 1, 6 and 4 in percentage values of 1.33, 0.603, 0.531 and 0.521, respectively. while the lowest ash content was found in blends 5 and 7 with a value of 0.411%. kiwi fruit/ broccoli 50:50 (blend 4) had the highest crude fiber content with a value of 2.76%.

Table 2. proximate composition of prepared blending beverages

Analysis	Blending beverages						
	1	2	3	4	5	6	7
Moisture %	91.96^{d}	88.41^{g}	90.35^{f}	91.21^{e}	92.21^{c}	93.42^{b}	93.98^{a}
Ash %	0.603^{c}	1.33^{b}	1.441^{a}	0.521^{e}	0.411^{f}	0.531^{d}	0.411^{f}
Fat %	0.13^{d}	0.24^{b}	0.16^{c}	0.05^{e}	0.05^{e}	0.02^{f}	0.52^{a}
Protein %	0.531^{g}	0.791^{f}	0.961^{d}	0.881^{e}	1.051^{c}	1.141^{b}	1.231^{a}
Crude Fiber %	1.39^{b}	1.29^{c}	0.67^{e}	2.76^{a}	0.091^{g}	0.29^{f}	1.13^{d}

Carbohydrate %	5.39^{d}	7.95^{a}	6.42^{b}	4.58^{f}	6.19^{c}	4.60^{e}	2.72^{g}
Caloric value Kcal/100ml	24.85^{d}	37.12^{a}	30.96^{b}	22.29^{f}	29.41^{c}	23.14^{e}	20.48^{g}

Data are mean, n=3, means with the same letter in the same raw are not significantly different at 5%; Blends from 1 to 7 are kiwi fruit/ broccoli by blending ratio with 0.1 % black seeds extract; 0.2 % ginseng extract and sweetened by stevia sugar.

Whereas kiwi fruit/ broccoli 20:80 (blend 7) had the highest lipid and protein content in percentage values of 0.52 and 1.23, respectively. Meanwhile, the highest carbohydrate percentage and caloric value observed in blend 2, kiwi fruit/ broccoli 70:30 with values of 7.95 and 37.12 Kcal/100g, respectively. These differences in the values can be attributed to the differences in type and amount of each fruit and vegetable in each type of blending beverage. Aiding in disease prevention, such as reducing the risk for cardiovascular diseases, neurological conditions, depression or cancer. Supporting gut health and therefore enhancing the immune system. These findings are in accordance with those reported by **(Sajith and Riya .2023, Wadmare et al., 2019)**.

Physicochemical characteristics of prepared blending beverages:

Data presented in Table (3) showed the physicochemical properties of different prepared blending beverages including total soluble solids (TSS), pH, acidity, density and sugar/acid ratio. The result of brix (TSS) revealed that first blend had highest value (12%), while by decreased the kiwi fruit/ broccoli blending ratio led to the lowest value (7.0%) in blend 7. Concerning pH values were observed within different types of beverages ranging from 4.30 as the lowest pH value found in ratio one to 5.48 as the highest pH value observed in ratio seven.

Table 3. Some chemical and physical characteristics of prepared blending beverages

Parameters / Blends	TSS (°Brix) %	pH value	Acidity % (Citric acid)	Density (gm/cm^3)	Sugar/acid ratio
1	$12.0^{a}\pm0.0577$	$4.30^{g}\pm0.0057$	$1.629^{a}\pm0.0203$	$1.048^{a}\pm0.0003$	$7.37^{b}\pm0.0624$
2	$11.0^{b}\pm0.0577$	$4.35^{f}\pm0.012$	$1.595^{a}\pm0.0176$	$1.044^{b}\pm0.0003$	$6.90^{c}\pm0.065$
3	$10.0^{c}\pm0.0577$	$4.51^{e}\pm0.0057$	$1.498^{b}\pm0.0103$	$1.039^{c}\pm0.0003$	$6.68^{c}\pm0.0617$
4	$9.0^{d}\pm0.0577$	$4.69^{d}\pm0.0057$	$1.422^{c}\pm0.0165$	$1.035^{d}\pm0.0003$	$6.33^{d}\pm0.0404$
5	$8.5^{e}\pm0.0057$	$4.87^{c}\pm0.0057$	$1.115^{d}\pm0.0178$	$1.033^{e}\pm0.0003$	$7.63^{a}\pm0.1241$
6	$7.8^{f}\pm0.0057$	$5.17^{b}\pm0.0115$	$1.077^{de}\pm0.0103$	$1.031^{f}\pm0.0003$	$7.27^{b}\pm0.0717$
7	$7.0^{g}\pm0.0577$	$5.48^{a}\pm0.0088$	$1.038^{e}\pm0.0103$	$1.027^{g}\pm0.0003$	$6.74^{c}\pm0.0881$

Data are mean ± SE, n=3, means with the same letter in the same column are not significantly different at 5%; Blends from 1 to 7 is kiwi fruit/ broccoli by blending ratio with 0.1 % black seeds extract; 0.2 % ginseng extract and sweetened by stevia sugar.

This relationship between the pH of different beverage ratios is reflected in the acidity measurements, which decrease with the addition of broccoli extract from blends one to seven with value ranged from 1.629% to 1.038%. Slight changes in density values were observed within different types of beverages ranging from 1.048 as the highest value to 1.027 as the lowest value. However, the Sugar/ acid ratio, it was observed that the blend 5 which consisted of kiwi fruit/ broccoli with ratio 40:60 had the most favorable and acceptable in taste as this finding confirmed in sensory evaluation, these findings are supported by the observation of **(Daniel et al., 2022)**. These beverages contain biologically active substances such as antioxidants that may lower the risks from certain diseases associated with aging.

Bioactive and functional components of prepared blending beverages:

Phytochemical components of prepared blended beverages are presented in Table (4). Total phenol content can be used as an important indicator of antioxidant capacity and can be used as a preliminary screen for any functional beverage intended for use as a natural source of antioxidants. The results revealed that blend 1 recorded the highest amount of total phenolic in a value of 0.498 mg GAE/100 ml and gradually decline with decreasing kiwi fruit/ broccoli ratio, the lowest phenolic compound contents were observed in last blending ratio seven with value of 0.102 mg GAE/100ml. These results agreed with those obtained by **(Daniel et al., 2022 & Paganga et al., 1999)** who determined the phenolic contents in some fruits, vegetables and various beverages. Also, concerning total flavonoids, the obtained results showed that the same trend in the results of total phenolic compound which the highest total flavonoids with value of 0.089 mg QE/100 ml in blend 1, while beverage ratio seven contain the lowest content of 0.022 mg QE/ml, respectively. These results were in accordance with those stated by (**Paganga et al., 1999)**. Demand for fruit and vegetables has increased in recent years in several countries in response to increased public awareness of the benefits of healthy eating. It has been suggested that consumption of antioxidant-rich foods may delay or prevent many diseases. Therefore, the guide to health promotion and disease prevention in the USA and around the world includes recommendations for daily consumption of a variety of fruits and vegetables, since they have significant amounts of bioactive compounds, especially vitamins, minerals and fiber. To help ingest adequate amounts of fruits and vegetables, juices are a practical alternative.

Data in Table (4) showed the vitamin C content in prepared blending beverage and from these results it can be noticed that the vitamin C content ranged from 102.02 to 5.59 mg/100 ml among the seven blends beverage which the highest amount of vitamins C observed in first ratio comprising 80% kiwi and 20% broccoli. while the lowest value of vitamin C was found in the ending ratio consists of 20% kiwi and 80% broccoli **(Chwil et al., 2023)**.

The antioxidant activities of all blending beverages were expressed in terms of percentage of inhibition and IC_{50} values (concentration of sample required to scavenge 50% of free radicals). The findings indicated that the lower the IC_{50} values were, the higher the antioxidant capacity of the blends became. Data showed that the best radical scavenging activity was observed for the first ratio (6.577 µg/ml) comprising 80% kiwi and 20% broccoli, and gradually decline through decreasing kiwi fruit/ broccoli ratio down to the lowest ratio seven (1314.83 µg/ml) consists of 20% kiwi and 80% broccoli. Based on these findings it can be inferred that this beverage may play a significant role as an immunomodulator, the previous results were approximately in agreement with the results obtained by **(Maheshwari et al., 2022, Elisabetta et al.,2018)**. A functional beverage can be defined as dietary item that, besides providing nutrients and energy, beneficially controls one or more targeted functions in the body by improving certain physiological responses and/or by reducing the risk of diseases.

Table 4. Bioactive and functional components of prepared blending beverages

Parameters **Blends**	**Total Phenolic** **mg GAE/100mL**	**Total Flavonoids** **mg CE/100mL**	**Ascorbic acid** **mg /100ml**	**Antioxidant activity** **(IC_{50}) µg/mL**
1	0.498^{a}±0.0001	0.089^{a}±0.0002	102.02^{a}±1.81	6.577^{g}±0.004
2	0.303^{b}±0.0008	0.088^{b}±0.0002	90.74^{b}±1.58	10.67^{f}±0.006
3	0.282^{c}±0.0005	0.063^{c}±0.0002	88.68^{b}±1.24	58.83^{e}±0.006
4	0.228^{d}±0.0004	0.058^{d}±0.0002	79.68^{c}±0.92	76.25^{d}±0.006

5	0.213[e]±0.0005	0.042[e]±0.0006	38.53[d]±2.62	111.02[c]±0.006
6	0.122[f]±0.0006	0.038[f]±0.0003	20.54[e]±0.92	130.74[b]±0.006
7	0.102[g]±0.0006	0.022[g]±0.0003	5.59[f]±0.35	1314.83[a]±0.441

Data are mean ± SE, n=3, means with the same letter in the same raw are not significantly different at 5%; Blends from 1 to 7 are kiwi fruit/ broccoli by blending ratio with 0.1 % black seeds extract; 0.2 % ginseng extract and sweetened by stevia sugar.

Minerals content of prepared blending beverages

The data in Figure (1) showed that seven blending beverages contain different values of iron, copper, and zinc, blends seven consists of 20% kiwi and 80% broccoli contains highest amount of iron in a value of 39.62 mg/l followed by blends ratio 5; 3 with approximately equal values of 26.44 and 20.26 mg/l, respectively. The least values were found in ratio 4, 1, 2 and 6 with a little difference between them in values of 7.47, 8.2, 8.81 and 9.57 mg/l, respectively. Also, beverages with ratio no., 7 showed the highest content of copper in value of 0.910 mg/L, while, blending ratio no., 5 observed the highest zinc concentration at 3.711 mg/L followed by blends ratio 7 and 3 with values of 2.211 and 1.581 mg/L, respectively. The comparison showed that blend 5 may cover around 30% from RDA of Zinc, so, it can be helpful to improve human health via stimulation of immune cells response against to pathogens.

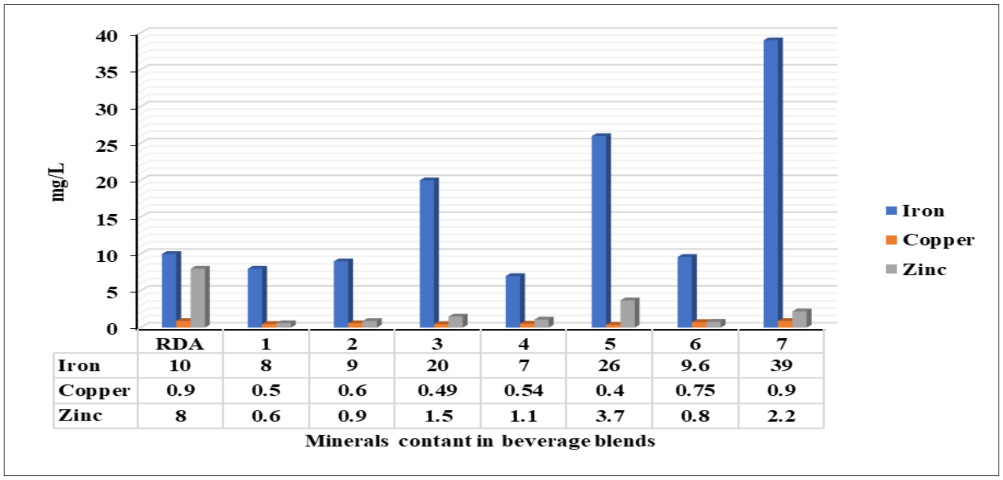

Figure 1. Minerals content of prepared blending beverages comparing with the recommended dietary allowance (RDA)

Data are mean, n=3, Blends from 1 to 7 are kiwi fruit/ broccoli by blending ratio with 0.1 % black seeds extract; 0.2 % ginseng extract and sweetened by stevia sugar

The Recommended Dietary Allowance (RDA) is a daily nutrient intake level that satisfy the nutrient requirements of ~98% of healthy individuals in an age- and sex-specific population, among seven blending beverages when comparing their minerals content with the RDA, a 250 ml serving of these functional beverages could potentially fulfil a significant portion of daily mineral needs. This is consistent with nutrient adequacy score and nutrient density calculated based on the methods of **(Darmon et al., 2005),** thus these beverages could be considered as healthy beneficial functional beverages and playing a vital role in boosting the immunity.

Sensory attributes of prepared blending beverages:

Data in Figure (2) depicts the sensory evaluation attributes for fresh prepared blending beverages. The mean score values for all parameters of sensory evaluation including appearance, color, taste, odors, mouthfeel and over acceptability. Beverages can provide energy and key nutrients to improve health and prevent chronic diseases. From a physiological perspective, beverages fulfill unique roles in the diet by fulfilling hydration needs, quenching thirst, and assisting with food mastication and digestion. The results showed that there were no significant differences in some parameters like appearance, color, and odor among the seven blends. However, when other parameters such as taste, mouthfeel, and over acceptability, found that blends ratio from 2 to 5 were favored by the most panellists. Also, blending five (kiwifruit: broccoli with ratio 40:60) received best acceptance for the panelists, which can be attributed to the sugar/acid ratio, as previously explained. The panelists also observed a high acidity in the first blending ratio due to the substantial proportion of kiwi, and a distinct vegetable flavor in the seventh blend due to its high broccoli content. Nevertheless, all blends except blending ratio seven (kiwifruit: broccoli with ratio 20:80) were generally accepted by the panelists **(Daniel et al., 2022)**.

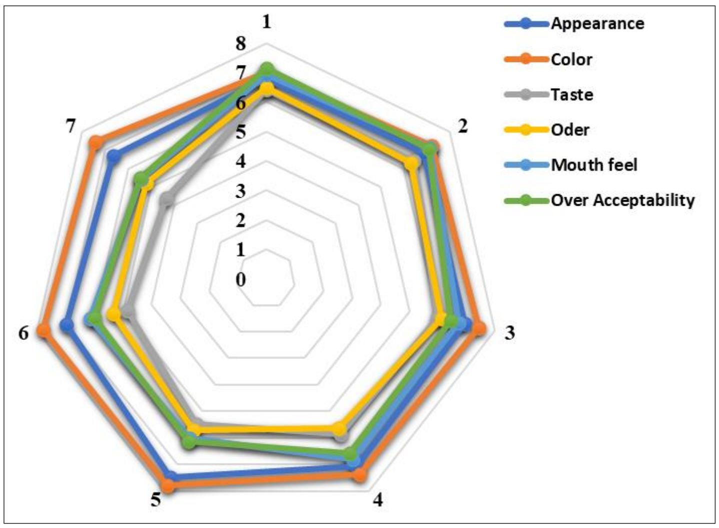

Figure 2. Sensory attributes of prepared blending Beverage

Blends from 1 to 7 is kiwi fruit/ broccoli by blending ratio with 0.1 % black seeds extract; 0.2 % ginseng extract and sweetened by stevia sugar.

Immunomodulatory effect of prepared blending beverages:

Immunomodulation tests were performed on seven different beverages as a complementary test to ensure that they had an immune-boosting effect as shown in (Table 5). Two key immunological tests were selected, phagocytic response of macrophages and lymphocytes population. It was noticed that the best result in both immunity parameters was observed in blending beverage five (kiwi fruit/ broccoli with ratio 40:60) with value (46.03, 74.0 %), followed by blend 6 (42.04, 68.0%), Blend 7 (40.04, 66.0%), then Blend 4 (39.04, 65.0), respectively.

Blending beverage 5 (kiwi fruit/ broccoli with ratio 40:60) showed a great ability to stimulate phagocytic cells and enhance their efficiency in the phagocytosis test, as well as enhance lymphocytes in the lymphocytic population test. It also had the highest value of zinc, as previously shown in (Figure 1), in addition had potential bioactive compounds as antioxidant activity, as previously shown in (Table 4), and due to these components, which play a main role in boosting immune system, as explained in **(Daniel et al., 2022)**. These findings are in a closed agreement with the results obtained by previous studies highlighting the immunomodulatory properties of kiwi, broccoli, ginseng and black seed with an emphasis on their role in enhancing the immune response **(Sajith and Riya, 2023; Mahn and Castillo, 2021; Ciesielska-Figlon et al., 2023; Valdés-González et al., 2023).**

Table 5. Immunomodulatory effect of prepared blending beverages on phagocytic response of macrophages and Lymphocytic population

Blends	Phagocytosis %	Lymphocytic population %
1	32.03^{g}	53.0^{g}
2	35.03^{f}	57.0^{f}
3	38.03^{e}	61.0^{e}
4	39.04^{d}	65.0^{d}
5	46.03^{a}	74.0^{a}
6	42.04^{b}	68.0^{b}
7	40.04^{c}	66.0^{c}

Means (n=3) value in the same column with different superscripted letters are significantly different ($p \leq 0.05$); Blends from 1 to 7 are kiwi fruit/ broccoli by blending ratio with 0.1 % black seeds extract; 0.2 % ginseng extract and sweetened by stevia sugar.

CONCLUSION

Based on the previous results, it could be concluded that all blends of beverage prepared from kiwi fruit, broccoli, ginseng and black seeds distinguished with potent antioxidants and rich in ascorbic acid, phenol and flavonoid compounds. Moreover, all blends were accepted through sensory evaluation attributes especially blend 5 and except blending ratio 7 which observed the lowest acceptability. Accordingly, it could be encouraged to consume these functional blends that support health, wellness and it is effective in immunoenhancing.

REFERENCES

Alkhatib A. (2020). Antiviral Functional Foods and Exercise Lifestyle Prevention of Coronavirus. Nutrients, 12,1-16; Doi: 0.3390/nu12092633.

Ansari A.Q., Ahmed.S. A, waheed. M.A and Juned. S. (2013). Extraction and determination of antioxidant activity of Withania somnifera Dunal. European Journal of Experimental Biology, 3(5):502-507.

AOAC, (2019). Official Method of Analysis. 17th Edition, Association of Official Agricultural Chemists (AOAC), Rockville M.D., USA

Bousquet, J., Le Moing, V., Blain, H., Czarlewski, W., Zuberbier, T., de la Torre, R. and Anto J.M. (2021). Efficacy of broccoli and glucoraphanin in COVID-19: From hypothesis to proof-of-concept with three experimental clinical cases. World Allergy Organization Journal, 14(1), 100498.

Budny, J.L.: Grotke, N.C. and Mac Gillivory (1976). Phagocytes M: N.Y. Stote J.Med. 76:877-898

Caroline E.C., Philip C.C. and Elizabeth A.M. (2019). Diet and Immune Function. Nutrients, 11(8):1-11; Doi: 10.3390/nu11081933.

Chwil, M.; Matraszek-Gawron, R.; Kostryco, M. (2023). Rubi idaei fructus as a source of bioactive chemical compounds with an important role in human health and comparison of the antioxidant potential of fruits and juice of three repeat-fruiting rubus idaeus L. cultivars. Metabolites, 13:1124. https://doi.org/10.3390/ metabo13111124.

Ciesielska-Figlon, K.; Wojciechowicz, K.; Wardowska, A.; Lisowska, K.A. (2023). The Immunomodulatory Effect of Nigella sativa. Antioxidants, 12:1340. https://doi.org/10.3390/ antiox12071340

Cundra L.B., D'Souza M.D., Parekh, P.J. and Johnson D.A. (2020). The impact of kiwifruit on human health. Gut and Gastroenterology, 3, 001-010.

Daniel Felipe Toro Suárez, Luciana Leite de Andrade Lima, Thayza Christina Montenegro Stamford, Dayanne Consuelo da Silva, Tiago Gomes Santos, Gerlane Souza de Lima, Vivianne Montarroyos Padilha and Tânia Lúcia Montenegro Stamford (2022). Physicochemical and sensorial characterization of yacon mixed juice with bioactive properties, Ciência Rural, Santa Maria, v.52:6, e20210140.

Darmon N., Darmon M., Maillot M. and Drewnowski A. (2005). A Nutrient Density Standard for Vegetables and Fruits: Nutrients per Calorie and Nutrients per Unit Cost. J. Am. Diet Assoc.; 105:1881-1887.

David D. Kitts, Arosha N. Wijewickreme and Chun Hu (2000). Antioxidant properties of a North American ginseng extract , Molecular and Cellular Biochemistry 203: 1–10 .

Elisabetta T., C.-Y. Oliver Chen, M. Palmery, D.V. Valencia, and Peluso I. (2018). Non-Provitamin A and Provitamin A Carotenoids as Immunomodulators: Recommended Dietary Allowance, Therapeutic Index, or Personalized Nutrition? , Oxidative Medicine and Cellular Longevity Volume 2018, Article ID 4637861, https://doi.org/10.1155/2018/4637861

Farhadi S. and Ovchinnikov R.S. (2018). The relationship between nutrition and infectious diseases: A review. Biomed Biotechnol. Res. J., 2,168-172.

Fatima A., Harshitha C. and Priyanka A. (2020). Food and Nutrition as natural immune boosters: An Elaborative Review. International Journal of Innovative Science, Engineering & Technology, 7(6)105-120.

Heny, N. J. and Claman (1989). Immune enhancing T cell and B cell J Allergy clim. Immunology 84(6) : pp1012-1014

Karacabey K. and Ozdemir N. (2012). The Effect of Nutritional Elements on the Immune System. Journal of Obesity & Weight Loss Therapy, 2(9),1-6; https://doi:10.4172/2165-7904.1000152

Kim, J.H.; Kismali, G. and Gupta, S.C. (2018). Natural Products for the Prevention and Treatment of Chronic Inflammatory Diseases: Integrating Traditional Medicine into Modern Chronic Diseases Care. Evid. Based Complementary Altern. Med., 2018, 9837863.

Li, H., Xia, Y., Liu, H. Y., Guo, H., He, X. Q., Liu, Y. and Gan R.Y. (2022). Nutritional values, beneficial effects, and food applications of broccoli (*Brassica oleracea* var. italica Plenck). Trends in Food Science & Technology, 119, 288-308.

Maheshwari S., V. Kumar, G. Bhadauria and Mishra A. (2022). Immunomodulatory potential of phytochemicals and other bioactive compounds of fruits: A review. Food Frontiers; 3:221–238.

Mahn, A. and Castillo, A. (2021). Potential of Sulforaphane as a Natural Immune System Enhancer: A Review. Molecules, 26, 752, https://doi.org/10.3390/ molecules26030752.

Manfred Schmolz, Reinhard W. März, Marco Schaudt, Cornelia Schaudt and Carola Lauster (2014). Immunomodulatory activities of a concentrated fruit and vegetable juice tested in a randomized, placebo-controlled, double-blind clinical trial in healthy volunteers, Food and Nutrition Sciences, 5, 341-350.

Martin KussMann (2010). Nutrition and immunity, RSC Food Analysis Mongraphs. No.9, chapter 9, pp: 268-299, The Royal Society of chemistry.

Mensor L.L, F. S. Menezes, G.G. Leitao, A. S. Reis, T. C. Dos Santos, and Coube C.S. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., 15:127-130

Mukherjee Pulok K., Neelesh K. Nema, Santanu Bhadra, and D. Mukherjee, Fernão C. Braga and Motlalepula G. Matsabisa (2014). Immunomodulatory leads from medicinal plants, Indian Journal of Traditional Knowledge Vol. 13 (2), April 2014, pp. 235-256

Paganga, G.; N. Miller and Rice-Evans C.A. (1999). The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? Free Radical Res., 30(2):153–162.

Ragana, (1979). Manual of analysis of fruit and vegetable products. Tata Mc Grow Hil publishing (New Delhi) pp: 94-96.

Ramesh K. S., Ravindranath S. Kambimath and Venkatesan N. (2016). Study of immunomodulatory activity of aqueous extract of Carica papaya in Wistar rats, National Journal of Physiology, Pharmacy and Pharmacology, Vol 6, Issue 5, p: 442:444

Richardson, D. P., Ansell, J. and Drummond L.N. (2018). The nutritional and health attributes of kiwifruit: A review. European Journal of Nutrition. doi.org/10.1007/s00394-018-1627-z

Sajith M. and Riya Farha P. (2023). Therapeutic potential of kiwi fruit. The Pharma Innovation Journal; 12(7):28-37.

Sang Myung Lee , Bong-Seok Bae, Hee-Weon Park, Nam-Geun Ahn, Byung-Gu Cho, Yong-Lae Cho and Yi-Seong Kwak (2015). Characterization of Korean Red Ginseng (Panax ginseng Meyer): History, preparation method, and chemical composition , Journal of Ginseng Research V:39, I:4, P: 384-391

SAS, (1999). Statistical Analysis system. SAS user’s guide: for personal computers, version 8.2 Edition SAS Institute, Cary, 5136 p.

Singleton V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic , phophotungstic acid reagents. Am. J. Enolo. Viticul, 16:144-158

Soliman M.D.E. and Attia M.Y. (2007). Specific phagocytic inhibition test (A new assay) in diagnosis of foods, aspirin, and inhalants allergy. Sc. J. Az. Med. Fac. (Girls), 28(3):559-575.

Valdés-González, J.A.; Sánchez, M.; Moratilla-Rivera, I.; Iglesias, I.; Gómez-Serranillos, M.P. (2023). Immunomodulatory, Anti-Inflammatory, and Anti-Cancer Properties of Ginseng: A Pharmacological Update. Molecules, 28:3863, https://doi.org/ 10.3390/molecules28093863

Wadmare V.B., Gadhe K.S. and Joshi M.M. (2019). Studies on physical and chemical composition of Broccoli (*Brassica oleracea* L.). International Journal of Chemical Studies; 7(2):825-828.

Watts B.G., Ylimaki L., Jeffery and Elias L. (1989). Basic sensory methods for food evaluation. Ottawa: The International Development Research Center. 160 p.

Zhishen, J., Mengcheng. T. and Jianming W. (1999). the determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chem, 64: 555-559.