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Research article

Efficiency of newly formulated functional instant soup mixtures as dietary supplements for elderly

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ABSTRACT

Healthy diet for elderly not only provides them with their needs from macro and micronutrients but also help preventing and treating age-related disorders including non-communicable diseases. So, the present study established to evaluate physical, sensory, chemical and biological characteristics of newly formulated functional instant soup mixtures as dietary supplements for elderly. Lyophilized chickpea, some vegetables and some by-products (at 5% and 10%) were incorporated in the preparation of two instant soup mixtures. The biological effects of the mixtures were studied using a geriatric animal model. The results revealed a reasonable acceptance of the two mixtures even after storage period (4 months) in addition to their contents from protein, fat, crude fiber and carbohydrates (16.62, 6.20, 6.60 and 65.89%, respectively in mixture I; 16.89, 6.30, 6.30 and 54.16%, respectively in mixture II). Mixture II was more promised in flavonoids content and scavenging radical activity than mixture I. Feeding the geriatric rats on the two mixtures did not produce any change in either liver or kidney functions and suggested the ability of these mixtures to prevent the hyperglycemia and hyperlipidemia and improve bone health. A slight decrease in brain lipid peroxidation, although not statistically significant, of rats has been observed upon feeding on these mixtures. Also, the two mixtures increased feces weight of rats which indicates to the beneficial effects of these mixtures in prevention of constipation. In conclusion the formulated instant soup mixtures with high acceptability and antioxidant activity can provide elderly people with high percent of their requirements from macro and micronutrients.

1. Introduction

It is very important for older people to maintain a healthy diet. This will help them in staying fit and keeping them active during the day. Avoiding malnutrition is crucial especially in elderly people since it leads to multiple health problems, like non-communicable diseases (Zahangir et al., 2017). Other health issues facing elder people include chewing and swallowing problems as a result of loss of teeth. So, swapping gristly food with soft food makes chewing and swallowing much easier (Okamoto et al., 2015). One way of avoiding malnutrition and solving the swallowing problem in elderly is providing nutrients enriched foods that are easy to eat and to cook, for example instant soup powder which has many advantages like flavor stability across a long period of time at room temperature and protection from enzymatic and oxidative spoilage (Farzana et al., 2017). The nutritional quality can be improved by introducing macro and micro nutrient sources which is appropriate for the elderly.

Chickpea (*Cicer arientum L.*) contain powerful bioactive compounds among them saponins which promote great antioxidants activity and calcium which reduces the risk of osteoporosis in addition to its role in prevention of tooth decay (Bar-El Dadon et al., 2017). Chickpea also are great sources of protein rich in tryptophan amino acid (Çevikkalp et al., 2016) that organizes the appetite, sleep and mood (Friedman, 2018).

Mushrooms are healthy, nutritious and low in calories also rich in proteins, minerals and vitamins but low in fats (Valverde et al., 2015). Mushrooms are excellent sources of vitamin b, which plays an important role in nervous system and help maintaining healthy red blood cell (Enas et al., 2016).

Parsley and dill are used to elevate the flavor of dishes like soup and salads. Also they are low in calories and packed with minerals, vitamin (A, K and C) and nutrients that improve heart health and immune system (Karklelienė et al., 2014; El Gindy et al., 2017).

Celery leaves and stems, either fresh or dried, have been used in the food, pharmaceutical and cosmetic industries. It contains a lot of

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minerals and vitamins, and it is recommended for the treatment of cardiovascular, digestive and cognitive problems (Salehi et al., 2019). Also it contains flavonol glycosides (quercetin, apiol, myristicin and luteolin) as reported by Moreno-Luna et al. (2012).

Olive oil is rich in monounsaturated fatty acids, oleic acids in addition to containing modest amount of vitamin E and K (Casado-Díaz et al., 2019). Olive oil can reduce the risk of chronic diseases due to its potent antioxidant effect and richness in biologically active compounds (Šarolić et al., 2014).

Moure et al. (2001) suggested that vegetables by-product valorized as sources of natural antioxidants. Lettuce leaves are sources of vitamins (such as vitamin C) and minerals including calcium, phosphorus, iron and copper (Kim et al., 2016). Onion peels contain a large quantity of polyphenol quercetin and exhibited many beneficial effects such as anti-obesity, anti-diabetes, antihypertensive in animal models and human (Kim and Yim, 2015). Banana peels can be incorporated in functional foods as a source of dietary fiber which has shown beneficial effects in the prevention of several diseases such as cardiovascular diseases, diverticulitis, constipation, irritable colon, colon cancer and diabetes (Rodrigues et al., 2006).

Spent brewer's yeast is a good cheap protein source in addition to its plenty of nutritious and healthy ingredients such as vitamin B (Jarmołowicz et al., 2013; Waszkiewicz-Robak, 2013). Whey protein is the most common by-product of cheese industries. The amino acids profile of whey protein is similar to those of human skeletal muscle, which makes them absorb more rapidly than other proteins (Ronghui, 2015).

The objective of the present study was to evaluate the physical, sensory and chemical characteristics of newly formulated functional instant soup mixtures from the above mentioned ingredients in addition to the biological effects and reasonable safety of these mixtures via evaluation of them in geriatric animal model.

2. Materials and methods

2.1. Materials

Chickpea, vegetables (mushroom, parsley, dill and celery), olive oil and the by-products (outer leaves of lettuce, onion peels, banana peels, whey protein and brewery yeast) were purchased from local market**.**

2.2. Animals

Male Wistar rats of 247.8 ± 16.86g (geriatric rats, 18 weeks old) were used in the present study. Animals were obtained from the animal house of National Research Centre, Cairo, Egypt. Animals were kept individually in metabolic stainless steel cages; water and food were given *ad-libitum*. This study has been carried out according to the animal welfare guidelines established by the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt, and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.3. Methods

2.3.1. Preparation of the instant soup mixtures

Chickpeas with hull were soaked in water for 12 h then boiled for 90 min, leaves and peels were washed with distilled water. Mushroom were sliced and rinsed in water for 1 min then in o.2% sodium metabisulphite for 15 min. Boiled chickpea, vegetables (mushroom, parsley, dill and celery) and the by-products (outer leaves of lettuce, onion peels, banana peels) were freeze-dried at 10^{-1} mbar and 30 °C for 72h (Edwards Moduly Freeze Dryer, United Kingdom). The materials were ground then stored individually in polyethylene bags at 24 °C. Two mixtures of instant soup powders were prepared (Table 1) by mixing the lyophilized ingredients, olive oil and corn starch as thickening agent. Then the mixtures were sealed in translucent polyethylene bags at 24 °C until used.

Table 1. Chemical composition of the instant soup powder mixtures.

Ingredients	Mixture I 5% by-products (g/100g)	Mixture II 10% by-products (g/100g)
Chickpea	25	22.5
Mushroom	25	22.5
Parsley	5	5
Dill	5	5
Celery	15	15
Olive oil	5	5
Corn starch	15	15
By-products		
Outer leaves of Lettuce	1	2
Onion peels	1	2
Banana peels	1	2
Brewery yeast	1	2
Whey protein	1	2

2.3.2. Physical and sensory evaluation of the instant soup mixtures

The instant soup powder mixtures were evaluated at the zero time of preparation and after storage period (4 months) after dissolving in hot water (25 g instant soup mixture/100mL water) for its sensory characteristics (appearance, and thickness, flavor, creaminess, taste and color). The evaluation was carried out by trained 10-member panelist from the staff members of food technology department, National Research Centre, Dokki, Cairo, Egypt. Each panelist was provided with the sample in an unlabeled transparent cup under white lights and asked to cleanse the palate with water before tasting the second sample. The general acceptability was calculated. Viscosity of soup mixtures were measured according to Brookfield manual by using Brookfield viscometer at 50 rpm. The SC4-21 spindle was selected for the measurement. pH of the two mixtures were determined using pH meter.

2.3.3. Chemical analysis of the instant soup mixtures

The Proximate analysis (moisture, ash, crude fat, crude fibers and nitrogen content) was carried out according to the methods of AOAC (2004). Amino acids were determined using amino acid analyzer (Biochrom 30) Germany, according to the method of AOAC (2012). Minerals (zinc, iron, phosphorus, sodium, potassium, magnesium, calcium and selenium) were determined according to the methods described by A.O.A.C. (2000). Vitamins (B12, Folic Acid, A, C, D and E) were determined by HPLC as described by Pyka and Sliwiok (2001). Total phenolic contents, total flavonoid contents and radical scavenging activities of the instant soup mixtures were determined at the 0,1,2,3 and 4 months of the storage period. Samples of the two mixtures (20g of each sample) were extracted with 300mL ethanol (70%), shaken for 1 h and then filtered with Whatman No.1 paper. The extracts were labeled and kept for determining total phenolic contents, total flavonoid contents and radical scavenging activities. Total phenolic contents were determined by spectrophotometer at 760 nm using the Foline Ciocalteau colorimetric method described by Singleton et al. (1999). The total flavonoids were determined by the colorimetric method of Zhuang et al. (1992). The radical scavenging activity was assayed using stable 2,2-diphenil-1-picrylhydrazyl (DPPH) free radical according to Brand-Williams et al. (1995).

2.3.4. Evaluation of the instant soup mixtures in geriatric rats

2.3.4.1. Preparation of diets for the animal experiment. Diets were formulated as described in Table 2. Balanced diet, salt and vitamin mixtures were prepared in accordance with AIN-93 (Reeves et al., 1993). Twenty grams from powder of mixture I or II were mixed with the balanced diet to give diets supplemented with 20% of either mixture I or II respectively. The contents of protein, fat, crude fiber and carbohydrate

Table 2. Composition of different experimental diets (g/100 g).

Ingredients	Diets		
	Balanced	Mixture I	Mixture II
Protein			
Casein	12*	8.68	8.62
Fat			
Corn oil	10	8.76	8.74
Carbohydrate			
Sucrose	10	10	10
Starch	58.5	44.38	44.40
Salt mixture	3.5	3.5	3.5
Vitamin mixture	1	1	1
Cellulose (fiber)	5	3.68	3.74
Mixture I	-	20	-
Mixture II	-	-	20

* 12 g casein has been estimated to contain 10 g protein. 20g of mixture I provide 3.32 g protein, 1.24 g fat, 1.32 g fiber and11.38g carbohydrate and 20g of mixture II provide 3.38 g protein, 1.26 g fat, 1.26 g fiber and10.83g carbohydrate.

of the 20 g powder of mixture I or II were reduced from casein protein, corn oil, cellulose and starch.

2.3.4.2. Animals' treatments. Eighteen rats were divided into three groups (each of six rats based on Mead's resource equation). The first group was considered as the control group where rats received a balanced diet. The second group was named mixture I where rats were fed on balanced diet supplemented with 20% of mixture I. The third group was named mixture II where rats were fed on balanced diet supplemented with 20% of mixture II. During the experiment, feces weight, body weight and food intake were recorded weekly. After four weeks (end of the study) total food intake, body weight gain and feed efficiency ratio (Body weight gain/total food intake) were calculated.

2.3.4.3. Analysis of feces, blood and tissues. Feces moisture was determined according to AOAC (2000). Blood samples were collected from all rats after an overnight fast. A portion of the whole blood was analyzed for haemoglobin (Hb) concentration according to Drabkin (1949). The remaining blood was centrifuged and the plasma was analyzed for fasting blood glucose levels according to Trinder (1969), total cholesterol according to Watson (1960), high-density lipoprotein cholesterol (HDL-C) using the method of Burstein et al. (1980), low-density lipoprotein cholesterol (LDL-C) according to Schriewer et al. (1984) and triglycerides according to the method of Megraw et al. (1979). Cholesterol/HDL-C ratio was calculated. The levels of creatinine and urea were determined depending on Larsen (1972) and Fawcett and Scott (1960) in succession as indicators of kidney functions. The activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to Reitman and Frankel (1957). The activity of alkaline phosphatase (ALP) was determined according to Bessey et al. (1946). The levels of bilirubin, total protein and albumin were determined according to Jendrassik and Grof (1938), Rheinhold (1953) and Doumas et al. (1971) in succession. Plasma sodium, potassium, calcium (total and ionized), phosphorus and magnesium were determined according to Trinder (1951), Terri and Sesin (1958), Gindler and King (1972), El-Merzabani et al. (1977) and Smith (1955) in succession. After blood sampling rats were dissected and the brain, liver, kidney, femur and abdominal fat were immediately separated from each rat and weighed then the brain immediately analyzed for Malondialdehyde (MDA) according to Ohkawa et al. (1979). Femur of each rat was burned at 600 °C using muffle to be converted into ash, which was later used in the estimation of calcium, magnesium and phosphorus by the atomic absorption spectrophotometer apparatus (model IL atomic absorption/air ethylene).

Table 4. Proximate analysis of the two mixtures of instant soup on dry weight basis.

Nutrients	Mixture I	Mixture II
	g/100g	
Moisture[1]	3.95 ± 0.17	3.65 ± 0.14
Protein[1]	16.62 ± 0.25	16.89 ± 0.11
Fat[1]	6.20 ± 0.70	6.30 ± 0.44
Crude fiber[1]	6.60 ± 0.60	6.30 ± 0.53
Ash[1]	9.74 ± 0.14	12.70** ± 0.62
Carbohydrate[2]	56.89 ± 1.55	54.16 ± 1.23
Calories[3]	350.00* ± 0.83	341.00 ± 5.51

* significant at $p \leq 0.05$, ** significant at $p \leq 0.01$.
[1] With freeze dried.
[2] By difference: 100 - (moisture + protein + fat + crude fiber + ash).
[3] Calculated (fat x 9 + protein x 4 + carbohydrate x 4),

2.3.4.4. Statistical analysis. Results of chemical analysis (Proximate analysis, amino acids, minerals, vitamins, total phenolic contents, total flavoniods and the radical scavenging activity) of the two mixtures were expressed as mean ± SD and compared statistically using T-test by SPSS program version 16. Results of the animal experiment were expressed as mean ± SE and analyzed statistically using GraphPad Prism version 7.01 (San Diego, California). The Kruskal Wallis test was used to identify significant differences within the experimental groups and Dunn's multiple comparisons post-test was used to assess the significance of differences between experimental groups. In all cases p ≤ 0.05 was used as the criterion of statistical significance.

3. Results

3.1. Physical and sensory evaluation of the instant soup mixtures

The highest apparent viscosity was found in the mixture I (886 cp), followed by mixture II (679 cp). The pH of the mixture I and II were 6.5 and 6.8, respectively. Sensory scores (Table 3) of the instant soup

Table 3. Sensory evaluation of the instant soup mixtures during the storage period.

		Sensory attributes						
		Appearance	Thickness	Flavor	Creaminess	Taste	Color	General acceptability
Mixture I	Zero time	8.05	7.85	7.90	7.65	8.75	7.95	8.35
Mixture II		9.10	8.45	9.15	9.10	9.85	9.25	9.60
Mixture I	After 4 months storage	8.10	7.95	8.10	7.55	8.65	7.85	8.70
Mixture II		8.70	7.90	8.60	8.35	9.05	8.80	9.00

Table 5. Amino acids profile of the two mixtures of instant soup compared with the requirement according FAO/WHO/UNU.[1]

Essential amino acids	Requirement (mg/g protein)	Mixture I (mg/g protein)	%[2]	Mixture II (mg/g protein)	%[2]
Threonine	23	14.4 ± 0.36	62.61	16.35** ± 0.33	71.1
Valin	39	19.5 ± 0.44	50	23.98** ± 0.23	61.5
Isoleucine	30	15.75 ± 0.13	52.5	18.75** ± 0.11	62.5
Leucine	59	26.1 ± 0.30	44.24	30.3** ± 0.36	51.4
Total aromatic amino acids	38	29 ± 0.36	76.32	35** ± 0.70	92.11
Lysine	46	24 ± 0.46	52.17	25* ± 0.36	54.35
Total sulfur amino acids	22	14.82 ± 0.09	67.4	17.7** ± 0.66	80.45
Tryptophan	6	2.1 ± 0.20	35.00	2.9** ± 0.17	48.33

* significant at $p \leq 0.05$, ** significant at $p \leq 0.01$.
[1] From FAO/WHO/UNU Expert Consultation (2007).
[2] % that provided by g protein.

Table 6. Minerals content of the two mixtures of instant soup in comparison with Dietary Reference Intake (DRI) for elderly men.[1]

Minerals	RDA/AI [2] (mg/day)	Mixture I (mg/100g)	%[3]	Mixture II (mg/100g)	%[3]
Calcium	1200	109 ± 1.4	9.1	112.5 ± 1.8	9.4
Phosphorus	700	128.62 ± 2.12	18.4	130.54 ± 2.85	18.65
Iron	8	3 ± 0.17	37.5	4.03** ± 0.07	50.4
Sodium	1200	91.085 ± 1.73	7.6	92.49** ± 0.91	7.71
Potassium	4700	387.6 ± 3.97	8.25	393.3 ± 3.82	8.37
Zinc	11	1.27 ± 0.07	11.54	1.42* ± 0.03	12.91
Magnesium	2300	402.5** ± 2.18	17.5	393.35 ± 1.85	17.1
Selenium	55	6.77* ± 0.07	12.31	7.3 ± 0.26	13.27

* significant at $p \leq 0.05$, ** significant at $p \leq 0.01$.
[1] According to National Academies of Sciences, Engineering, and Medicine. (2019).
[2] RDA = Recommended Dietary Allowance; AI = Adequate Intake.
[3] % that provided by 100g mixture.

mixtures with regard to appearance, thickness, flavor, creaminess, taste, color, and overall acceptability were found to be more acceptable in mixture II than mixture I. Sensory scores of the two mixtures were almost stable during the storage.

3.2. Chemical analysis of the instant soup mixtures

The obtained results of macro and micronutrients of the two mixtures were compared to the nutritional requirements recommended by the Dietary Reference Intake (DRI) for elderly males. This gender was chosen as a recommendation of macro and micronutrients, the two mixtures of instant soup contained macro and micronutrients greater than those required for females; thus, they are able to cover the needs of both genders. It is clear (Table 4) that the two mixtures showed high content of protein (16.62 and 16.89 g/100g, respectively). Regarding DRI of protein for males aged >70 years (56 g protein/day), 100g of each mixture (I and II) provide 29.7% and 30.16%, respectively of daily requirements. The carbohydrate contents of the two mixtures were 56.89 and 54.16 g/100g. Regarding DRI of carbohydrate, each 100g of mixture I and mixture II provide 43.8% and 41.7%, respectively of daily requirements of carbohydrate. The fat contents of the two mixtures were 6.2 and 6.3 g/100g, respectively. Regarding DRI of fat, each 100g of mixture I and mixture II provide 20.7% and 21%, respectively of daily requirements of fat.

The essential amino acids profile of the two mixtures in comparison with the requirements is presented in Table 5. It was shown that the highest recorded amount of amino acids present in the mixture I and II was tyrosine and phenylalanine (aromatic amino acids). The mixture I and II provide 76.32 and 92.11%, respectively, of the daily requirement from aromatic amino acids. While the lowest recorded amount in the mixture I and II was for tryptophan. The mixture I and II provide 35 and 48.33%, respectively, of the daily requirement from tryptophan.

As regards to the results of the minerals content of the two mixtures (Table 6), the mixture I provide 9.1, 37.5, 17.5, 18.4, 12.31, 11.54, 8.25 and 7.6% of the RDI for calcium, iron, magnesium, phosphorus, selenium, zinc, potassium and sodium, respectively while mixture II provides 9.4, 50.4, 17.1, 18.65, 12.37, 12.91, 8.37, and 7.71% of the RDI for calcium, iron, magnesium, phosphorus, selenium, zinc, potassium and sodium, respectively.

Table 8. Total phenolic compounds, total flavonoids and radical scavenging activities of the instant soup mixtures during the storage period.

	Total Phenolic compounds (mg GAE/100g)	Total Flavonoids (mg/100g)	DPPH (%)
Mixture I			
Zero time	1222.54** ± 2.21	254.89 ± 1.84	72.33 ± 1.60
One month	1211.60** ± 2.41	249.27 ± 1.94	72.05 ± 0.98
Two months	991.17** ± 1.09	230.05 ± 0.99	71.00 ± 0.87
Three months	986.00** ± 1.25	229.36 ± 1.76	69.38 ± 0.90
Four months	906.34** ± 2.42	224.75 ± 2.00	61.11 ± 1.10
Mixture II			
Zero time	745.65 ± 1.44	326.84** ± 1.77	87.99** ± 1.72
One month	744.66 ± 1.61	324.03** ± 1.23	86.98** ± 1.03
Two months	742.47 ± 1.95	316.68** ± 1.66	85.88** ± 1.08
Three months	699.77 ± 1.89	312.06** ± 2.00	77.82** ± 1.58
Four months	573.12 ± 1.97	279.55** ± 2.18	75.95** ± 1.00

Total phenolic, total flavonoids and radical scavenging activity of the two mixtures were statistically compared at each time of storage. ** significant at $p \leq 0.01$.

Table 7. Vitamins content of the two mixtures of instant soup in comparison with Dietary Reference Intake (DRI) for elderly men.[1]

Vitamins	RDA[2]	Mixture I	%[3]	Mixture II	%[3]
A	900 (μg RAE[4]/day)	663 ± 2.85 (μg/100g)	73.7	793** ± 1.32 (μg/100g)	88.1
C	90 (mg/day)	31.5 ± 0.40 (mg/100g)	35	38.4** ± 0.95 (mg/100g)	42.7
E	15 (mg/day)	7.6 ± 0.46 (mg/100g)	50.7	6.86 ± 0.14 (mg/100g)	45.73
B_{12}	2.5 (mg/day)	0.1 ± 0.01 (mg/100g)	4	0.2** ± 0.02 (mg/100g)	8
Folate	400 (μg/day)	115.5** ± 0.62 (μg/100g)	28.9	101.1 ± 0.85 (μg/100g)	25.3
D	15 (μg/day)	2.5 ± 0.20 (μg/100g)	16.7	2.25 ± 0.05 (μg/100g)	15

** significant at $p \leq 0.01$.
[1] According to National Academies of Sciences, Engineering, and Medicine. (2019).
[2] RDA = Recommended Dietary Allowance.
[3] % that provided by 100g mixture.
[4] RAE = Retinol Activity Equivalent.

Table 9. Nutritional parameters as well as feces' weight and moisture of different studied groups.

Parameters	Control	Mixture I	Mixture II	*p*-value
Initial B.W. (g)	247.83 ± 8.80	247.83 ± 6.54	247.67 ± 6.40	0.9776
Final B.W. (g)	295.67 ± 5.31	292.83 ± 6.46	279.50 ± 9.67	0.4929
B.w. gain (g)	47.83 ± 4.51	45.00 ± 2.57	31.83 ± 6.57	0.0932
Total food intake (g)	549.83 ± 15.73	576.17 ± 10.46	572.67 ± 14.97	0.3527
Food Efficiency Ratio	0.09 ± 0.010	0.08 ± 0.004	0.05 ± 0.010	0.0876
Feces weight (g/day)	1.16 ± 0.04	2.46** ± 0.05	2.42* ± 0.03	0.0003###
Feces moisture (%)	21.29 ± 0.64	25.05* ± 0.75	25.33* ± 0.90	0.0089##

The data are expressed as mean values ±standard error. The confidence level is 95%. Kruskal Wallis test *p*-value < 0.05 (##, significant; ###, high significant) means significant difference; > 0.05 means non-significant difference.
*$p \leq 0.05$; **$p \leq 0.01$; both significantly different from control group (Dunn's Multiple Comparison test).

Vitamin contents of the two mixtures are presented in Table 7. Results showed that 100g of each tested mixture contained suitable amounts of vitamins when compared with RDI. Because these mixtures are considered a dietary supplement, they will be taken with the original meal. The results showed that mixture I provide 4, 28.9, 16.7, 50.7, 35 and 73.7% of the RDI for vitamin B_{12}, folate, Vitamin D, vitamin E, vitamin C and vitamin A, respectively while mixture II provides 8, 25.3, 15, 45.73, 42.7 and 88.1% of the RDI for vitamin B_{12}, folate, Vitamin D, vitamin E, vitamin C, and vitamin A, respectively.

As illustrated in Table 8, total Phenolic compounds showed a slight decrease in content during storage at 24 °C, about 89% was still present after 4 months. Flavonoids and antioxidant capacity of the two mixtures were almost stable during the storage. The results indicated that mixture II was promised in total flavonoids content and radical scavenging activity than mixture I.

3.3. Effect of dietary supplementation with the instant soup mixtures on the nutritional parameters as well as weight and moisture of rats' feces

As illustrated from Table 9 that the dietary supplementation with mixtures I and II, more extremely mixture II, resulted in a slight reduction in the body weight gain in comparison to the control rats group. Additionally, rats fed on the balanced diet supplemented with either mixture I or II recorded values of feces' weight and moisture significantly higher than those of the control rats group.

Table 10. Organs' weights of different studied groups.

Parameters	Control	Mixture I	Mixture II	*p*-value
Brain weight (g)	1.45 ± 0.07	1.50 ± 0.06	1.40 ± 0.07	0.6570
Brain relative weight	0.49 ± 0.03	0.51 ± 0.02	0.50 ± 0.02	0.7975
Kidney weight (g)	2.27 ± 0.09	2.21 ± 0.15	2.21 ± 0.10	0.8719
Kidney relative weight	0.77 ± 0.04	0.76 ± 0.05	0.79 ± 0.03	0.8496
Liver weight (g)	7.26 ± 0.37	7.32 ± 0.14	7.21 ± 0.16	0.9379
Liver relative weight	2.45 ± 0.10	2.50 ± 0.04	2.59 ± 0.09	0.4235
Abdominal fat weight (g)	3.74 ± 0.33	2.98 ± 0.20	2.41* ± 0.27	0.0155#
Abdominal fat relative weight	1.28 ± 0.13	1.02 ± 0.08	0.85 ± 0.08	0.0685
Femur weight (g)	1.02 ± 0.07	1.24 ± 0.05	1.20 ± 0.05	0.0427#
Femur relative weight	0.34 ± 0.02	0.42 ± 0.02	0.43 ± 0.02	0.0343#

The data are expressed as mean values ±standard error. The confidence level is 95%. Kruskal Wallis test *p*-value < 0.05 (#) means significant difference; > 0.05 means non-significant difference.
*$p \leq 0.05$; significantly different from control group (Dunn's Multiple Comparison test).

Table 11. The biochemical parameters of different studied groups.

Parameters	Control	Mixture I	Mixture II	*p*-value
Hb (g/dL)	14.34 ± 0.35	15.20 ± 0.23	15.03 ± 0.29	0.2044
Glucose (mg/dL)	79.48 ± 5.05	82.86 ± 4.11	81.77 ± 5.16	0.8808
Brain MDA (nmol/g tissue)	14.83 ± 0.94	14.35 ± 0.27	13.87 ± 1.30	0.5097
T. Cholesterol (mg/dl)	73.52 ± 5.33	75.27 ± 3.63	74.36 ± 4.93	0.9844
T.G (mg/dL)	77.05 ± 3.65	78.21 ± 4.44	75.99 ± 4.52	0.9379
HDL-Ch (mg/dL)	37.67 ± 1.05	39.50 ± 0.76	40.25 ± 0.65	0.1649
LDL-Ch (mg/dL)	21.77 ± 1.07	20.93 ± 0.94	20.75 ± 1.26	0.7018
Cholesterol/HDL ratio	1.96 ± 0.15	1.90 ± 0.08	1.84 ± 0.10	0.8968
Urea (mg/dL)	23.56 ± 1.09	23.47 ± 1.56	22.64 ± 1.70	0.9130
Creatinine (mg/dL)	0.41 ± 0.03	0.40 ± 0.03	0.38 ± 0.02	0.8539
AST (U/L)	19.50 ± 1.61	18.67 ± 1.89	17.50 ± 1.06	0.6629
ALT (U/L)	52.33 ± 1.78	50.50 ± 1.06	51.33 ± 2.18	0.8110
ALP (IU/L)	209.08 ± 6.11	207.08 ± 6.39	204.06 ± 4.94	0.9690
T. Bilirubin (mg/dL)	2.65 ± 0.07	2.42 ± 0.10	2.45 ± 0.07	0.1271
D. Bilirubin (mg/dL)	1.45 ± 0.04	1.42 ± 0.06	1.42 ± 0.06	0.9464
Ind. Bilirubin (mg/dL)	1.20 ± 0.07	1.00 ± 0.11	1.02 ± 0.12	0.2901
T. Protein (g/dL)	4.95 ± 0.46	5.16 ± 0.38	5.22 ± 0.25	0.5782
Albumin (g/dL)	5.03 ± 0.21	5.08 ± 0.28	5.54 ± 0.20	0.2901

The data are expressed as mean values ±standard error. The confidence level is 95%. Kruskal Wallis test *p*-value < 0.05 means significant difference; > 0.05 means non-significant difference.

3.4. Effect of dietary supplementation with the instant soup mixtures on the organs' weights of rats

Regarding organs' weights (Table 10), there were no significant changes in the weights of the brain, kidney and liver among the different studied groups. While, the abdominal fat weight measured from rats fed on either mixture I or II, more significantly mixture II, was less than that measured from control rats group. The weight and relative weight of femur measured from rats fed on either mixture I or II were higher than that measured from the control rats group.

3.5. Effect of dietary supplementation with the instant soup mixtures on the biochemical parameters of rats

As evident from Table 11, the dietary supplementation with mixtures I and II did not produce a significant change in blood Hb nor glucose. Regarding brain MDA, as the most plentiful lipid peroxidation-specific aldehyde, there were no significant changes in this parameter among the different studied groups. As for the lipid profile, it was demonstrated from the results tabulated in Table 11 that neither mixture I nor II caused alteration in the lipid profile though there was a slight increase in HDL-Ch in the rats fed on either mixture I or II in compared to the control rats group. Also, neither mixture I nor II produced any significant changes in either kidney or liver functions when compared to the control group.

3.6. Effect of dietary supplementation with the instant soup mixtures on the plasma minerals as well as weight and minerals of rats' femur

The groups supplemented mixture I or II did not show a significant difference in plasma values of sodium, calcium, ionized calcium and phosphorus compared to the control group (Table 12). While the plasmatic values of potassium and magnesium recorded by rats fed on a balanced diet supplemented either mixture I or II were slightly higher, but not statistically significant, than those recorded by the control group. A slight increase (non-significant, Kruskal Wallis test $p > 0.05$) in femur calcium, phosphorus and magnesium recorded by rats fed on a balanced diet supplemented either mixture I or II when compared to the control group.

Table 12. Plasma and femur minerals content of the rats supplemented mixture I and II.

Parameters	Control	Mixture I	Mixture II	*p*-value
Plasma				
Sodium (mEq/L)	158.15 ± 3.99	156.93 ± 2.71	156.22 ± 3.44	0.9663
Potassium (mEq/L)	5.95 ± 0.19	6.53 ± 0.29	6.83 ± 0.30	0.1252
Calcium (mg/dL)	9.56 ± 0.35	9.76 ± 0.36	9.93 ± 0.27	0.7761
Ionized calcium (mg/dL)	4.74 ± 0.25	4.77 ± 0.31	4.70 ± 0.21	0.9928
Phosphorus (mg/dL)	7.58 ± 0.42	7.91 ± 0.30	7.72 ± 0.39	0.9130
Magnesium (mg/dL)	3.30 ± 0.27	3.71 ± 0.12	3.96 ± 0.09	0.2329
Femur				
Calcium (mg/g)	282.30 ± 8.14	285.78 ± 8.77	285.35 ± 8.92	0.9379
Phosphorus (mg/g)	117.57 ± 7.90	133.09 ± 9.51	134.18 ± 9.08	0.5622
Magnesium (mg/g)	1.79 ± 0.09	1.89 ± 0.16	1.94 ± 0.27	0.8776

The data are expressed as mean values ±standard error. The confidence level is 95%. Kruskal Wallis test *p*-value < 0.05 means significant difference; > 0.05 means non-significant difference.

4. Discussion

The elevated viscosity of mixture I than mixture II may be owing to the increase of chickpea and mushroom content in mixture I. The great acceptability of the prepared instant soup mixtures was due to their ingredients. The stability of this acceptability during the storage period may be due to the incorporation of lyophilized ingredients in the preparation of the instant soup mixtures. Fissore and Pisano (2015) observed that freeze-drying reduces the water content to very low levels without affecting the stability of composition and the characteristics. It also contributes to the low moisture contents of the instant soup mixtures. This low moisture content played an important role in maintaining the quality of the instant soup mixtures during the storage period. The high protein content of instant soup mixtures is associated with the presences of chickpea, mushroom, whey-protein and brewer's yeast, which are good sources of protein. Bauer et al. (2013) observed that the addition of these ingredients to soup had greater potential in overcoming protein calorie-malnutrition in addition to the prevention of sarcopenia in the elderly. The fat contents of instant soup mixtures associated with the presence of olive oil. The fiber content of instant soup mixtures is associated with the presence of banana peel, onion peel, whole chickpea with outer layer and vegetables.

The good amino acid profiles of instant soup mixtures may be due to the presence of chickpea, mushroom and whey protein which are considered excellent sources of essential amino acids. It should be emphasized that the chickpea and mushroom proteins are complete which means that they contain all essential amino acid (Margier et al., 2018; Rana et al., 2015). It is important also to note that whey protein consists of branches amino acids (lysine, phenylalanine, methionine and tryptophan) which essential to tissue growth and promote the healing of bone, skin and muscle.

Micronutrient (minerals and vitamins) deficiencies are not associated only with malnutrition but also with many age-related disorders such as Alzheimer's disease, hypertension and heart diseases (Hoffman, 2017). Additionally, vitamins and minerals (such as potassium and sodium) play important roles in the biological system (Jayasinghe et al., 2016); also, zinc improves the mental health and immune function of elderly. In the present study, the two instant soup mixtures recorded high contents of minerals and vitamins which may be attributed to the ingredients of these mixtures. Abdel-Haleem et al. (2014); Roy et al. (2010); Moharram et al. (2008); Kurtzman (2005) reported that chickpea, mushroom, brewer's yeast and vegetables are excellent sources of minerals especially calcium, phosphorus, iron, zinc, magnesium, potassium and sodium. Slavin and Lloyd (2012) reported that plant-based foods contain considerable amounts of antioxidant vitamins. Celery and outer layers of lettuce have antioxidant vitamins such as vitamin C (ascorbic acid) and vitamin E (tocopherols), which are associated with cognitive decline in the elderly (Heo and Lee, 2004; Zanotta et al., 2014). Also, parsley and dill contain vitamin C and vitamin E (Laribi et al., 2015). Also, chickpea, mushroom and brewers yeast are good sources of vitamin B_{12}, and mushroom is an excellent source of vitamin D (Cardwell et al., 2018).

The antioxidant activities of the instant soup mixtures can be attributed to the presence of antioxidant vitamins in addition to flavonoids and polyphenols which are abundant in chickpea and vegetables. The elevated antioxidant activity of mixture II than mixture I may be due to the increase of by-products (banana and onion peels as well as lettuce leaves and whey protein) in the formulation especially that these ingredients have potent antioxidant activities as confirmed by Padilla--Camberos et al. (2016); Park et al. (2007); Park et al. (2018); Mohamed et al. (2019).

In this work, the reduction in body weight gain and the abdominal fat of rats fed on either mixture I or II compared to control rats can be attributed to the fiber contents of these mixtures. Commonly, fibers contribute to the improvement of intestinal function for elderly who suffer from constipation. This may interpret the significant elevation of feces' weight of rats fed on either mixture I or II. So, the prepared mixtures may be a good solution to constipation, a compliant disorder to the elderly (Dore et al., 2018).

The geriatric rats fed on either mixture I or II exhibited a slight increase (non-significant $p > 0.05$) in Hb compared to the control rats. That improvement in Hb may indicate to the benefits of these mixtures in preventing anemia that develops in the elderly due to deficiency of iron, folate and vitamin B_{12} (Patel, 2008). Feeding rats on the two mixtures did not produce any change in either liver or kidney functions. Also, neither hyperglycemia nor hyperlipidemia was observed after feeding on these mixtures. On the contrary, these mixtures could contribute to the prevention of hyperglycemia and hyperlipidemia owing to not only chickpea and vegetables but also olive oil that is considered potent antioxidant due to its phenolic compounds, improving the lipoprotein profile, glucose metabolism, blood pressure and antithrombotic profile (Moreno-Luna et al., 2012). In addition to the presence of fibers and polyphenols in these mixtures which improve postprandial glycemic response and cholesterol level as supported by Basu et al. (2019) and Cory et al. (2018). In older people high brain fat content makes it more susceptible to oxidation. Lipid peroxidation products are responsible for the oxidative brain (https://www.frontiersin.org/articles/10.3389/fnmol.2019.00132/full Zhu et al., 2006) and cognitive impairment in the elderly (Kim et al., 2015). In the present study, a slight (not significant) decrease in brain lipid peroxidation of rats has been observed in feeding on the two instant soup mixtures. The antioxidant activities of the prepared mixtures may be responsible for this slight decrease in the brain lipid peroxidation. This data indicate the preventive role of instant soup mixtures against neurological disorders such as Alzheimer's and Parkinson's disease as well as stroke once Shichiri (2014) reported that lipid peroxidation is involved in these disorders.

Rats fed on either mixture I or II recorded elevation in plasma potassium and magnesium, although not statistically significant, owing to the contents of these mixtures from these minerals. Elevated serum magnesium indicates the ability of the instant soup mixtures to improve bone health that emphasized by Orchard et al. (2014) who declared that magnesium has an important role in healthy bone remodeling. Also, the calcium contents of the two mixtures may help to avoid osteoporosis, which has been confirmed to be linked to calcium deficiency (Kruger and Wolber, 2016). De Jonge et al. (2018) concluded that a high intake of vegetables might be contributed to the improvement of bone mineral density.

The major limitation of this study was the inability to conduct the experiment directly on volunteers. Although the prepared instant mixtures were formulated from edible constituents as a dietary supplement for the elderly and evaluated regarding their acceptability and nutritive values, the pre-study on animals was required to obtain adequate data about the probable biological effects and reasonable safety of these newly formulated instant soup mixtures. Therefore, further studies focusing on the effect of these mixtures on either healthy elderly or those who are suffering from age-related diseases are required.

5. Conclusions

The developed instant soup mixtures recorded great acceptability up to 4 months of storage. It was revealed that the prepared instant soup mixtures high in protein, fiber, essential amino acids, minerals, and vitamins and can provide reasonable percent of the elderly requirements from macro and micronutrients. The instant soup mixtures also recorded antioxidant activity and high content of total phenolics and flavonoids. Additionally, the instant soup mixtures elevated the feces' weight and afforded reasonable safety, which was evident from the unaffected liver and kidney functions of rats. Also neither hyperglycemia nor hyperlipidemia was observed in feeding rats on these mixtures.

Declarations

Author contribution statement

Rasha S. Mohamed, Safaa S. Abozed: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

S. El-Damhougy, Manal F. Salama, Mona M. Hussein: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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