

## Nutritional characteristics and bioactive compounds of different ovo-vegetarian diets supplemented with spirulina

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### Abstract

In human nutrition, innovative research during the past few years recommended the contribution of functional vegetarian diets to improve human health and minimize risk diseases. In present study, three innovative ready-to-use (RTU) and ready-to-eat (RTE) chickpea-based ovo-vegetarian diets (OVDs) incorporating different vegetables (pea, taro, and broccoli) at 15% were prepared. These formulated OVDs with 34% chickpea were either supplemented with 1% spirulina as food supplement or formulated without spirulina. Herein, the nutritional and bioactive compounds of those OVDs could be investigated. Chemical composition, minerals content, bioactive compounds and antioxidant activity as well as amino acids content of OVDs before and after cooking were determined. RTE OVDs with or without spirulina were organoleptically tested after frying cooking. Results of composite analysis were 62.46 to 68.54, 17.52 to 20.57, 5.54 to 6.19, 2.47 to 2.69, 5.06 to 6.65 and 61.50 to 63.84% for moisture, crude protein, lipids, ash, fiber and carbohydrate contents in RTU OVDs, respectively. Significant differences ( $p < 0.05$ ) were found in chemical composition in RTU and RTE as well as caloric value of OVDs. RTU OVDs exhibit appropriate content of ascorbic acid, total phenolic compounds (TPC) flavonoids and flavonols which basically depends on their ingredients and possess a valuable antioxidant activity. However, frying process dramatically reduced the ascorbic acid, flavonoids and flavonols contents. Addition of spirulina had increased the minerals, phytochemicals and amino acids contents in OVDs. High organoleptic acceptability of RTE OVDs was noticed to confirm the consumer attractiveness. In conclusion, the possibility to produce healthy RTU and RTE OVDs incorporated with common consumed vegetables supplemented with spirulina could provide a promising approach for improving the human health and dietary pattern practices.

**Keywords:** Spirulina, bioactive compounds, amino acids, cooking, antioxidant activity, ovo-vegetarian diets, health benefits.

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### 1. Introduction

Recently, there has been a renewed interest in vegetarian diets and healthy eating. The vegetarian diets are often diverse formulated in composition and shape, comprising a wide range of dietary components for numerous and individual dietary requirements. Practically, adopting a vegetarian dietary pattern is traditionally interpreted to mean an absence of meat [1, 2].

Basically, vegetarians are distinguished by high consumption of fruit, vegetables, legumes, nuts, grains and soy protein-food components, and each of these may independently be associated with positive health impacts [1, 3-5]. Particularly, vegetarian diets were classified into (i) lacto-ovo-vegetarians (includes dairy and eggs), (ii) lacto-vegetarians (includes dairy), (iii) ovo-vegetarians (includes eggs), and (iv) vegan which have further restrictions

imposed and exclude all animal origin foods. Additionally, the meat substituting industry was highly encouraged to reduce the meat consumption and thereby reduce the risk of related disease. Obviously, substituting the meat consumption by alternative protein rich products made from plant proteins, so-called Novel Protein Foods, would be an attractive choice [6]. *The vegetarian diet could significantly reduce people's risk of heart disease as suggested by Oxford University. The vegetarians have up to 32% less risk of developing heart disease than non-vegetarians* [2, 7, 8]. Actually, it could encourage the processed meat consumers to change their nutritional style and prevent themselves from 42% higher risk of heart disease, a 19% higher risk of type 2 diabetes and bladder cancer as previously mentioned [4, 9].

Promising sources for new products and applications are Microalgae, a biological resource that enhance the nutritional value of food products, by enrich the polyunsaturated fatty acids, pigments, sterols, vitamins, and other biologically active compounds [10]. Spirulina had been used as a nutrient-dense food many countries. Indeed, nowadays microalgae are mainly marketed as food supplements with growing market for food products either as nutritious supplement, or as a source of natural food colourant as well as unique and attractive tastes [11]. Spirulina had been used in the traditional food products, like biscuits [12], pasta [13], mayonnaises/salad dressings [14] and puddings/gelled desserts [15] have been previously studied as vehicles to microalgae addition.

Expressively, vegetarians tend to have lower overall cancer rates, lower body mass index (BMI), adjustable blood pressure by eating diets lower in saturated fats, have higher levels of dietary fiber, magnesium, iron and potassium, vitamins E and folate, carotenoids, flavonoids and other phytochemicals [16, 17, 4, 9, 8]. Practically, vegetables are commonly eaten as fresh or cooked for improving its sensory properties. The phytochemicals have been described to possess antimutagenic or even anticarcinogenic activity [16, 18]. The Egyptian cuisine is notably conducive to ready-to-use and ready-to-eat vegetarian diets, as it rely heavily on vegetable dishes. However, several commonly consumed vegetables such as cauliflower, green pea, green

bean, spinach and green zucchini were favorable for Egyptian consumers over the years ago. There are many studies reviews the health benefits of mentioned vegetables considering their phytochemicals content and potential antioxidant, anticarcinogenic, antimicrobial activities [19-26]. Indeed, carefully planned vegetarian and vegan diets can provide adequate nutrients for optimum health [1]. Clearly, evidence suggests that infants and children can be successfully reared on vegan and vegetarian diets [27, 28].

Vegetarian and vegan diets need to ensure a balance of nutrients from a wide variety of foods, especially for vulnerable groups. Improving dietary habits is a societal, not just an individual problem. Thus it demands a population-based, multisectoral, multidisciplinary, and culturally relevant approach. Accordingly, the main objectives of this study were to investigate the possibility to prepare innovative VDs from different vegetables incorporated with chickpea as protein source supplemented with spirulina. Studying the effect of frying cooking method as well could be investigated. To achieve this purpose, six ovo-vegetarian diets have been developed by incorporating 3 different vegetables with chickpea and spirulina as well as some other edible ingredients. Proximate chemical composition, minerals, bioactive compounds and their antioxidant activity, amino acids as well as the organoleptic properties for prepared diets were carried out.

## 2. Materials and methods

### 2.1. Ingredients

Three different vegetables namely, pea (*Pisum sativum* L.), taro corms (*Colocasia esculenta* L.), and broccoli (*Brassica oleracea* L.) were obtained in fresh status from the central local vegetable market at El-Obour City, Egypt. Dry edible chickpea (*Cicer arietinum* L.), sweet potato (*Ipomoea batatas* L.), wheat flour, carrot (*Daucus carota* L.), green leafy vegetables mix [fresh coriander (*Coriandrum sativum* L.), dill (*Anethum graveolens* L.), parsley (*Petroselinum crispum* L.)], red pepper (*Capsicum annum* L.), onion (*Allium cepa* L.), garlic (*Allium sativum* L.) eggs and edible salt were obtained from the local supermarket, Egypt. In addition, traditional spices were brought from Ragab El-Attar's local spices supermarket, Egypt then was formulated according to [29]. Spirulina was obtained from

Aquaculture Research Center at Arab Academy for Science, Technology & Maritime Transport, Arab League, Egypt.

## 2.2. Ingredients preparation

All mentioned vegetables were washed, sorted and prepared as follow: green pea was peeled, taro corms were peeled manually by sharp knife then chopped into 1x1x1cm cubes. The green leaves of fresh broccoli plants were removed; the florets were cut into 1.5–2 cm parts. All prepared vegetables were washed and blanched for appropriate time (3, 7 and 3 min, respectively) using live steam blancher then cooled down and kept until use under freezing conditions. Unpeeled chickpeas were washed and soaked in water for 12 h. Then, excessive water was drained and chickpeas were peeled manually and ground for 3 min using a conventional kitchen machine. Sweet potato and carrots were peeled, washed, chopped in 1 cm slices, and blanched using live steam blancher for 6 and 4 min, respectively. Subsequently, the blanched materials were immediately cooled down and homogenized to a homogeneous puree. Sweet red pepper was washed

and chopped in small cubes after removing the initial seeds. Further ingredients such as fresh onion and garlic were peeled, washed and then chopped immediately before preparing the vegetarian diets. To prepare the green leafy vegetables mix, fresh coriander, dill and parsley were washed, ripped then mixed as (2:1:1) respectively. The dried spices mixed then used immediately.

## 2.3. Different innovative ready-to-use OVDs preparation

Six ovo-vegetarian diet formulas were prepared from the previously prepared ingredients according to recipes in **Table 1**. Two kilograms from each formula were prepared using a kitchen machine. Each ready-to-use ovo-vegetarian formula was filled in 2 polyethylene bags as 0.3 kg for chemical analysis of fresh diet and 1.7 kg for stir-frying process and chemical analysis of fried diets. The big diets bags were kept for homogeneity of all ingredients for 12–18 hr under cooling conditions before frying, while small diet bags were subjected immediately for analysis. The whole experiment and analysis were done in triplicates.

**Table 1.** Innovative chickpea-based ready-to-use vegan diet recipes

Ingredients <sup>a</sup>	Ready-to-use OVD recipes (%)					
	CP-S	CP+S	CT-S	CT+S	CB-S	CB+S
Peeled soaked chickpea	34	34	34	34	34	34
Green pea	16	15	–	–	–	–
Taro	–	–	16	15	–	–
Broccoli	–	–	–	–	16	15
Spirulina	–	1	–	1	–	1
Fixed ingredients <sup>b</sup>	50	50	50	50	50	50

<sup>a</sup>: All mentioned ingredients were obtained on fresh status (see materials),

<sup>b</sup>: Fixed ingredients were mixed as [18% blanched sweet potato, 20% wheat flour, 12% blanched carrot puree, 12% green leafy vegetables mix (coriander: dill: parsley; 2:1:1), 10% red pepper paste, 10% fresh onion, 2.5% salt, 1.5% fresh garlic and 2% dried spices.

## 2.4. Different innovative ready-to-eat OVDs preparation

Ready-to-eat OVDs were left at room temperature for 5 min then mixed with 0.1% sodium bicarbonate amount immediately before frying. The vegetarian diet mixture was shaped using a frame and wide knife which were designed especially for this purpose.

Appropriate amounts of each prepared diet mixture was put into the frame, and then cut with the knife as symmetrical bars as (10 x 0.8 x 0.6 cm: L x W x T) prior to frying. The vegetarian bars were fried at 180–190°C for 3–5 min in preheated sun flower oil. The OVDs were served immediately after frying for the panelists to evaluate its organoleptic characteristics. However, appropriate samples have been taken off for chemical and phytochemicals analysis.

## 2.5. Proximate chemical composition and minerals content

Ready-to-use and ready-to-eat OVDs were subjected to chemical analysis (moisture, crude protein, crude lipids, ash and crude fibre contents according to methods of A.O.A.C. [30]. However, the available carbohydrates content was determined by difference according to Merrill and Watt [31]. The minerals content including sodium, potassium, calcium was determined in both prepared fresh and fried vegetarian diets using flame photometry while magnesium, iron, copper, manganese and zinc contents were determined by atomic absorption spectroscopy according to A.O.A.C. [30]. A standard colorimetric method was employed for phosphorus as mentioned by Borah *et al.* [32].

## 2.6. Ascorbic acid determination

The ascorbic acid content in various OVDs either ready-to-use and ready-to-eat applying the 2,6-dichloroin-dophenol titrimetric method was determined according to A.O.A.C. [30]. Vitamin C content is expressed as mg 100 g<sup>-1</sup> fw. A pure ascorbic acid (Sigma) was used to prepare a standard solution as (1 mg ml<sup>-1</sup>).

## 2.7. Total phenolic content (TPC) determination

One g of dried ready-to-use and ready-to-eat OVDs was mixed with 25 ml of 70% methanol (v/v). The mixes were shaken vigorously in a dark bottle for 100 min at 100 rpm. After centrifugation at 3,225  $\times$  g for 10 min, the supernatant was collected and the residue was re-extracted twice with 15 ml 70% methanol for total phenolic content and antioxidant activity determination. To avoid oxidation, all extracts were stored in the dark at -20°C and analyses were performed within 48 h. The TPC of ready-to-use CVDs as well as ready-to-eat CVDs was determined according to Folin-Ciocalteu spectrophotometric method [33]. The measurements were compared to a standard curve of prepared gallic acid (GA) solution, and the total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample (mg of GAE g<sup>-1</sup> dw).

## 2.8. Determination of antioxidant activity

The radical scavenging activity using DPPH reagent (1,1-Diphenyl-2-picrylhydrazyl) for ready-to-use and ready-to-eat OVD extracts have been carried out using modified method by Lu *et al.* [33]. Each extract from fresh and fried diets (0.1 ml) was added to 2.9 ml of 6 $\times$ 10<sup>-5</sup> mol methanolic solution of DPPH. The absorbance at 517 nm was measured after the solution had been allowed to stand in the dark for 60 min. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE) per gram ( $\mu$ mol TE g<sup>-1</sup> dw).

## 2.9. Analysis of phytochemicals

### 2.9.1. Total carotenoids determination

According to Yuan *et al.* [34]; 5 g of each freeze-dried OVDs were extracted with a mixture of acetone and petroleum ether (1:1, v/v) repeatedly using the mortar and pestle until a colorless residue was obtained. The upper phase was collected and combined with crude extracts after washed for several times with water. The extracts were made up to a known volume with petroleum ether. Total carotenoids content was determined by recording the absorbance at 451 nm with a spectrophotometer. Total carotenoids were estimated as mg g<sup>-1</sup> dw.

### 2.9.2. Flavonoids and flavonols determination

The total flavonoids content of ready-to-use and ready-to-eat OVDs were determined according to the method of Mohdaly *et al.* [35]. A 0.5 ml aliquot of 2% AlCl<sub>3</sub> ethanolic solution was added to 0.5 ml of the extracts and mixed well. After keeping for 1 h at room temperature, the absorbance at 420 nm was measured. A yellow color indicates the presence of flavonoids. The total flavonoids content were expressed as mg quercetin equivalent (QE) per 100 g dw. The total flavonols content were determined according to Kumaran and Karunakaran [17]. A 0.6 ml aliquot of 2% AlCl<sub>3</sub> ethanolic solution was added to 0.6 ml of each extract and 0.8 ml of a 5% aqueous sodium acetate solution were added. After mixing and keeping for 2.5 h at room temperature, the absorbance at 440 nm was measured. Total flavonols content were expressed as mg quercetin equivalent (QE) per 100 g dw.

### 2.10. Amino acids determination

The amino acids profile was carried out after hydrolysis by 6.0 N HCl for 24 h at 110 °C in evacuated ampoules. Quantitative determination of amino acids were carried out by Biochrome 30 instruction manual (Analyzer used), 2005. EZ chrome manual (software for data collection and processing), 2004 according to A.O.A.C. [36].

### 2.11. Organoleptic evaluation

Organoleptic evaluation of the ready-to-eat OVDs immediately after preparation of the six OVDs incorporating different vegetables and spirulina was carried out. Fifteen panelists of the staff members and students from the Food Technology Department, Faculty of Agriculture, Benha University, in the age range of 20 to 57 years were asked to evaluate the fried OVD bars towards appearance, color, taste, odor, texture, oiliness, and overall acceptability. A 7-point hedonic scale (7 being like extremely, 4 like accepted and 1 dislike extremely) was used to select the best recipe for a wide scale production. Results were subjected to analysis of variance and average of the mean values of the aforementioned attributes and their standard error were calculated according to Wilson *et al.* [37].

### 2.12. Statistical analysis

The statistical analysis was carried out using SPSS program with multi-function utility regarding to the experimental design under significance level of 0.05 for the whole results. Multiple comparisons applying LSD were carried out according to Steel *et al.* [38].

## 3. Results and discussion

### 3.1. Proximate chemical composition of innovative ready-to-use and ready-to-eat OVDs

The chemical composition of prepared OVDs and their caloric value were significantly differed between RTU and RTE regardless spirulina supplement as illustrated in **Table 2**. Practically, obtained results of proximate chemical composition concluded that prepared diets are considered as valuable source of crude protein, lipid, fiber and carbohydrates both RTU and RTE OVDs which may have appropriate health benefits

[39, 2, 21, 4, 40, 41]. The moisture content had peaked in RTU CT+S and RTE CB+S to be 68.54 and 56.00%, respectively. Although, it had bottomed in RTU CP+S and RTE CT+S reaching to 62.46 and 52.77%, respectively. However, a slight difference between selected vegetables with or without spirulina and vice versa after cooking. That's meaning, around 10% reduction in the moisture content of fried OVD diets was recorded according to their means values. Spirulina incorporation into different vegetables directed to increasing the crude protein, lipids content and ash content in CP+S, CB+S and CT+S, respectively. Thus, these formulas were the highest mentioning parameters as presentable in **Table 2**. Moreover, the frying process was negatively affected on crude protein and fiber as well as carbohydrates contents for some formulas. Conversely, it was clearly increased the lipids, ash and caloric values for OVDs as shown by Barakat [42].

Frying cooking increased the lipid content in all diets by double times when compared to fresh diets. All formulated diets seems to have adequate fiber content which was not significantly different in the most of prepared diets. According to Dietary Reference Intakes [43], the formulated OVDs, 100 g dw could provide at least 40-50% of the RDA for adults and at least 25-35% of the RDA for pregnant and lactating women daily. In context, Adequate Intake (AI) of dietary fiber could be compensated. Moreover, RDA of carbohydrates is 130 g d<sup>-1</sup> for age ranging from 9-70 years of both genders, which increased to 210 g d<sup>-1</sup> for females in pregnancy and lactation. Consuming about 100 g dw CVD could provide at least 25-50% of the RDA for adults (about 90% absorbance efficiency). Accordingly as shown, 100 g of ready-to-eat OVDs fw could provide about 187-198 kcal which is cover the requirements of adult person (70 kg) for about 2.5 - 4 h [43, 44]. These results are in agreement with [42, 45, 29].

### 3.2. Minerals content of innovative ready-to-use and ready-to-eat OVDs

The minerals content (sodium, potassium, calcium, phosphorus, magnesium, iron, copper, manganese and zinc) in (mg 100g<sup>-1</sup> dw) of ready-to-use and ready-to-eat OVDs are given in **Table 3**. Generally, minerals content was changed after frying with different reduction rates. Significant difference ( $p < 0.05$ ) was establish between both RTU and RTE



OVDs among all minerals. CP+S showed higher sodium content while the lowest content was recorded in CB+S. After frying, the sodium content was ranged from 1698.5 mg 100g<sup>-1</sup> dw in CP-S to 1990.67 mg 100g<sup>-1</sup> dw in CB-S. Potassium content in formulated OVDs with different vegetables was ranged from a low of 1410.2 mg 100g<sup>-1</sup> dw in CP-S to a high of 1970.37 mg 100g<sup>-1</sup> dw in CB-S. The CB-S showed the highest calcium content in both RTU and CB+S for RTE, while the lowest calcium content was recorded in both fresh and fried CP-S. The formulated CB+S and CP-S having higher phosphorus content than other formulated vegetables, while the lowest phosphorus content had been recorded in both fresh and fried CP-S and CT-S. High magnesium content was found in fresh and fried CT-S followed by CP-S diets while iron content in different formulated diets is given in the same table, which was ranged from 287.9 mg 100g<sup>-1</sup> dw in CT-S to 663 mg 100g<sup>-1</sup> dw in CP-S while, it was ranged from 385.63 mg 100g<sup>-1</sup> dw in CB-S to 501.47 mg 100g<sup>-1</sup> dw in CP-S fried OVDs. These results were in agreement with [42, 45, 32, 46]. Our presented results of minerals content may compatible with vegetables and legumes minerals content which were reviewed by Gebhardt and Thomas [44]. For human requirements, the presented minerals content in 100 g dw of prepared OVDs could provide available content of ADI and RDA. As mentioned in Dietary Reference Intakes [43], the presented OVDs seem to rich in some minerals content and supplementing with spirulina slightly increased the minerals but sufficient supplement level may further needed.

### 3.3. Ascorbic acid, phytochemicals components and their antioxidant activity of innovative ready-to-use and ready-to-eat OVDs

Recently, research has confirmed a strong relationship between the amount of available biologically active compounds in vegetables and their antioxidant properties [47-55]. Data in Table 4 displays some phytochemicals content of various innovative RTU and RTE OVDs. All fresh diets demonstrated appropriate content of vitamin C which basically depends on the initial ingredients especially with Spirulina addition. However, the average levels of vitamin C were affectedly and significantly decreased in RTE OVDs which were

influenced by frying cooking [56]. Moreover, TPC, flavonoid, flavonols and antioxidant activity of both RTU and RTE-CVDs are also obtainable in the same table. Similarity, the TPC, antioxidants, flavonoids and flavonols of fresh prepared OVDs were higher than RTU regardless the vegetable kind. The TPC was a ranged from 52.11 mg GAE g<sup>-1</sup> for RTE CB-S to 81.70 mg GAE g<sup>-1</sup> for RTE CT+S [42, 57]. The antioxidant activity was recorded the highest value in RTU CP+S to be 70.42 μmol TE g<sup>-1</sup>. However, the lowest rate was attained with spirulina and before frying cooking as portrayed in CB+S formulas to be 28.36 μmol TE g<sup>-1</sup>. The content of TPC and relative antioxidant activity in different OVDs shows different significances pattern in both RTU and RTE OVDs as a result of cooking method impact [42, 45, 29, 48, 57]. A significant loss of flavonoids content was observed in RTE CVDs when compared to RTU ones. The total flavonoids loss was varied according to the kind of vegetables. However, they were losing with half degree of deterioration after frying. Carotenoids have been extensively studied for their potential protection against numerous cancer diseases. In recent years, several reports on the retention of total carotenoids in cooked vegetables are available [58, 34, 55]. In all diets, the formulation of OVDs incorporating different vegetables exhibit rich carotenoids content, a result of increasing the carotenoids content in chickpeas grains [59]. It is presented herein that, flavonoids and flavonols were retained by 66 and 64%, respectively. The retained content may depends on initial flavonoids and flavonols content, vegetable structure or diet matrix, and leaching of flavonoids and flavonols and its derivatives into the oil followed by thermal degradation during frying cooking, being similar to reports by [42, 45, 29, 60, 34, 55].

### 3.4. Amino acid content of spirulina and innovative ready-to-use and ready-to-eat OVDs

The amino acid composition of spirulina and some selected RTU and RTE OVDs is given in Table 5. It is clear that the amount of many EAA in spirulina matched (Isoleucine) or exceeded (Therionine, Valine, Leucine and Tyrosine) their corresponding quantities in egg's protein as a standard. This property make these spirulina an excellent source of protein supplementing for other foods which are low in Isoleucine, Therionine, Valine, Leucine and Tyrosine. The addition of spirulina with 1% to RTU

OVDs led to an increase in all EAA. From the obtained results, it could be observed that CP+SR was the highest in all EAA than the other treatments. Then leusine, phenylalanine, and valine recorded the highest EAA amount of all treatments. The aspartic and glutamic amino acids constituted

the major portion in spirulina and all treatments, it shows a value of 0.638 and 0.825, 0.539 and 0.893, 0.641 and 1.037, 0.574 and 0.920 and 0.602 and 0.991 g g<sup>-1</sup> nitrogen in spirulina, CP-SR, CP+SR, CP-SF and CP+SF, respectively.

**Table 2.** Chemical composition of innovative read-to-use and read-to-eat chickpea-based ovo-vegetarian diets incorporating different vegetables and supplemented with spirulina (mean  $\pm$  SD)

Recipes <sup>‡</sup>	Chemical composition (%)													
	Moisture content		Crude protein content		Lipids content <sup>dw</sup>		Ash content <sup>dw</sup>		Crude fiber content <sup>dw</sup>		Carbohydrates content <sup>dw</sup>		Caloric value kcal/100 g <sup>fw</sup>	
	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE
CP-S	64.03 $\pm 0.42aB$	54.53 $\pm 0.38aA$	19.93 $\pm 2.8bB$	17.09 $\pm 0.67bA$	5.56 $\pm 1.15aA$	6.46 $\pm 0.28aB$	2.51 $\pm 0.01bA$	2.89 $\pm 0.02aB$	5.85 $\pm 0.28bB$	4.33 $\pm 0.04cA$	61.68 $\pm 1.8aB$	58.01 $\pm 0.59bA$	132.67 $\pm 2.14bA$	191.47 $\pm 2.73aB$
CP+S	62.46 $\pm 1.66aB$	55.76 $\pm 0.22aA$	20.57 $\pm 1.37bB$	19.17 $\pm 2.01bA$	5.65 $\pm 0.06aA$	6.37 $\pm 0.36aB$	2.69 $\pm 0.03aA$	2.92 $\pm 0.07aB$	5.12 $\pm 0.44aB$	3.07 $\pm 0.02aA$	61.50 $\pm 0.76aB$	56.75 $\pm 2.38abA$	139.51 $\pm 7.21bA$	188.57 $\pm 2.63aB$
CT-S	63.81 $\pm 0.26aB$	53.6 $\pm 0.74aA$	17.52 $\pm 2.1aB$	16.96 $\pm 3.28abA$	5.47 $\pm 0.02aA$	6.92 $\pm 0.35bB$	2.55 $\pm 0.02cA$	2.92 $\pm 0.04aB$	6.12 $\pm 0.22cB$	3.75 $\pm 0.06bA$	63.84 $\pm 2.28bB$	58.09 $\pm 2.69bA$	132.70 $\pm 0.81bA$	198.24 $\pm 3.38aB$
CT+S	68.54 $\pm 9.03bB$	52.77 $\pm 0.45aA$	18.60 $\pm 2.1abB$	14.15 $\pm 0.34aA$	5.54 $\pm 0.04aA$	6.07 $\pm 0.49aB$	2.62 $\pm 0.02dA$	3.09 $\pm 0.01cB$	6.65 $\pm 0.29dB$	3.34 $\pm 0.03aA$	60.28 $\pm 3.33aA$	63.13 $\pm 1.07dB$	113.26 $\pm 35.26aA$	196.88 $\pm 4.28aB$
CB-S	65.63 $\pm 0.37abB$	53.04 $\pm 0.26aA$	17.79 $\pm 0.98aB$	16.06 $\pm 1.04aA$	6.11 $\pm 0.11bA$	6.66 $\pm 1.19abB$	2.47 $\pm 0.01aA$	2.91 $\pm 0.02aB$	6.34 $\pm 0.59cB$	3.39 $\pm 0.02aA$	62.57 $\pm 1.35abB$	60.16 $\pm 3.4bcA$	126.71 $\pm 2.13bA$	199.60 $\pm 6.11aB$
CB+S	64.8 $\pm 0.27aB$	56.00 $\pm 0.22aA$	18.00 $\pm 0.76aB$	16.25 $\pm 1.25aA$	6.19 $\pm 0.28bA$	6.99 $\pm 0.29bB$	2.69 $\pm 0.01aA$	2.98 $\pm 0.01bB$	5.09 $\pm 0.28aB$	4.66 $\pm 0.03dA$	63.06 $\pm 0.66bB$	56.43 $\pm 1.74abA$	130.97 $\pm 1.08bA$	187.76 $\pm 0.9aB$

<sup>‡</sup>: see materials and methods, table 1; <sup>dw</sup>: values were calculated on dry weight basis,

<sup>fw</sup>: values were calculated on fresh weight basis,

RTU: ready-to-use, RTE: ready-to-eat,

a, b, c, ...: means with the same letter in the same column are not significantly different ( $p > 0.05$ ),

A, B, C, ...: means with the same letter in the same row into each parameter are not significantly different ( $p > 0.05$ ).

**Table 3.** Minerals content of innovative read-to-use and read-to-eat chickpea-based ovo-vegetarian diets incorporating different vegetables and supplemented with spirulina (mean  $\pm$  SD)

Recip es <sup>‡</sup>	Minerals content (mg 100g <sup>-1 dw</sup> )																	
	Sodium		Potassium		Calcium		Phosphorus		Magnesium		Iron		Copper		Manganese		Zinc	
	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE
CP-S	1767.2 $\pm 0.5aB$	1698.5 $\pm 0.5aA$	1410.2 $\pm 2.3aA$	1496.47 $\pm 1.78aA$	1413.77 $\pm 0.45aB$	1237.6 $\pm 0.21aA$	141.83 $\pm 0.21dB$	173.13 $\pm 1.58dB$	116.57 $\pm 1.05eA$	91.93 $\pm 1.3eB$	663 $\pm 6.64dA$	501.47 $\pm 2.25bA$	31.7 $\pm 2.16bA$	31.38 $\pm 1.35aA$	27.73 $\pm 1.35bA$	72.63 $\pm 1.35dB$	79.23 $\pm 1.35dB$	70 $\pm 1.3bA$
CP+S	2117.47 $\pm 0.55cB$	1811.57 $\pm 0.55dA$	1868.1 $\pm 1.1dB$	1653.67 $\pm 1.72bA$	1851.97 $\pm 0.45dB$	1466.93 $\pm 0.45bA$	168.5 $\pm 0.26cB$	159.47 $\pm 2.12bA$	101.43 $\pm 1.05bB$	39.47 $\pm 1.27aA$	676.23 $\pm 1.35fB$	420 $\pm 1.3bA$	39.2 $\pm 2.76cA$	37.42 $\pm 2.12cA$	52.83 $\pm 1.35bA$	178.3 $\pm 1.3dB$	69.57 $\pm 1.99aA$	73.97 $\pm 1.35cB$
CT-S	1909.47 $\pm 0.29bB$	1831.03 $\pm 0.45dA$	1601.37 $\pm 1.15cA$	2081.87 $\pm 1.58dB$	1634.53 $\pm 0.23cB$	1446.07 $\pm 0.29bA$	148.63 $\pm 0.5aA$	145.43 $\pm 0.97aA$	97.9 $\pm 4.97bA$	132.07 $\pm 1.48fB$	287.9 $\pm 1.3aA$	468.13 $\pm 19.81cB$	27.73 $\pm 1.35aB$	24.45 $\pm 1.48aA$	83.2 $\pm 1.3cA$	99.87 $\pm 4.38cB$	73.97 $\pm 1.35bB$	62.57 $\pm 2.91aA$
CT+S	1960.93 $\pm 0.29bB$	1701.17 $\pm 0.55cA$	1961.97 $\pm 1.15eB$	1410.63 $\pm 1.33aA$	1678.53 $\pm 0.29cB$	1490.13 $\pm 0.29bA$	157.13 $\pm 0.7bB$	147.67 $\pm 0.55aA$	97.93 $\pm 1.58bB$	43.33 $\pm 1.05bA$	589.93 $\pm 13.38dB$	467.57 $\pm 1.35cA$	30.4 $\pm 1.3bA$	29.94 $\pm 1.4bA$	81.9 $\pm 1.3cB$	33 $\pm 1.3aA$	76.6 $\pm 1.3cB$	70.87 $\pm 0.75bA$
CB-S	2208.87 $\pm 0.55dB$	1990.67 $\pm 0.55eA$	1970.37 $\pm 1.78eA$	1928.77 $\pm 1.15cA$	1890.77 $\pm 0.45dB$	1419.73 $\pm 0.45bA$	165.73 $\pm 0.6cB$	145.6 $\pm 0.61aA$	82.43 $\pm 1.85aB$	63.07 $\pm 1.63cA$	311.7 $\pm 1.3bA$	385.63 $\pm 3.5aB$	27.73 $\pm 1.35aA$	26.86 $\pm 1.46aA$	232.47 $\pm 1.35dB$	90.67 $\pm 0.75cA$	73.53 $\pm 0.81bB$	71.3 $\pm 1.3bA$
CB+S	1792.03 $\pm 0.55bB$	1773 $\pm 0.5bA$	1529.3 $\pm 2.3bA$	1697.53 $\pm 1.15bB$	1533.97 $\pm 0.45bA$	1533.97 $\pm 0.45cA$	177.8 $\pm 0.53dB$	160.33 $\pm 0.84cA$	103.2 $\pm 2.66cB$	70.77 $\pm 1.05dA$	494.83 $\pm 13.03cA$	540.2 $\pm 1.3eB$	42.7 $\pm 4.03dA$	40.06 $\pm 3.12dA$	44.9 $\pm 1.3bA$	88.5 $\pm 2.25cB$	83.2 $\pm 1.3eB$	70 $\pm 1.3bA$

<sup>‡</sup>: see materials and methods, table 1; <sup>dw</sup>: values were calculated on dry weight basis,

RTU: ready-to-use, RTE: ready-to-eat,

a, b, c, ...: means with the same letter in the same column are not significantly different ( $p > 0.05$ ),

A, B, C, ...: means with the same letter in the same row into each parameter are not significantly different ( $p > 0.05$ ).

**Table 4.** Ascorbic acid, total phenolic compounds and antioxidant activity, total flavonoid and total flavonols of innovative read-to-use and read-to-eat chickpea-based ovo-vegetarian diets incorporating different vegetables and supplemented with spirulina (mean  $\pm$  SD)

Recipes <sup>‡</sup>	Ascorbic acid [mg 100 g <sup>-1</sup> fw]		TPC [mg g <sup>-1</sup> dw]		Antioxidant activity [ $\mu$ mol TE g <sup>-1</sup> dw]		Total flavonoid [mg QE g <sup>-1</sup> dw]		Total flavonols [mg QE g <sup>-1</sup> dw]	
	RTU	RTE	RTU	RTE	RTU	RTE	RTU	RTE	RTE	RTU
CP-S	54.00 $\pm$ 0.63aB	3.73 $\pm$ 1.27aA	64.68 $\pm$ 5.04aB	56.61 $\pm$ 4.43aA	51.04 $\pm$ 6.77bA	61.65 $\pm$ 12.01bB	25.73 $\pm$ 1.04aB	16.21 $\pm$ 0.14aA	11.03 $\pm$ 0.3aB	9.46 $\pm$ 0.09bcA
CP+S	69.40 $\pm$ 6.87aB	5.16 $\pm$ 0.62aA	65.87 $\pm$ 1.53abB	58.11 $\pm$ 3.68aA	70.42 $\pm$ 2.75dB	61.14 $\pm$ 9.53bA	23.41 $\pm$ 0.3aA	20.61 $\pm$ 3.57aA	10.83 $\pm$ 0.16aB	9.49 $\pm$ 0.35bcA
CT-S	44.85 $\pm$ 6.89aB	4.94 $\pm$ 1.28aA	59.84 $\pm$ 2.25aB	52.81 $\pm$ 2.04aA	47.77 $\pm$ 3.26bB	39.58 $\pm$ 9.78aA	18.47 $\pm$ 0.99aA	19.16 $\pm$ 3.88aA	11.39 $\pm$ 0.15bB	9.42 $\pm$ 0.02bcA
CT+S	108.10 $\pm$ 44cB	4.59 $\pm$ 1.47aA	81.70 $\pm$ 28.56bcB	56.6 $\pm$ 1.4aA	64.04 $\pm$ 31.36cA	66.5 $\pm$ 3.41bA	65.59 $\pm$ 22.12cB	37.14 $\pm$ 1.14bA	11.61 $\pm$ 0.18bcB	8.76 $\pm$ 0.96aA
CB-S	51.27 $\pm$ 8.65aB	3.84 $\pm$ 1.28aA	75.45 $\pm$ 3bB	52.11 $\pm$ 0.55aA	34.54 $\pm$ 2.93aA	46.33 $\pm$ 6.19aB	53.4 $\pm$ 0.91bB	30.38 $\pm$ 0.44bA	11.38 $\pm$ 0.28bB	9.06 $\pm$ 0.11bA
CB+S	62.96 $\pm$ 8.14bB	3.67 $\pm$ 0.64aA	65.48 $\pm$ 5.06abA	70.62 $\pm$ 4.51bA	28.36 $\pm$ 5.88aA	39.87 $\pm$ 6.54aB	56.81 $\pm$ 11.18bB	38.16 $\pm$ 2.86bA	10.9 $\pm$ 0.08aB	9.25 $\pm$ 0.09bA

<sup>‡</sup>: see materials and methods, table 1; <sup>dw</sup>: values were calculated on dry weight basis,

<sup>fw</sup>: values were calculated on fresh weight basis,

RTU: ready-to-use, RTE: ready-to-eat,

a, b, c, ...: means with the same letter in the same column are not significantly different ( $p > 0.05$ ),

A, B, C, ...: means with the same letter in the same raw into each parameter are not significantly different ( $p > 0.05$ ).

**Table 5.** Amino acid composition of spirulina and innovative RTU and RTE OVDs incorporating different vegetables and supplemented with spirulina (g g<sup>-1</sup> N) compared with standard protein and amino acids scores.

Amino acids	Spirulina	Recipes <sup>‡</sup>				Hens egg FAO (1970)
		CP-SR	CP-SF	CP+SR	CP+SF	
Therionine	0.326	0.191	0.202	0.220	0.231	0.320
Valine	0.452	0.292	0.304	0.355	0.334	0.428
Isoleucine	0.380	0.229	0.242	0.250	0.246	0.393
Leucine	0.577	0.379	0.414	0.443	0.423	0.551
Tyrosine	0.341	0.220	0.238	0.256	0.237	0.260
Phenylalanine	0.323	0.310	0.326	0.366	0.344	0.358
Lysine	0.320	0.235	0.255	0.275	0.237	0.436
Histidine	0.112	0.122	0.131	0.150	0.122	0.112
Aspartic	0.638	0.539	0.574	0.641	0.602	0.601
Serine	0.323	0.245	0.263	0.286	0.280	0.796
Glutamic	0.825	0.893	0.920	1.037	0.991	0.478
Proline	0.267	0.354	0.303	0.311	0.341	0.260
Glycine	0.336	0.210	0.235	0.242	0.243	0.207
Alanine	0.563	0.263	0.284	0.286	0.280	0.370
Argenine	0.460	0.379	0.440	0.516	0.377	0.381
Total of EAA	2.841	1.978	2.112	2.325	2.174	3.218
Total of N-EAA	3.422	2.883	3.020	3.319	3.114	3.093
Total of amino acids	6.263	4.861	5.132	5.644	5.288	6.311
% protein	11.20	10.72	9.16	12.35	11.89	12.78

EAA: Essential amino acids; N-EAA: Non-essential amino acid.

Data in **Table 6** shows the nutritional evaluation of spirulina and some selected RTU and RTE OVDs. Total essential amino acids (TEAA), total nonessential amino acids (TNEAA) (g 16 g<sup>-1</sup> N), ratio of EAA:protein and EAAI (%) in spirulina

were higher than those of egg, which contained 45.46, 54.75 [g 16<sup>-1</sup> g N], 0.46 and 103.33% in spirulina compared with 44.06, 47.04 [g 16 g<sup>-1</sup> N], 0.44 and 100% in egg. From the results, it could be observed that CP+SR and CP+SF had the highest



amount of EAA, ratios of E:N, E:P, E:T and EAAI (%), which contained 37.20 and 34.79 g 16<sup>-1</sup> g N, 0.701 and 0.698 for E:N ratio, 0.372 and 0.348 for E:P ratio, 0.412 and 0.411 for E:T and 85.00 and 79.34% for EAAI. Thus, it was recorded lower than egg protein according to FAO (1970).

Assessment of amino acids level individually to total essential amino acids had been illustrated in **Table 7**. As recommended by FAO and WHO, there are two categories being very important for determining the protein quality, one of them depends on calculating individual (A) to essential (E) amino acids ratios. Ratios for tested protein as well as these for different FAO patterns, hen's egg [FAO 1970] (control ratio) are presented in **Table 7**. From these results, it could be noticed that lysine was the most deficient amino acid in spirulina and both RTU and RTE OVDs. The lower ratios than control were observed for threonine and isoleucine in all treatments, while the phenylalanine and histidine in spirulina were lower than control. Although differed greatly, the ratios for other essential amino acids matched or exceeded the corresponding ratios of control.

Data in **Table 8** illustrate the certain amino acids of spirulina and some selected RTU and RTE OVDs and compared to FAO pattern mg g<sup>-1</sup> protein. With regard to limiting amino acids, it could be noticed that the histidine is the most deficient and the first limiting amino acid in spirulina, while the first limiting amino acid was lysine in all treatments. The second limiting amino acid in spirulina was lysine, while it was threonine in all treatments. The third limiting amino acid was leucine in CP-SR and CP-SF treatments, while it was histidine in CP+SF treatments.

### 3.5. Organoleptic properties of innovative ready-to-eat chickpea-based OVDs

Organoleptic properties of food products is an important criterion by which its consumer acceptability can be assessed. The organoleptic properties of RTE OVDs based on a seven-point hedonic scale showed that most of fried diets recorded mean scores higher than 4 (acceptable score) for all tested parameters, **Table 9**. However, the incorporation of spirulina led to decrease the acceptability of them to be low than 4 in CB+S and CP+S. The appearance of RTE OVDs showed higher mean scores for CB-S, CT-S and CP-S. That's present the effect of spirulina in appearance also. The most preferable color for the panelists was recorded for the same appearance preferable formulas. While, lowest score was recorded for CP+S, CT+S and CB+S. Results for taste, as the most important organoleptic property showed that CB-S and CP-S were the best favored significantly. Odor fascinates the consumer and is able to increase his appetite. The highest score was recorded for CB-S that flowed by CP-S formulas. The cooking method affected the texture of those innovative diets, where the negligible difference was found either spirulina or without spirulina integration. Oiliness reflects the oil retaining after cooking and panelists were asked to give higher score for lower oil content after pressing the bars between their fingers. The lowest retentive oil level had been noted for CB+S while the highest retaining oil level had been recorded for CP-S, significantly. Moreover, the overall acceptability scores indicated that the different diets could be arranged as CB-S > CT-S > CP-S > CT+S > CP+S > CB+S. These results are more or less agreement with [42, 45, 29, 61].

**Table 6.** The nutritional evaluation of spirulina and innovative RTU and RTE OVDs's proteins

Recipes <sup>‡</sup>	Item					
	EAA g 16 g N	N-EAA g 16 g N	E:N ratio	E:T ratio	E:P ratio	EAAI (%)
Spirulina	45.456	54.752	0.830	0.454	0.455	103.33
CP-SR	31.648	46.128	0.686	0.407	0.316	71.13
CP-SF	33.792	48.320	0.699	0.412	0.338	77.82
CP+SR	37.20	53.104	0.701	0.412	0.372	84.85
CP+SF	34.784	49.824	0.698	0.411	0.348	79.34
Egg FAO (1970)	44.060	47.040	0.940	0.484	0.440	100.00
Beef FAO (1970)	42.724	57.276	0.746	0.427	0.427	79.55

E:N-Ratio of essential amino acids to non-essential amino acids; E:T-Ratio of essential amino acids to total amino acids; E:P-Ratio of essential amino acids to protein; EAAI-Essential amino acid index according to Oser (1959)

**Table 7.** Assessment of individual amino acids to references essential amino acids in hen's egg protein [mg individual AA g<sup>-1</sup> TEAA]

Amino acids	Spirulina	Recipes <sup>‡</sup>				Hens egg score (FAO 1970)
		CP-SR	CP-SF	SP+SR	CP-SF	
Therionine	119.4	96.56	95.64	94.49	106.30	110.42
Valine	151.03	147.62	143.94	152.67	153.70	147.69
Isoleucine	135.04	115.77	114.58	111.99	113.21	135.61
Leucine	205.05	191.61	196.02	190.29	194.66	190.13
Tyrosine	121.18	111.22	112.69	110.24	109.07	89.72
Phenylalanine	114.78	156.72	154.36	157.48	157.85	123.53
Lysine	113.72	118.81	120.74	118.11	109.07	150.45
Histidine	39.80	61.68	62.03	64.74	56.14	52.45

A/E Ratio: mg amino acid per gram of total essential amino acids.

**Table 8.** Scores of essential amino acids to limiting the three essential amino acids responsible for limiting the quality of protein of formulated OVDs.

Amino acids	Spirulina	Recipes <sup>‡</sup>				Pattern mg g <sup>-1</sup> protein*
		Cp-SR	Cp-SF	Cp+SR	Cp+SF	
Therionine	<b>134.45</b>	<b>76.48</b>	<b>80.90</b>	<b>87.77</b>	<b>92.37</b>	40
Valine	<b>144.65</b>	93.28	97.08	<b>113.52</b>	106.95	50
Isoleucine	151.97	91.52	96.56	103.86	98.44	40
Leucine	<b>131.88</b>	<b>86.69</b>	<b>94.69</b>	<b>101.15</b>	<b>96.53</b>	70
Tyrosine	<b>156.02</b>	<b>100.30</b>	<b>108.86</b>	<b>117.03</b>	<b>108.34</b>	35
Phenylalanine	<b>107.75</b>	103.44	108.73	<b>121.90</b>	114.45	48
Lysine	92.91	<b>68.39</b>	<b>74.02</b>	<b>79.79</b>	<b>68.94</b>	35
Histidine	85.37	93.14	99.41	114.24	<b>92.60</b>	21
First AA	Histidine	Lysine	Lysine	Lysine	Lysine	
Second AA	Lysine	Therionine	Therionine	Therionine	Therionine	
Third AA	-	Leucine	Leucine	-	Histidine	

\* According to FAO/WHO AD HOC Committee (FAO, 1973); Under line preferred that the lowest percentage compared to FAO pattern.

Amino acid score according to FAO (1973) =  $\frac{\text{mg amino acid in 1 g protein}}{\text{mg amino acid suggested by FAO/WHO}} \times 100$

**Table 9.** Organoleptic properties of innovative read-to-eat chickpea-based ovo-vegetarian diets incorporating different vegetables and supplemented with spirulina (mean  $\pm$  SE)

Recipes <sup>‡</sup>	Organoleptic properties						Overall acceptability
	Appearance	Color	Taste	Odor	Texture	Oiliness	
CP-S	5.46 $\pm 1.05c$	5.46 $\pm 1.39b$	4.23 $\pm 1.42ab$	4.58 $\pm 1.35a$	5.54 $\pm 0.88b$	5.31 $\pm 0.95ab$	4.62 $\pm 1.19ab$
CP+S	2.92 $\pm 1.75a$	2.38 $\pm 1.80a$	3.19 $\pm 1.97a$	3.69 $\pm 1.97a$	4.92 $\pm 1.19a$	4.77 $\pm 1.09a$	3.69 $\pm 1.32a$
CT-S	5.35 $\pm 0.90c$	5.50 $\pm 0.91a$	3.62 $\pm 1.71a$	4.65 $\pm 1.38a$	5.31 $\pm 0.95b$	5.00 $\pm 1.00ab$	4.85 $\pm 1.07ab$
CT+S	3.08 $\pm 1.61ab$	2.46 $\pm 1.71a$	4.46 $\pm 1.51ab$	4.27 $\pm 1.79a$	5.08 $\pm 1.12ab$	4.54 $\pm 1.13a$	4.23 $\pm 1.42ab$
CB-S	5.58 $\pm 1.08c$	5.50 $\pm 1.08b$	5.62 $\pm 0.96c$	5.00 $\pm 1.29b$	5.38 $\pm 0.77b$	5.23 $\pm 1.01ab$	5.46 $\pm 0.97c$
CB+S	3.27 $\pm 1.27b$	2.46 $\pm 1.39a$	3.81 $\pm 1.84a$	3.92 $\pm 1.85a$	4.62 $\pm 1.61a$	4.23 $\pm 1.42a$	3.85 $\pm 1.72a$

<sup>‡</sup>: see materials and methods, table 1,

a,b,c,.....: Means with the same letter in the same column are not significantly different ( $p > 0.05$ ).

## Conclusion

Reduced risk of many diseases in health-conscious are strongly associated with consuming vegetarian and functional diets. Accordingly, moderation, variety and appealing in individual diets is recommended. The current study concluded the potential applicability of different innovative OVDs incorporating different vegetables and supplemented with spirulina. Obtained results could provide sufficient information about macro- and micronutrients, phytochemicals content and their antioxidant activity, amino acids as well as sensory attractiveness of prepared OVDs. Highly consumer acceptability could be an encourage motive for large scale applications. However, studies about formulating different functional diets with spirulina could be an innovative issue to establish novel functional vegetarian diets for vegetarians. The present investigation could also provide a valuable impact of cooking method to optimize the cooking procedure for designing novel functional foods for specific group.

**Compliance with Ethics Requirements.** Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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