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Randomized Control Trials

Protein bioavailability of *Wolffia globosa* duckweed, a novel aquatic plant – A randomized controlled trial☆

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SUMMARY

Background & aims: While the world is extensively looking for alternatives to animal protein sources, it is not clear which plant sources can provide the requisite full complement of essential amino acids (EAAs). *Wolffia globosa* is an aquatic, edible duckweed, the smallest plant on earth, and it offers all nine EAAs, dietary fibers, polyphenols, iron, zinc and B_{12} vitamin. This work was designed to evaluate Mankai (a newly developed high-protein strain of *W. globosa*) as an optional bioavailable source of EAAs for humans (primary outcome), and of further nutrients such as vitamin B_{12}, in comparison to well-established animal and plant protein sources; cheese and peas, respectively.

Methods: 36 men, subjected for 3 days to a stable diet and subsequent overnight (12 h) fast, were randomized to consume one of three iso-protein (30 g) based test-meals (soft cheese, green peas, Mankai). Blood samples were collected at 0, 30, 90 and 180 min.

Results: The 3 h blood concentrations of the EAAs: histidine, phenylalanine, threonine, lysine, and tryptophan, triggered by intake of Mankai, was essentially significant as compared to baseline ($p < 0.05$) and similar to that of soft cheese and pea changes ($p > 0.05$ between groups). Although branched-chain-amino-acids (leucine/isoleucine, valine) increased significantly by Mankai within 3 h ($p < 0.05$ vs. baseline), the change was relatively higher for cheese as compared to Mankai or peas ($p < 0.05$ between groups). The increase in vitamin B_{12} by Mankai was higher as compared to changes induced by either cheese ($p=0.007$) or peas ($p=0.047$, between groups).

Conclusions: Mankai may provide a high-quality substitute source for animal protein, and a potential bioavailable source of vitamin B_{12}.

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1. Introduction

Plant protein sources are considered qualitatively inferior to those of animals in terms of their essential amino acid (EAA) composition. Plant sources exhibit less protein digestibility and thus amino acid (AA) bioavailability, some due to their inclusion of trypsin inhibitors or hemagglutinins. In animal models, trypsin inhibitors caused pancreatic hypertrophy and increased synthesis and secretion of proteases (such as trypsin, chymotrypsin and elastase). As a result, the endogenous amino acids are diverted to synthesis of enzymes due to their increased production [1–3]. However, unlike plant food sources, red meat products, and particularly processed ones, have been implicated in increased cardio-metabolic risks and diabetes [4–10]. The above and other observations have led to an increased movement towards plant-based diets [11], either as a sole nutritional source (e.g. vegetarians) or with occasional inclusion of meat (e.g. "flexitarians"). The

Abbreviations: Amino acid, AA; branched chain amino acids, BCAA; essential amino acid, EAA; general estimating equation, GEE; protein digestibility corrected amino acid score, PDCAAS.

☆ This study registered in clinicaltrials.gov (NCT03020225).

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question that arises is whether plant sources on their own can provide the requisite EAAs for human nutrition.

An emerging aquatic plant protein introduced to the Western diet is from the duckweed family [12,13], *Wolffia*, a genus within the duckweed family, has been characterized as a rich source of certain EAA compared to seeds [14]. Moreover, *Wloffia* has a long history in Southeast Asia as a natural food source or "vegetable meat ball" [13]. The morphological structure of *Wolffia globosa* [15,16] is a simple, rootless thalus 0.4–0.9 mm long (the smallest plant on earth), without veins, having a budding pouch at its basal end. Clonal clusters are arranged either solitary or two connected, consisting of a mother plant and a daughter plant. The nutritional properties of Mankai, a cultivated strain of *Wolffia Globosa*, is reflected in high protein content (more than 45%) of the dry matter, the presence of 9 essential and 6 conditional AAs and demonstrable protein digestibility corrected amino acid score [PDCAAS] of 89%. In this study we aimed to explore the EAAs bioavailability of Mankai intake in healthy humans, compared to intake of iso-protein containing meals of well-established animal (soft cheese) and plant (peas) protein sources.

2. Materials and methods

2.1. Study population

This study, registered in clinicaltrials.gov (NCT03020225), enrolled 37 apparently healthy men who signed a written informed consent. The study was conducted in an isolated research center workplace in Israel (the Nuclear Research Center, Dimona), as a preparation to an ongoing long-term clinical trial (DIRECT PLUS: NCT03020186), which aims to address the effectiveness of plant enriched diets. Inclusion criteria were men, age >30 and moderate abdominal obesity. Exclusion criteria included ≥2 mg/dl serum creatinine, impaired liver function [i.e. ≥threefold the upper level of normal alanine transaminase (ALT) and aspartate transaminase (AST)], active cancer, participation in another trial, or major illness. The study protocol was approved by the Medical Ethics Board and the Helsinki Committee of the Soroka University Medical Center. The test meal was conducted on February 1, 2017 in three randomized groups. Of the 37 initial participants, 36 completed the study. One dropped out at the onset due to technical difficulties in withdrawing venous blood samples. The participants received a gift card for their participation.

2.2. Experimental design

Three days prior to the test meal, the participants were instructed to maintain a stable diet that consisted of detailed recipes tailored to provide 2000 kcal/day, comprised of 55% carbohydrates, 15% protein, and 30% fat from total energy. Baseline blood samples were taken after a 12 h overnight fast by four paramedics and, following meal administration, after 30, 90 and 180 min. To keep exact time intervals, each randomized intervention group was further divided into three time groups, with baseline sampling at 8 am, 8.15 am, 8.30 am, and the exact time sequence was maintained throughout the entire study. This study was a single-phase trial.

Each test meal provided 30 g of protein from the respective source: 1. Cheese protein (333 g of soft white cheese, 9% fat) 2. Green peas (cooked, cutlet) 3. *W. globosa* (Mankai, cooked, cutlet). A chef prepared the cutlets that included 30 g protein from either peas (600 g) or Mankai (410 g) with an additional minimal list of ingredients. The final recipe included the following additional ingredients for both cutlets: onions (280 g), mushrooms (200 g), canola oil (21 g for peas, 58.2 g for Mankai), protein-free stabilizer (16.2 g for peas, 13.4 g for Mankai), salt and pepper. The cheese meal also included one slice of bread. In addition to the 30 g equivalent protein content of each protein specific source, the estimated amount of protein provided in each dish was as follows: cheese (39.7 g), peas (39.3 g) and Mankai (39.3 g). While other nutritional values/components varied across groups, the 3 test meals groups had a complete profile of all the 9 EAAs.

The nutritional characteristics of the test meals are provided in Table 1. All the test meals were served with a 250 ml bottle of mineral water. To maintain an exact time frame, the participants were required to complete their meal within 15 min at their allocated food tables.

2.3. Measurements of blood parameters

Blood samples were taken at baseline, and at 30, 90, and 180 min post administration of the test meals for the EAAs measurements. Other parameters were measured only in 3 h interval. Samples were stored after centrifugation at −80 °C. The profiling of AA concentrations in serum was carried out by using electrospray tandem-mass spectrometry [17]. Serum vitamin B_{12} was analyzed with a competitive ElektroChemiLumineszenz-ImmunoAssay "ECLIA" (Cobas 8000, Roche Diagnostics, Mannheim, Germany) using Intrinsic Factor as antibody. All biochemical analyses were performed at the laboratories of the University of Leipzig, Germany.

2.4. Statistical analysis

The primary outcome was the change of the EAAs concentration in the blood. The secondary outcomes were selected blood biomarkers, as B12 vitamin, which is primarily derived by animal protein sources. Computer-generated random numbers were used to assign the subjects to the three different meals randomly. All the statistical analyses were based on non-parametric tests. Differences in blood measures at various time points, as compared to baseline, were analyzed by the Friedman or Wilcoxon test, depending on the number of time points. To examine the differences between the three meal groups, we used the Kruskal–Wallis test. In case of significant differences between groups, we used Mann–Whitney test as a post-hoc test. In a sensitivity analysis, the data were further tested by using general estimating equation (GEE) model with a random effect for subjects and fixed effect of meal groups. The results were similar to the non-parametric tests. The sample

Table 1
Nutritional values of the provided test meals.[a]

	Soft cheese	Green peas	Mankai
Protein (g)	39.7	39.3	39.3
Fat (g)	35.3	21.0	58.2
Carbohydrates (g)	11.0	118.2	92.0
Energy (kcal)	520.2	820.4	1055.3
Dietary fibers (g)	15.7	52.4	39.4
Vitamin B12 (μg)	1.16	0.00	2.81
Essential amino acids			
Methionine (g)	0.73	0.45	0.6
Histidine (g)	0.88	0.59	0.61
Phenylalanine (g)	1.56	1.11	1.59
Threonine (g)	1.35	1.13	1.24
Lysine (g)	2.52	1.76	2.12
Tryptophan (g)	0.45	0.21	0.63
Leucine (g)	3.01	1.79	2.51
Isoleucine (g)	1.59	1.08	1.33
Valine (g)	2.02	1.30	1.70

[a] The calculated nutritional values are based on USDA database (or Eurofins labs for Mankai) using the entire ingredients of each test meal. Essential amino acids calculations were based solely on 30 g protein from soft cheese, green peas, or Mankai.

size analysis for this study was based on findings from a previous short-term food intake trial that addressed the bioavailability of AAs [18]. In that study, the difference in peak leucine concentration between animal and plant protein was 48.7 μmol/L with a SD of 16.3 μmol/L for the animal protein and 27.3 μmol/L plant protein. Thus, the sample size calculation was 14 participants (7 participants in each group), suggesting that our sample size of 11–13 per group should be considered adequate. Power calculation was preformed. For leucine as an example, power was 95.33% [18]. We hypothesized that the change of the EAAs and other biomarkers will be similar across different test meals. Results are presented as means ± SD unless otherwise stated. Statistical analyses were performed with IBM SPSS (version 24). All *P*-values were two-sided, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics (population and meals nutritional profile)

We randomized 37 participants (36 completed) into 3 groups according to the food intake source: cheese (n = 12), peas (n = 11) and Mankai (n = 13). The participants were men of 48.8 ± 10.4 years of age with mean body-mass-index (BMI) of 31.1 ± 4.1 kg/m^2. Age, adiposity, blood pressure, and blood parameters for glycemic control, lipids, liver function, and vitamin B_{12}, were approximately equally distributed across the randomized groups (Table 2). Participants' fasting plasma concentrations of the 9 EAAs were comparable across the groups (Table 2).

3.2. Changes in blood concentrations of amino acids

Plasma AAs were measured at times 0, 30, 90, and 180 min post-test meal administration. The 3 h blood concentrations profile of EAA was similar across the 3 protein sources and specific to each AA (Fig. 1 and Table 3). In at least one-time point within 3 h, the food sources significantly increased EAA concentrations above baseline: Mankai in seven of the EAAs (lysine and methionine-exceptions), cheese in six of the nine EAAs (lysine, methionine, and tryptophan-exceptions), and peas in six of the EAAs (lysine, methionine and valine-as exceptions); $p < 0.05$ as compared to baseline.

The blood concentrations of methionine, histidine, phenylalanine, threonine, lysine, and tryptophan showed no significant differences ($p > 0.05$) between the 3 food groups, essentially at all time points (Fig. 1). In the cheese meal group, the branched chain amino acids (BCAA) (leucine/isoleucine and valine) plasma concentrations attained from the baseline were significantly higher compared to the equivalent in Mankai: $p = 0.016$ for leucine/isoleucine at 30 min and $p < 0.001$ at 90 min; for valine, $p = 0.016$ at 90 min. For peas compared to cheese: at 30 min, $p = 0.037$ for leucine/isoleucine and at 90 min, $p = 0.001$ and $p = 0.011$ for valine at 90 min.

3.3. Changes in blood levels of selected biomarkers

Vitamin B_{12}: The increase in serum vitamin B_{12} concentrations after 180 min relative to baseline was higher in the Mankai meal group as compared to those of cheese ($p = 0.006$ between groups) or peas ($p = 0.049$ between groups) which were essentially null (Table 3). However, the vitamin B_{12} was not significantly elevated compared to baseline.

Triglycerides concentrations were significantly higher after 180 min as compared to baseline in all three meal groups with no significant differences between groups. ALT liver enzyme levels, taken as potential hepatic stress indicators, remained unchanged after 180 min, i.e. as compared to baseline, with no significant differences between groups (Table 3).

4. Discussion

As there is a growing need to identify new nutritious plant protein sources for both health [19] and sustainability reasons [20], the present study aimed to evaluate the bioavailability of EAAs in Mankai, a cultivated strain of *W. globosa*, an aquatic plant of the duckweed family, compared to well-established animal and plant protein sources [21–23]. To our knowledge, there have been no reported studies that examined the nutritional bioavailability values of duckweed, as compared to other protein sources, in humans. The results indicate that Mankai has demonstrable nutritional value as a high-quality protein source, since Mankai

Table 2
Baseline characteristics of the study population across the intervention groups.[a,b]

	Soft cheese (n = 12)	Green peas (n = 11)	Mankai (n = 13)	p-value	Entire
Age (y)	48 ± 8.8	49.5 ± 12.1	48.9 ± 11	0.93	48.8 ± 10.4
Weight (kg)	98.7 ± 11.5	100.7 ± 15.8	98.1 ± 11	0.98	99.1 ± 12.5
Height (cm)	180.6 ± 7.1	175 ± 6.1	177.1 ± 5.1	0.16	177.6 ± 6.4
BMI (kg/m^2)	3.1 ± 30.3	33 ± 5.8	31.2 ± 2.9	0.62	31.5 ± 4.1
Systolic blood pressure (mmHg)	138 ± 17	130 ± 16	136 ± 13	0.28	134.7 ± 15.2
Diastolic blood pressure (mmHg)	88 ± 14	81 ± 13	82 ± 11	0.34	83.9 ± 12.3
Fasting glucose (mg/dL)	97.5 ± 13.4	96 ± 9.2	96.7 ± 9.4	0.98	97.7 ± 10.5
HDL-c (mg/dl)	45.3 ± 9.9	41.9 ± 9.3	44.4 ± 7.8	0.76	44 ± 8.9
LDL-c (mg/dl)	131.3 ± 30.2	124.5 ± 40.6	124.6 ± 26.4	0.83	126.7 ± 31.6
ALT (U/l)	43.2 ± 13.2	42.3 ± 21.1	46 ± 19.5	0.79	43.9 ± 17.5
Vitamin B12 (pg/mL)	371.1 ± 146.4	473 ± 144.1	423 ± 220.4	0.48	419.5 ± 176.7
Essential amino acids (μmol/L)					
Histidine	31.1 ± 4.1	31.3 ± 4.1	30 ± 2.9	0.78	30.7 ± 3.7
Tryptophan	14.2 ± 2.9	13.8 ± 3.5	12.4 ± 2.5	0.4	13.4 ± 3.0
Phenylalanine	67.4 ± 13.5	60.3 ± 10.2	63.4 ± 8.5	0.51	63.8 ± 10.9
Methionine	30.3 ± 7.4	25.6 ± 4.2	28.2 ± 6.1	0.25	28.1 ± 6.2
Threonine	31.2 ± 6.7	29.9 ± 6.8	30.2 ± 5.7	0.94	30.5 ± 6.2
Lysine	81.0 ± 24.9	73.2 ± 22.2	72.7 ± 19.5	0.44	75.6 ± 21.9
Valine	187.1 ± 31.2	179.3 ± 36.3	184.4 ± 25	0.68	183.7 ± 30.2
Isoleucine/leucine	141.8 ± 27.2	129 ± 26.6	139.7 ± 19.6	0.33	137.1 ± 24.5

[a] The values are means ± SDs.
[b] Group comparisons were performed with the use of Kruskal–Wallis test.

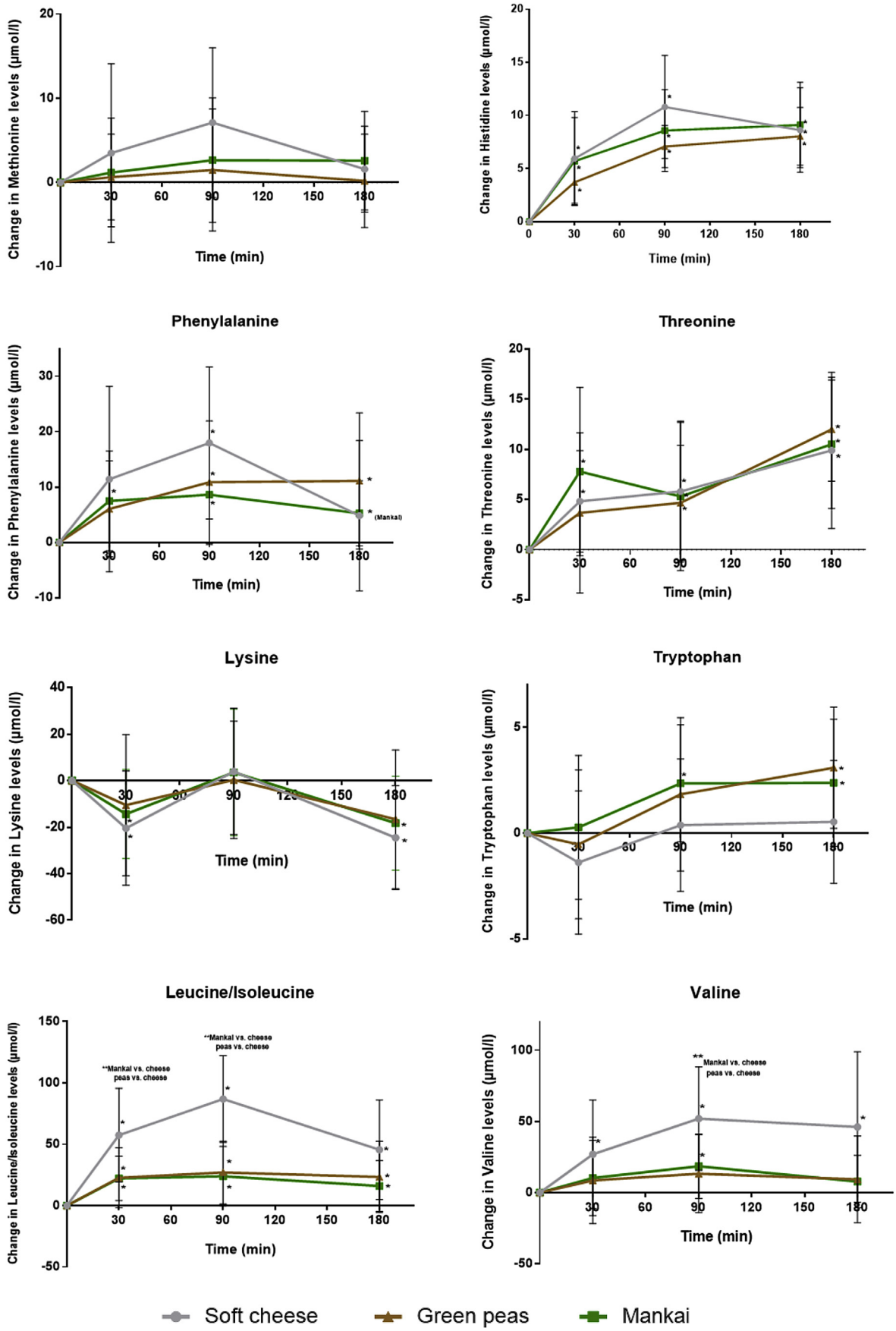

Fig. 1. Changes in essential amino acids concentrations at 30, 90 and 180 min after administration of the test meal. Vertical bars indicate ±1 SD; * indicates $p < 0.05$ vs. baseline; ** indicates $p < 0.05$ for difference between the intervention groups. Values were analyzed as repeated measurements by using the Friedman test. Differences between groups were analyzed by using the Kruskal–Wallis test, in case of significance, by using the Mann–Whitney test.

Table 3
Trajectory of essential and conditional amino acids over 180 min following the test meals administration.

	Test meal			p-value between groups
	Soft cheese	Green peas	Mankai	
Essential amino acids (μmol/L)				
Histidine				
ΔTime 30	5.94 ± 4.4*	3.71 ± 2.0*	5.67 ± 4.1*	0.204
ΔTime 90	10.80 ± 4.8*	7.70 ± 2.0*	8.58 ± 3.8*	0.066
ΔTime 180	8.62 ± 4.0*	8.03 ± 2.7*	9.11 ± 4.0*	0.906
Tryptophan				
ΔTime 30	−1.39 ± 3.4	−0.53 ± 3.5	0.27 ± 3.4	0.512
ΔTime 90	0.38 ± 3.1	1.83 ± 3.6	2.35 ± 2.7*	0.292
ΔTime 180	0.53 ± 2.9	3.09 ± 2.9*	2.38 ± 3.0*	0.143
Phenylalanine				
ΔTime 30	11.41 ± 16.7	6.04 ± 8.6	7.50 ± 9.0*	0.614
ΔTime 90	17.97 ± 13.7*	10.87 ± 11.1*	8.63 ± 9.0*	0.090
ΔTime 180	4.83 ± 13.6	11.13 ± 12.3*	5.26 ± 5.9*	0.372
Methionine				
ΔTime 30	3.50 ± 10.6	0.63 ± 5.1	1.17 ± 6.4	0.771
ΔTime 90	7.11 ± 8.9	1.48 ± 7.2	2.63 ± 7.4	0.446
ΔTime 180	1.58 ± 5.1	0.19 ± 5.5	2.60 ± 5.9	0.724
Threonine				
ΔTime 30	4.81 ± 5.0*	3.66 ± 8.0	7.77 ± 8.4*	0.406
ΔTime 90	5.79 ± 7.0*	4.66 ± 5.7*	5.29 ± 7.4*	0.846
ΔTime 180	9.89 ± 7.8*	11.98 ± 5.2*	10.50 ± 6.4*	0.807
Lysine				
ΔTime 30	−20.45 ± 24.6*	−10.56 ± 30.4	−14.36 ± 19.1*	0.545
ΔTime 90	3.91 ± 27.1	0.33 ± 25.2	3.57 ± 27.2	0.852
ΔTime 180	−24.55 ± 22.3*	−16.60 ± 29.8	−18.27 ± 20.1*	0.723
Valine				
ΔTime 30	26.91 ± 38.1*	8.52 ± 30.5	10.24 ± 26.4	0.241
ΔTime 90	51.99 ± 36.4[a]*	13.25 ± 24.7	18.57 ± 22.7[b]*	0.017
ΔTime 180	46.12 ± 52.9*	9.23 ± 30.5	7.80 ± 18.5	0.092
Isoleucine/leucine				
ΔTime 30	57.42 ± 38.2[a]*	22.63 ± 24.5*	22.26 ± 18.2[b]*	0.031
ΔTime 90	86.86 ± 35.5[a]*	27.08 ± 25.6*	23.89 ± 24.2[b]*	Pv < 0.0001
ΔTime 180	45.57 ± 40.5*	23.39 ± 29.1*	16.00 ± 20.6*	0.153
Vitamin B12				
ΔTime 180	−7.86 ± 20.0	3.57 ± 13.5[c]	11.78 ± 15.7[b]	0.015
Triglycerides				
ΔTime 180	55.75 ± 36.7*	77.47 ± 55.4*	55.23 ± 40.8*	0.718
ALT				
ΔTime 180	−0.50 ± 2.3	−0.93 ± 2.3	−0.12 ± 2.6	0.689

*p < 0.05 vs. baseline.
[a] p < 0.05 between groups difference of change of green peas vs. soft cheese.
[b] p < 0.05 between groups difference of change of Mankai vs. soft cheese.
[c] p < 0.05 between groups difference of change of Mankai vs. green peas.

increased the level of seven EAAs compared to peas and cheese that increased the level of six EAAs. As the differences in concentrations of the various AAs depends most likely mainly on the content in the proteins, we show in Table 2 that the AAs profile content was generally comparable across the protein sources.

As a first controlled test of Mankai in humans, the present study has several limitations. Primarily, fat and carb are known to affect protein digestibility and absorption kinetics and hence the non-matching makes it hard to interpret directly on the protein sources. As we aimed to compare original wholesome foods and not extracts, our rationale was to explore the effect of the essential amino acid bioavailability from iso food protein sources, given their natural complementary nutritional elements. Thus, we aimed to add minimal and similar ingredients, and those who will apparently have neutral/minimal effect on the primary outcome. Under these circumstances, it was, unfortunately, not feasible for our purposes, to match all the macronutrients content. The test meals based on iso-equivalent protein to each intervention group. The chosen protein content of >30 g per meal is based on previous availability trials [24–26] and based on the knowledge that the dose is physiologically relevant in terms of ability to stimulate protein synthesis [24–26]. Secondly, this is an acute trial assessing the protein source–related differences over the course of 3 h. Even though we have demonstrated significant peaks of AA blood concentrations within this time frame, acute trials cannot assess whether these 3-h changes would continue over the longer term. Moreover, the study design as a single-phase trial and not a cross-over trial can affect our results. We did not perform a cross-over trial due to the complex designed protocol and our strict time frame. However, the participants were randomly assigned with apparently comparable characteristic profile distribution, including amino acids blood levels. Thirdly, due to the workplace's constraints, the participants enrolled in the study were only men, which may not allow us to draw conclusions regarding women. Furthermore, the evaluation of vitamin B_{12} was in vitamin B_{12} sufficient patients and thus we cannot conclude our results on B_{12} deficient patients. Also, we cannot completely conclude on the absorption of AAs. The concentrations in circulation is a product of absorption and disappearance rate to tissues. Infusion of e.g. a stable isotope tracer could have provided much more in-depth information. The major strength of our study is the design as a single-phase study was carried out while maintaining a tightly controlled feeding schedule on parallel, and high adherence rate, which strengthened its accuracy.

By definition, high-quality protein sources are those that are readily digested and thereby provide sufficient amounts of EAAs for

protein synthesis [27]. Protein from animal sources, such as cheese, provide all nine EAAs and is therefore considered a “complete” protein source [28,29]. Previous analysis showed that duckweed is rich in most EAAs, particularly when compared to seed protein [14]. In the present study, the Mankai group showed significantly increase in all the EAAs except lysine and methionine. That results is in line with the relatively low abundance of some EAAs in plants, particularly lysine and methionine [28].

The increase in BCAA's (leucine/isoleucine and valine) concentrations was relatively higher in the cheese group as compared to those of Mankai and peas. This result was anticipated, as whey protein contains the highest content of BCAA (especially leucine) compared to any other dietary sources of protein [30]. Tryptophan, which was significantly elevated in both plant sources as compared to the cheese group, has an established role in the synthesis of serotonin [31], a neurotransmitter that modulates appetite [32] and ensuing satiety sensations [33].

Inadequate intake of B_{12} is common among vegans, as it is virtually absent in plant foods [34]. As recently reviewed [35] plants, indeed, do not contain vitamin B_{12} because they have no cobalamin-dependent enzymes. However, few aquatic plants as algae are rich in vitamin B_{12}, with some species, such as Porphyrayezoensis (Nori), containing as much cobalamin as liver. Yet, the role of the cofactor in the plant metabolism remains unknown, as does the source of the vitamin for these organisms. The source of cobalamin seems to be bacteria, indicating an important and unsuspected symbiosis. The B_{12} in the Mankai is not a bacterial contaminant (disinfecting and washing the surface of the plant do not reduce the B_{12} content). It is part of the plant biomass and cannot be removed by highly purified process. The mechanisms is still not fully understood but recent, not published yet, studies in the Mankai's plant suggest that endophytes bacteria [36], microbes residing symbiotically and grow inside the plant tissues, are probably those who dominate the B_{12} production. The *W. globosa*, though is a more complex plant, might be more similar to other aquatic photosynthetic eukaryotes i.e., algae and seaweeds that are known to contain vitamin B_{12}.

In summary, blood concentration of EAAs after Mankai consumption was similar to that of well-established animal and plant protein sources and it might serve as a plant-compatible protein source in order to abstain from meat due to ecological, ideological and health concerns issues.

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Primary responsibility for final content: Alon Kaplan, Hila Zelicha, Iris Shai.

All authors read and approved the final manuscript.

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The investigators were responsible for the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, and approval of the manuscript; and decision to submit the manuscript for publication.

Conflict of interest

Ioav Cabantchik and Iris Shai advise in the nutritional committee of Hinoman LTD.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2018.12.009.

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