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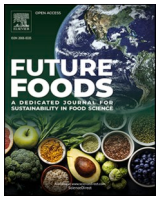

Effects of the consumption of algal biomass versus protein concentrate on postprandial satiety and metabolism

Jia Yee Wu [a,1], Rachel Tso [a,1], Yi Ning Yong [a,1,2], Susanna Poh Suan Lim [a], Thomas Wheeler [b], Arup Nag [c], Lirong Cheng [c], Md. Mahabubur Rahman Talukder [d], Lee Huffman [e], Siew Young Quek [f], Melvin Khee Shing Leow [a,g,h,i], Sumanto Haldar [a,j,*]

[a] *Clinical Nutrition Research Centre, Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology and Research (A*STAR), Singapore 117599, Singapore*
[b] *Food and Bioactives Group, Cawthron Institute, Nelson 7010, New Zealand*
[c] *Riddet Institute, Massey University, Palmerston North 4474, New Zealand*
[d] *Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology and Research (A*STAR), Singapore 138669, Singapore*
[e] *The New Zealand Institute for Plant and Food Research Ltd, Palmerston North 4442, New Zealand*
[f] *School of Chemical Sciences, The University of Auckland, Auckland 1010, New Zealand*
[g] *Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 636921, Singapore*
[h] *Department of Endocrinology, Division of Medicine, Tan Tock Seng Hospital, Singapore 308433, Singapore*
[i] *Cardiovascular and Metabolic Disorders Program, Duke-NUS Medical School, Singapore 169857, Singapore*
[j] *Faculty of Health and Social Sciences, Bournemouth University, Bournemouth, Dorset, BH8 8GP, UK*

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ABSTRACT

Algae are promising sources of nutritious and sustainable protein, but little is known about their metabolic health impact and acceptability as meal ingredients. This acute, randomized, controlled, five-way crossover trial compared whole algal biomasses and their corresponding protein concentrates to soy protein concentrate in terms of palatability, appetite, satiety, and metabolic response. Nineteen healthy Chinese males (21–50 years, 18.5–25.0 kg/m^2) consumed noodle meals supplemented with 10 g of nori biomass/protein concentrate (NB/NC), *Chlorella vulgaris* biomass/protein concentrate (CB/CC) or soy protein concentrate control (CON) in randomized order. At regular intervals, blood samples were collected to measure biochemical markers, while gastrointestinal tolerance, palatability, and appetite were assessed using questionnaires and visual analog scales (VAS). Results indicated that algae-enriched meals were well-tolerated and comparable to soy in both visual appeal and smell, with NB and CC outperforming soy in aftertaste ($p < 0.05$). There were no significant differences between treatments in glucose, insulin, C-peptide, appetite/satiety, plasma ghrelin, and GLP-1. However, exploratory analysis of serum triglycerides revealed significant time × treatment effects ($p < 0.004$) and differences in incremental area under the curve (iAUC$_{0\text{–}120}$, $p = 0.0249$). Our findings reveal that algal biomasses and protein concentrates are as comparable to soy protein concentrate in palatability, satiety, and metabolic outcomes, highlighting their potential as practical, sustainable, and nutritious ingredients.

1. Introduction

The growing interest in algae as a sustainable and nutrient-rich food source has catalyzed extensive research into its potential health benefits and practical applications. Its rich nutritional composition, including a high protein content with a wide spectrum of essential amino acids and fatty acids, positions algae as a highly promising candidate for enhancing human nutrition (Wu et al., 2023). In addition to the various anabolic effects of protein, it also has an important role in satiety and metabolism. In particular, protein has an important role in glucose homeostasis and different protein sources have been known to exert variable effects on postprandial glycemic response (Quek et al., 2016). Being

* Corresponding author.
E-mail addresses: shaldar@bournemouth.ac.uk, sumanto_haldar_from.bu@sifbi.a-star.edu.sg (S. Haldar).
[1] Jia Yee Wu, Rachel Tso, and Yi Ning Yong contributed equally to this work.
[2] Present address: Department of Nutrition, Dietetics and Food, Monash University, Notting Hill, VIC 3168, Australia

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rich sources of not only protein but also micronutrients, algae can potentially influence glucose homeostasis (Abo-Shady et al., 2023). For example, brown algae supplementation has shown distinct glucose metabolism responses in different ethnic groups (Murray et al., 2018).

To harness the full potential of this alternative food source, recent biotechnological advancements have emphasized protein extraction from harvested whole algae to create food-grade protein concentrates for use by the food industry (de Souza Celente et al., 2023; Espinosa-Ramírez et al., 2023). While these extracts from microalgae and seaweeds have demonstrated promise in preliminary consumption studies in the form of supplements (Ebrahimi-Mameghani et al., 2017; Hosseini et al., 2021; Murray et al., 2018; Takase et al., 2020; Vodouhè et al., 2022), their health impacts compared to whole algae require further exploration. Adding to the intricacy, the varied study designs and populations in existing research pose challenges in forming definitive conclusions about the overall health benefits of algae consumption (Wu et al., 2023).

Another significant gap in current research is the investigation of the feasibility of integrating substantial amounts of algae into meals. To date, studies have not thoroughly explored this aspect, particularly in a real food context as opposed to supplement forms. This gap is even more pronounced in the context of human trials (Kim et al., 2008). While there has been some work on brown seaweeds, studies on commonly edible algae, especially those known for their high protein content like microalgae *Chlorella spp*. and red seaweeds such as nori, are notably scarce.

Hence, this study intends to address these limitations by assessing the practicality, acceptability, and potential postprandial metabolic response of consuming pilot-scale protein concentrates from algae as part of a regular meal. We aim to compare these protein concentrates with their corresponding whole algae biomasses for their effects on gastrointestinal tolerance, satiety, and postprandial glycaemia in a group of healthy Chinese males. We hypothesize that the protein-rich algae protein concentrates will be a feasible and acceptable component of a meal.

In selecting the specific types of algae for this study, we have chosen two types of algae with high protein content: a red seaweed, nori (*Pyropia seriata*), and a green microalga, *Chlorella vulgaris*. Nori, widely known for its use in Asian cuisine, particularly in sushi, is a type of red seaweed that offers a unique nutritional profile, including high vitamin and mineral content (Todorov et al., 2022). Its popularity and culinary versatility make it an ideal candidate for assessing the feasibility of integrating seaweed into regular meals. On the other hand, *Chlorella*, a green microalga, is known for its high protein content and a range of health-promoting properties, including detoxifying effects and immune system support (Barkia et al., 2019). This selection not only allows us to explore the specific health benefits and culinary applications of these types of algae but also enables a comparative analysis of macroalgae and microalgae in terms of their nutritional impact and acceptability as food ingredients.

In addition, in this study we used soy protein concentrate to serve as a control, as soy is recognized as a nutritious and widely used plant protein source with well-documented benefits towards glycemic response (Azadbakht et al., 2007; Ferguson et al., 2014; König et al., 2012; Urita et al., 2012; van Nielen et al., 2014). Soy has been extensively studied and utilized in various food products due to its nutritional value, versatility, and positive impact on health. By incorporating soy protein concentrate as a control in our research, we aim to establish a benchmark for comparison with the selected algal types, nori and *Chlorella*. This approach allows us to assess these algae against a widely accepted and commonly used plant protein source like soy, which will help provide greater insights into their relative effects on glucose homeostasis, gastrointestinal tolerance, satiety, and overall suitability of the chosen algae for potential integration into everyday diets.

2. Materials and methods

2.1. Study population and recruitment

Twenty-three volunteers were recruited for this study via email and poster advertisements placed around the National University of Singapore, word of mouth, and from the Clinical Nutrition Research Centre (CNRC) mailing database of previous study participants agreeable to be contacted for future research studies. Inclusion criteria were as follows: male, Chinese ethnicity, aged between 21–50 years, and having a body mass index (BMI) between 18.5–25.0 kg/m^2. Exclusion criteria were: smoking, having diabetes (defined as hemoglobin A1c (HbA1c) $\geq$ 6.5 %); sustained elevated blood pressure ($\geq$140 mm Hg and/or $\geq$90 mm Hg for systolic and diastolic, respectively); having allergies, intolerances or strong dislikes to any of the study foods or common food ingredients; adhering to any special diets or dietary restrictions; unwilling to adhere to diet modifications as per the study protocol; taking part in strenuous physical activities and not willing to stop during the study duration; alcohol consumption on $>$ 4 days per week with $\geq$ 6 alcoholic drinks per week; having donated blood within 4 weeks of study commencement; having glucose-6-phosphate dehydrogenase (G6PD) deficiency; history of cardiovascular, metabolic, renal, gastrointestinal or blood disorders, thyroid dysfunctions or chronic infections (e.g. tuberculosis, HIV, Hepatitis B or C); having undergone any gastrointestinal surgery; taking any prescribed medications or dietary supplements likely to interfere with the study measurements; or having poor veins or history of severe vasovagal syncope from blood collection. Potential volunteers were invited to attend a screening and consent visit whereby oral and written information about the study objectives and protocol were provided to each individual and written informed consent was obtained prior to performing any study-related assessments. Eligibility was assessed using a health and lifestyle questionnaire and anthropometric measurements taken including height using a stadiometer (Seca 217, Hamburg, Germany), weight using a body composition analyzer (Tanita BC-418, Tokyo, Japan), seated resting blood pressure using an automated sphygmomanometer (Omron HEM907, Singapore) and a finger-prick blood HbA1c using an HbA1c analyzer (DCA Vantage, Erlangen, Germany).

The clinical trial was conducted at the Agency for Science, Technology and Research (A*STAR) CNRC in Singapore. The trial was reviewed and approved by the National Healthcare Group (NHG) Domain-Specific Research Board (DSRB), Singapore (DSRB Reference No. 2022/00909), and registered under ClinicalTrials.gov (ID: NCT05765448). The intervention phase of the study took place from 7 March 2023 to 20 June 2023.

2.2. Preparation of algal samples and study meals

Investigational meals comprised of a soy protein powder concentrate (Bulk Powders Pty Ltd, Australia) which served as the control, nori *(Pyropia seriata)* macroalgae biomass, nori *(Pyropia seriata)* protein concentrate, *Chlorella vulgaris* microalgae biomass and *Chlorella vulgaris* protein concentrate. Dried raw nori biomass was obtained from Geohae Co Ltd, Republic of Korea, through Pacific Harvest Ltd. Dried *Chlorella* biomass was obtained from GO Superfoods, Sheffield, U.K.

The post-harvest bioprocessing of algae to produce protein concentrates was carried out at the Riddet Institute, New Zealand. For nori, *Pyropia seriata* was subjected to disintegration to a particle size of 5 mm, subsequently hydrated in soft water with food-grade NaOH at pH 9.0 $\pm$ 0.2 for at least 45 min, followed by pH neutralization to pH 7.4 $\pm$ 0.2 with food-grade citric acid. It was then subjected to colloid mill processing for cell wall disruption and protein release, subsequently further buffered to pH 7.0 $\pm$ 0.2. Cell debris was removed by cascading, vibrating, sieving, and GAF filtration, resulting in the production of nori protein concentrate.

Similar extraction processing was applied to microalgae, *Chlorella*

vulgaris, with minor modifications to accommodate the differences in the material. As the particle size of microalgae is 85.2 ± 2.3 μm, the microalgae powder was used for protein extraction directly without a disintegration process. The alkaline hydration and extraction pH were set at pH 11.6 ± 0.1, 37 ± 2 °C to better loosen the cell wall structure. Two-stage high-pressure homogenization was employed for cell wall disruption with a control pressure of 50/8 Mpa and control inlet and outlet temperatures of 33 °C and 43 °C, respectively. Cell debris was removed by self-desludged centrifugation, and the pH was neutralized to pH 7.0 ± 0.2 using food-grade citric acid. Ultrafiltration with a membrane cut-off of 5 kDa was applied to extract liquids from both *Pyropia seriata* and *Chlorella vulgaris*, to remove salt and concentrate the extract liquid. The extract liquid was subsequently subjected to vat pasteurization at 70 ± 0.5 °C for 30 min and either freeze-dried or spray-dried. Both nori and *Chlorella* protein concentrate were stored at –20 °C until further analysis and experimentation.

All investigational materials used in the trial underwent microbial and nutritional analyses by Cawthron Laboratories (Nelson, New Zealand) and R J Hill Laboratories Limited (Hamilton, New Zealand) and were certified to be food-grade and thus safe for human consumption.

Study meals were prepared in the form of a noodle soup dish with 10 g of added investigational product (i.e., soy protein concentrate, nori biomass, nori protein concentrate, *Chlorella* biomass and *chlorella* protein concentrate). All study meals were prepared fresh on the morning of each study session. The noodle soup dish (Indomie Mi Goreng Original, 80 g, Indonesia) included one dried noodle block accompanied by noodle condiments consisting of a sachet of dried onions (~1 g), a sachet of seasoning powder (~3.6 g), a sachet of chili paste (~4 g), a sachet of seasoning oil (~5 g), and a sachet of sweet soy sauce (~3.2 g), which originally came with the noodle packet supplied by the manufacturer. 10 g of soy protein concentrate control, algal biomass, or algal protein concentrate was added with one packet of noodles and the noodle condiments into a microwaveable bowl, 400 mL of water was then added, and the bowl microwaved for 6 min. This noodle soup dish with added soy protein concentrate or algal biomass/protein concentrate was served to volunteers with one packet of plain crackers (Meiji Plain Crackers Original, 26 g, Japan) and 250 mL of drinking water. The components and nutritional composition of the 5 study meals are detailed in Tables 1a and b, respectively. The macronutrient composition and energy content of the algal biomasses and their respective protein concentrates were measured by the Cawthron Laboratories (Nelson, New Zealand), and the details of these are listed in Supplementary Table 1. The macronutrient composition and energy content of the study meals (e.g. noodles, noodles condiments, and crackers), as well as the soy protein concentrate used during the control session were obtained from the manufacturer's labels.

A standardized evening meal consisting of rice (CJ CheilJedang Cooked White Rice, 210 g) and chicken soup (Campbell's Instant Soup - Cream of Chicken, 22 g) was provided to all volunteers before each of their 5 study sessions to minimize any potential variations in baseline blood analytes due to carryover effects from their previous meal before the measurement visit.

Table 1a
Components of the study meals

Mass of ingredient used (g)	CON	NB	NC	CB	CC
Indomie Instant Noodles	80	80	80	80	80
Meiji Plain Crackers	26	26	26	26	26
Soy protein concentrate	10	-	-	-	-
Nori biomass	-	10	-	-	-
Nori protein concentrate	-	-	10	-	-
Chlorella biomass	-	-	-	10	-
Chlorella protein concentrate	-	-	-	-	10
Total	116	116	116	116	116

Abbreviations: CON – soy protein concentrate control; NB – nori (*Pyropia seriata*) biomass; NC – nori (*Pyropia seriata*) protein concentrate; CB – *Chlorella vulgaris* biomass; CC – *Chlorella vulgaris* protein concentrate.

Table 1b
Nutritional composition across study meals

Meal composition (g/meal)	CON	NB	NC	CB	CC	Range (Max–Min)
Energy (kcal)	531.5	530.0	525.0	533.6	529.8	8.6
Protein (g)	19.3	14.7	15.6	16.0	16.1	4.6
Total fat (g)	19.4	19.8	19.5	20.3	20.3	0.9
Saturated fat (g)	9.2	9.2	9.2	9.3	9.3	0.1
Carbohydrate (g)	67.7	72.0	70.3	70.4	69.4	4.3
Sugars (g)	7.5	7.9	8.1	8.4	8.0	0.9
Fiber (g)	2.7	6.3	4.0	4.0	3.4	3.6
Sodium (g)	1.1	1.0	1.2	1.0	1.4	0.4

Abbreviations: CON – soy protein concentrate control; NB – nori (*Pyropia seriata*) biomass; NC – nori (*Pyropia seriata*) protein concentrate; CB – *Chlorella vulgaris* biomass; CC – *Chlorella vulgaris* protein concentrate.
*Values shown are the overall nutritional composition of the study meals, including plain crackers and soup noodles supplemented with biomass or protein concentrates. Range is the difference between the maximum and minimum values observed across the meal types for each nutrient.

2.3. Study design and procedures

An acute, randomized, controlled, crossover study design was conducted which compared the effects of noodle-based meals with either whole algal biomass, their protein concentrates, or the soy protein concentrate control on postprandial metabolism and subjective satiety. A schematic of the study design is depicted in Fig. 1. Volunteers attended 5 study sessions, during which they consumed 1 of 5 investigational products according to their randomized treatment sequences, with each session separated by a minimum washout period of 7 days. The order of the 5 study meals was randomized by computer sequence generation (http://www.randomizer.org). Laboratory staff responsible for analyzing blood samples were blinded to treatment allocations until after the laboratory results reports were delivered. However, because of the visual appearance of the study products, volunteers and staff administering the meals could not be blinded during the consumption of meals.

Prior to each study session, volunteers were instructed to consume the standardized evening meal followed by an overnight fast of at least 10 h before arrival in the morning at the research clinic. Upon arrival, an intravenous cannula was inserted into a suitable upper extremity vein by a trained phlebotomist, for a duration of 4 h during which the volunteer remained at the research center. A baseline (0 min) fasting blood sample (6 mL) was then collected. Volunteers rated their baseline appetite (hunger, fullness, and prospective intake) using a series of validated 100 mm visual analogue scales (VAS) (Flint et al., 2000) with two ends describing each opposite extreme. The presence of gastrointestinal side effects experienced, if any, were recorded on a 7-point Likert scale from '0 (none)' to '6 (unbearable)' (Bovenschen et al., 2006), as listed in Supplementary Material, Appendix A. Volunteers were then served the study meal alongside 250 mL of plain drinking water and instructed to fully consume the meal within 15 min. Immediately upon finishing the meal, volunteers indicated their subjective palatability responses of the entire study meal on a series of 100 mm VAS for visual appeal, smell, taste, aftertaste, and overall acceptability. Throughout the 180 min postprandial period, volunteers were provided with up to a maximum of 250 mL drinking water to consume *ad libitum* for standardization in the assessment of satiety and fullness. Subsequent postprandial blood samples were collected through the intravenous cannula at 15-, 30-, 45-, 60-, 90-, 120-, 150-, and 180-min time points following the start of meal intake. Concurrently, volunteers also completed appetite ratings indicating their postprandial satiety (hunger, fullness, and prospective intake, each on 100 mm VAS) at each of these 8 time points, and postprandial gastrointestinal symptoms at the 15-, 60-, 120-, and 180-min time points. The intravenous cannula was removed after the 180 min

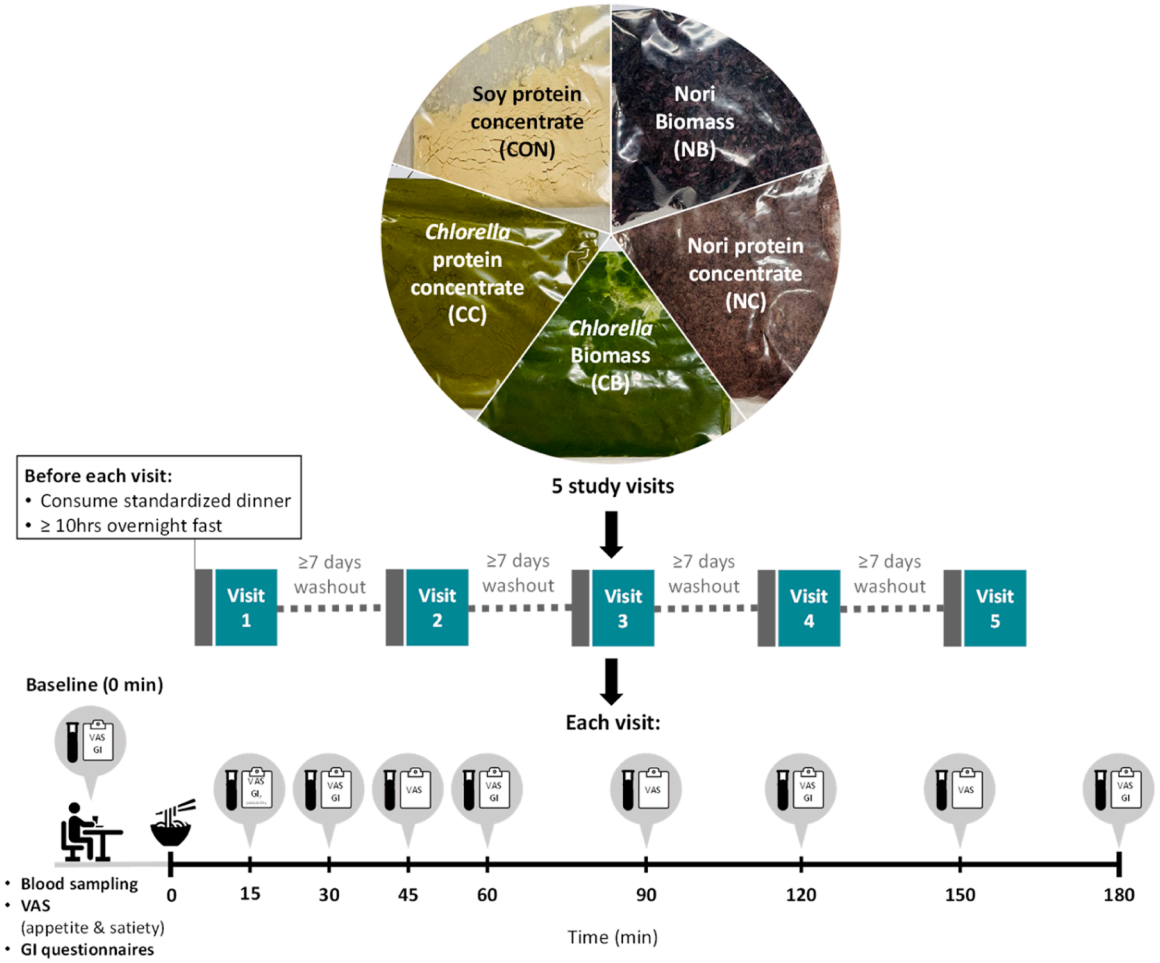

Fig. 1. Schematic of the study design. Each participant attended five separate study sessions during which they consumed five different study meals on separate occasions in a random order, with a minimum seven days washout period between each session. Participants were instructed to consume a standardized meal and fast for at least 10 h before each visit.

time point.

2.4. Blood sample collection and analysis

Blood samples from each time point were collected in a 3 mL K2 ethylenediaminetetraacetic acid (K2 EDTA) vacutainer (BD, Franklin Lakes, New Jersey, USA) and a 3 mL serum vacutainer (BD, Franklin Lakes, New Jersey, USA). K2 EDTA tubes were immediately stored on ice and centrifuged within 30 min after collection, while serum tubes were centrifuged between 30 min to 60 min of collection after clot formation. All tubes were centrifuged at 2000 x g for 10 min at 4 °C. Serum and plasma samples were directly aliquoted into 1.5 mL microtubes, with a quantity of 500 uL each. Plasma samples used for gut hormone analysis were treated with dipeptidyl peptidase IV (DPP-IV) inhibitor (Merck KGaA, Darmstadt, Germany) and aprotinin (Sigma Aldrich, St. Louis, Missouri, USA). All microtubes were stored at –80 °C until subsequent analyses.

Serum glucose, insulin and triglycerides (TG) were analyzed using standardized clinical chemistry methods by the National University Hospital Referral Laboratories, Singapore; glucose and TG were measured using an AU5800 clinical chemistry analyzer (Beckman Coulter Inc., Brea, California, USA) and an enzymatic colorimetric assay, and insulin was measured with UniCel DxI 800 Access Immunoassay System (Beckman Coulter Inc., Brea, California, USA). Plasma gut hormones active glucagon-like peptide-1 (GLP-1), active ghrelin, insulin, and C-peptide were quantified using customized panels from MILLIPLEX MAP Human Metabolic Hormone Magnetic Bead Panel (#HMHEMAG-34K, Merck KGaA, Darmstadt, Germany) according to the manufacturer's protocol.

2.5. Statistical analysis

Formal sample size power calculations were not possible since the investigational samples have not been previously studied in this manner to test the effects of algal biomass versus algal extracts, or algae versus soy, on postprandial nutrient metabolism and satiety. We therefore based the number of participants on previous comparable acute postprandial studies using alginate beverages versus soy protein (n = 12) (Huang et al., 2019), calcium alginate-enriched noodles in a study investigating postprandial blood glucose levels (n = 15) (Kato et al., 2018), and a postprandial glycemia study comparing two types of seaweed against a rice control (n = 12) (Tanemura et al., 2014). Hence, a pragmatic sample size of n = 20 participants was used. 23 participants were recruited, and 19 participants completed the study.

The primary outcome of this study was the acute effects of the investigational meals on postprandial glucose and insulin responses over 180 min post-meal. Secondary outcomes were the acute postprandial effects of the investigational meals on TG, active ghrelin, active GLP-1, C-peptide, and satiety over 180 min post-meal.

Statistical analyses were undertaken using Statistical Package for the Social Sciences (SPSS), version 27 (IBM, Chicago, Illinois, USA) and GraphPad Prism, version 9.5.1 (GraphPad Software, La Jolla, California,

USA). Data normality was assessed using the Kolmogorov-Smirnov test, with logarithmic or square-root transformations applied to correct for normal distribution where applicable, before subsequent statistical analyses. All Gaussian-distributed data were analyzed using two-way repeated measures ANOVA with Bonferroni adjustments applied to correct for multiple comparisons, while non-parametric data were analyzed using Friedman's test, using the Benjamini and Hochberg method to correct for multiple comparisons.

The 60 min, 120 min and 180 min incremental area under the curve ($iAUC_{0–60min}$, $iAUC_{0–120min}$, and $iAUC_{0–180min}$, respectively) were determined by subtracting the baseline value (at $t = 0$ min) from each value at all subsequent time points and calculating the total area between the postprandial curve above the baseline level within the specified time range. AUC values for all variables were calculated using GraphPad Prism, based on the trapezoidal rule (Allison et al., 1995). Between means and pairwise comparisons were conducted using one-way ANOVA with type III fixed effects and Tukey's post-hoc analysis for Gaussian distributed data, whereas the Friedman's test and Benjamini and Hochberg correction was applied for non-Gaussian distributed sets.

For each subjective VAS appetite/satiety dimension, the baseline-corrected change in postprandial scores was obtained by subtracting each individual's VAS score at each time point from that of the baseline ($t = 0$ min), in the determination of the 180 min baseline-corrected area under the curve (ΔAUC). Similarly, as above, two-way repeated measures ANOVA with Bonferroni corrections were used to test for time, treatment, and interaction effects, while one-way ANOVA with type III fixed effects with Tukey's post-hoc analysis was used to compare for differences in ΔAUC between groups. Spearman's test was used to assess for statistical correlation between plasma ghrelin levels and each subjective appetite rating.

3. Results

3.1. Participant characteristics

A total of 28 individuals were screened, and 23 were enrolled into the study – of which 19 completed all five treatment arms (Fig. 2). Baseline anthropometric and metabolic profiles of participants who completed the study are presented in Table 2. Twenty participants had HbA1c $\leq$ 5.6 % and 2 had readings between 5.7–6.4 % which would thus be classified as having prediabetes (American Diabetes Association, 2016). One participant was found to have elevated fasting TG of > 1.70 mmol/L, which is considered borderline high (Miller et al., 2011).

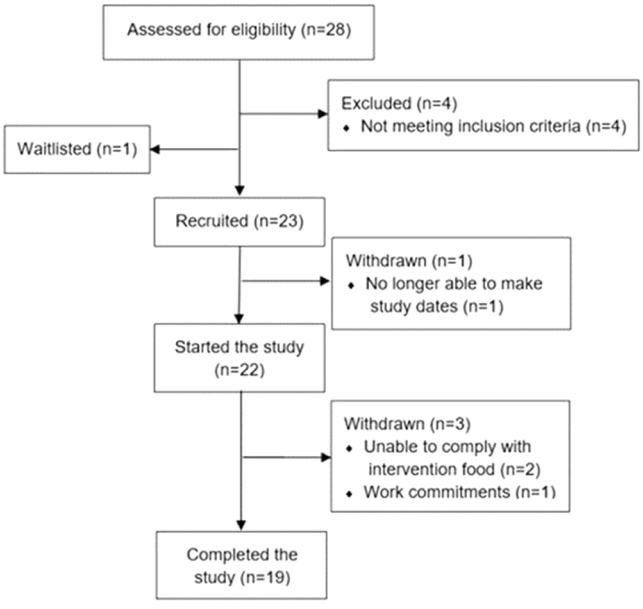

Fig. 2. Consolidated Standards of Reporting Trials (CONSORT) diagram. 28 volunteers were screened, of which 23 were recruited into the study and 19 participants completed the study. One participant was withdrawn before starting the study, while another three were withdrawn after commencing the study sessions.

3.2. Palatability scoring of test meals relative to the control meal

To explore the feasibility of incorporating algae into regular diets, this study investigated the sensory acceptability of algae-enriched foods. Acceptance was evaluated based on five subjective sensory attributes: visual appeal (Fig. 3a), smell (Fig. 3b), taste (Fig. 3c), aftertaste (Fig. 3d), and overall palatability (Fig. 3e). The findings indicated that the algae-enriched meals were generally well-received in terms of visual appeal and smell, showing comparable tolerance levels to those of the soy control (Fig. 3a and b). In taste and aftertaste (Fig. 3c and d), the algae-enriched meals tended to have a better trend than the soy protein concentrate control. The aftertaste scoring demonstrated a significant difference, with both NB and CC scoring significantly higher ratings than CON based on post-hoc tests (Fig. 3d, $p < 0.05$).

3.3. Self-reported gastrointestinal symptoms following consumption of study meals

Assessing gastrointestinal symptoms post-consumption is essential to understand the digestive tolerance of up to 10 g algae-enriched meals – a key factor in evaluating their suitability for regular dietary inclusion. Gastrointestinal effects following the consumption of the study meals were generally well-tolerated, with no significant time × treatment effects for all reported symptoms across the five treatment arms (data not shown; see Supplementary Material, Appendix A for gastrointestinal symptoms questionnaire).

3.4. Postprandial changes in subjective appetite ratings and plasma ghrelin

Mean postprandial changes in subjective hunger, fullness, prospective eating from baseline, and plasma ghrelin levels are detailed in Fig. 4. The gut hormone ghrelin, responsible for stimulating feelings of hunger, was measured to objectively compare the systemic regulation of appetite in response to meal intake, alongside the participants' perceived levels of satiety. The Spearman's coefficient (r) showed modest correlations between postprandial levels of plasma ghrelin and all of the following three subjective parameters – hunger ($r = 0.1088$, $p < 0.01$), fullness ($r = –0.1213$, $p < 0.001$) and prospective eating ($r = 0.2241$, $p < 0.0001$). In terms of 3-hour ΔAUC however, only prospective eating was negatively correlated with ghrelin ($r = –0.2161$, $p < 0.05$).

While there were sharp reductions in subjective hunger and

Table 2
Baseline anthropometric and metabolic measures of participants ($n = 19$).

Measurement	Mean ± SD
Age (years)	35.7 ± 10.3
Height (m)	1.7 ± 0.1
BMI (kg/m^2)	22.5 ± 1.8
Systolic Blood Pressure (mmHg)	125.6 ± 10.0
Diastolic Blood Pressure (mmHg)	79.2 ± 6.8
HbA1c (%)	5.4 ± 0.3
Fasting serum glucose (mmol/L)	5.22 ± 0.4
Fasting serum insulin (mU/L)	5.66 ± 2.1
Fasting serum triglycerides (mmol/L)	0.95 ± 0.4

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c. SD, standard deviation.

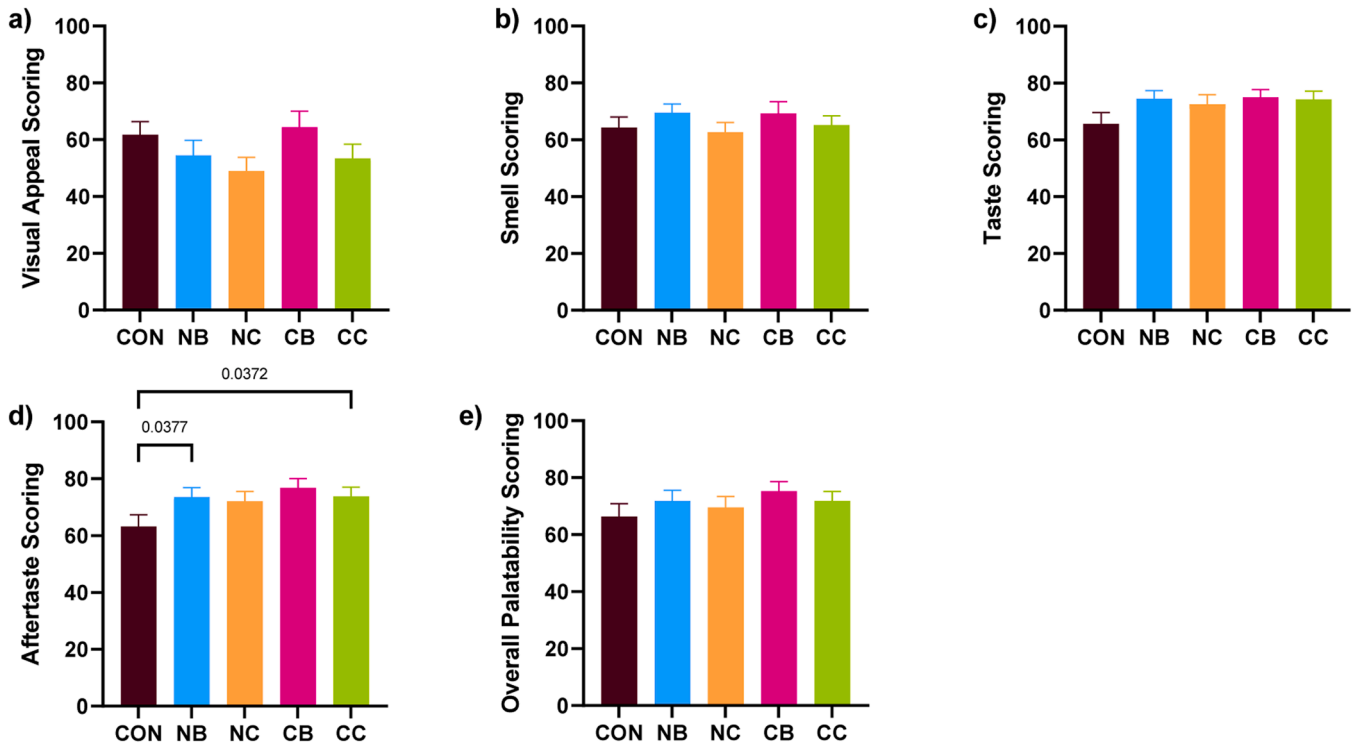

Fig. 3. Mean (± SEM) subjective scoring of each study meal based on sensory characteristics – (a) visual appeal, (b) smell, (c) taste, (d) aftertaste, and (e) overall palatability. A score of 0 indicates the least favorable perception, while 100 represents the most favorable. Abbreviations: CON – soy protein concentrate control; NB – nori (*Pyropia seriata*) biomass; NC – nori (*Pyropia seriata*) protein concentrate; CB – *Chlorella vulgaris* biomass; CC – *Chlorella vulgaris* protein concentrate.

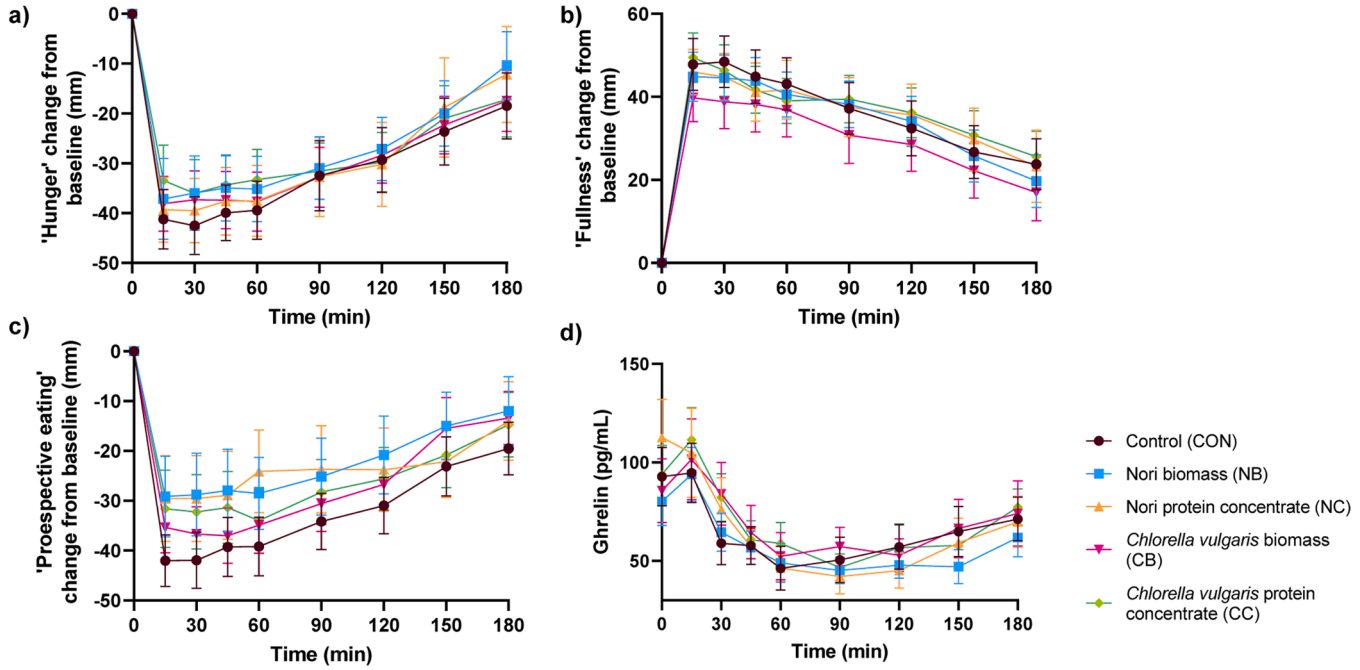

Fig. 4. Baseline-corrected mean (± SEM) ratings for subjective (a) hunger, (b) fullness, (c) prospective eating, and measured (d) plasma ghrelin over the 3-hour postprandial period among the four algal groups and the soy control group.

prospective eating – corresponding with sharp increases in fullness – between the baseline ($t = 0$ min) and 15 min after meal consumption, plasma ghrelin instead showed a more gradual decrease which spanned from 0 min until 60 min. Nevertheless, there were no significant differences in time × treatment effects within individual time points, nor in the ΔAUC for all dimensions of subjective appetite/satiety, as well as for plasma ghrelin between the five treatments.

3.5. Markers of postprandial glucose homeostasis

Serum glucose and insulin were measured to assess postprandial glucose homeostasis following the consumption of each test meal. C-

peptide is released alongside insulin following the post-translational modification of proinsulin and serves as a marker of pancreatic β-cell function (Okuno et al., 2013). The incretin hormone active GLP-1 is also known to potentiate insulin response, suppress glucagon release, reduce gastric emptying and prolong satiety during postprandial glucose metabolism (Eelderink et al., 2017), among its other functions. Postprandial changes in all of the above-mentioned biomarkers are shown in Fig. 5. No time × treatment differences were observed for repeated measures of serum glucose, insulin, active GLP-1 and C-peptide ($p > 0.05$). There were also no significant differences between groups either within any given time point, or in terms of $iAUC_{0–60}$, $iAUC_{0–120}$ nor $iAUC_{0–180}$ for the above-mentioned markers between the 5 treatment arms ($p > 0.05$) (Table 3).

3.6. Postprandial triglyceridemic response to control versus test meals

In addition to the above, we conducted exploratory analyses of postprandial changes in serum TG, a recognized risk factor of cardiovascular diseases (Kolovou et al., 2011), within the earlier postprandial phase (3h). Postprandial serum TG exhibited significant time × treatment effects ($p < 0.004$), as well as differences in $iAUC_{0–120}$ between treatments ($p = 0.0249$), although no significant pairwise differences were obtained after adjustment for multiple testing (Fig. 5, Table 3). The serum TG profile appeared similar for all interventions from 0 to 30 min, and then, subsequently, both nori samples (biomass and protein concentrate) showed attenuated responses in TG levels until 90 min (Fig. 5e). Interestingly, from 90 min onwards, NB and NC showed distinct trajectories in the graph – with NB maintaining a consistently low TG level among all the groups, whereas NC showed a steeper increase in TG levels, causing the gap between NB and NC to become more pronounced despite having similar nutritional compositions (Table 1b). This differentiation between the two nori treatments becomes a point of interest, suggesting that the form in which nori is administered (biomass versus concentrate) may have a significant impact on its lipid-modulating properties, with biomass appearing to be more effective in moderating the rise in TG.

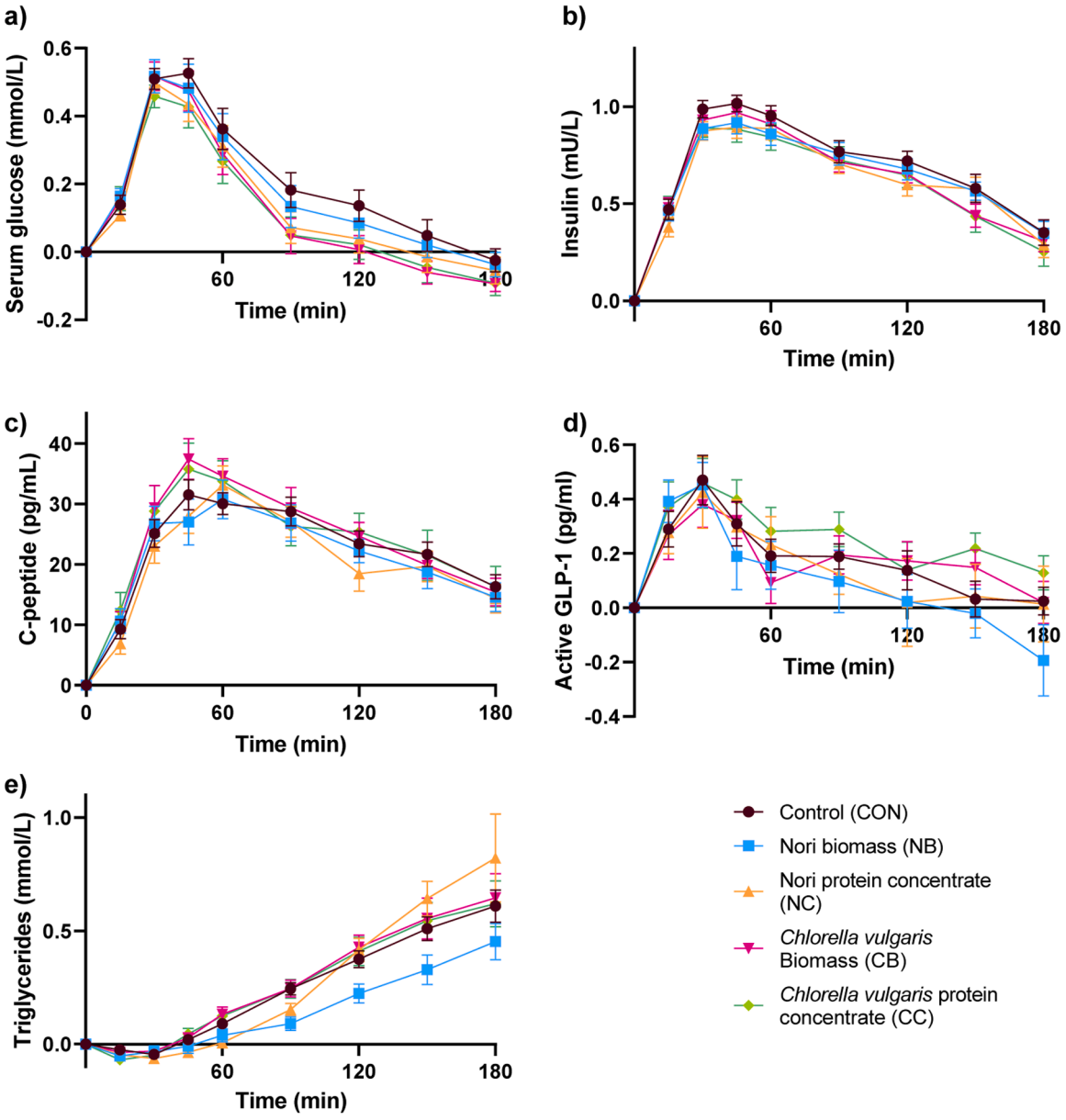

Fig. 5. Baseline-corrected 3-hour postprandial levels (Mean ± SEM) of (a) serum glucose, (b) insulin, (c) C-peptide, (d) active GLP-1, and (e) triglycerides in response to the test meals containing. Four algal groups and the soy control group.

Table 3
Postprandial incremental area under the curve (iAUC) of serum glucose, insulin, active GLP-1, C-peptide, and triglycerides.

Biomarker	Treatment	$iAUC_{0-60}$		$iAUC_{0-120}$		$iAUC_{0-180}$	
		Mean ± SEM	*P*	Mean ± SEM	*p*	Mean ± SEM	*p*
Serum glucose	CON	20.48 ± 1.45	> 0.05	34.67 ± 3.98	> 0.05	43.44 ± 5.05	> 0.05
	NB	20.67 ± 1.89		35.41 ± 4.05		44.32 ± 4.78	
	NC	18.13 ± 1.52		29.90 ± 2.93		37.96 ± 3.48	
	CB	19.78 ± 1.83		31.87 ± 3.31		39.97 ± 3.47	
	CC	18.21 ± 1.76		30.26 ± 3.00		39.50 ± 3.31	
Insulin	CON	44.24 ± 1.79	> 0.05	92.32 ± 4.15	> 0.05	126.67 ± 6.65	> 0.05
	NB	40.48 ± 2.38		86.22 ± 5.08		118.96 ± 7.24	
	NC	38.91 ± 2.07		82.3 ± 4.31		113.13 ± 5.99	
	CB	42.52 ± 2.47		87.35 ± 4.82		116.44 ± 6.32	
	CC	40.14 ± 2.48		84.19 ± 5.93		113.10 ± 8.56	
C-peptide	CON	1215 ± 78.41	> 0.05	2880 ± 158.6	> 0.05	4126 ± 1148	> 0.05
	NB	1206 ± 102.8		2807 ± 226.6		3933 ± 1417	
	NC	1121 ± 104.8		2717 ± 228.1		3809 ± 1095	
	CB	1420 ± 125.4		3190 ± 240		4387 ± 1532	
	CC	1414 ± 178.1		3091 ± 330.2		4363 ± 2225	
Active GLP-1	CON	929.5 ± 219	> 0.05	1411 ± 308.3	> 0.05	1696 ± 365.8	> 0.05
	NB	833.9 ± 190.7		1155 ± 221		1329 ± 246.7	
	NC	571.7 ± 131.5		845 ± 188.2		1054 ± 239.1	
	CB	716.5 ± 149.4		1088 ± 217.7		1339 ± 266.3	
	CC	793 ± 134.9		1305 ± 244.1		1683 ± 344.6	
Triglycerides	CON			16.99 ± 1.37	**0.0249**	45.8 ± 3.95	> 0.05
	NB			13.14 ± 2.00		34.22 ± 4.19	
	NC			14.88 ± 1.59		52.7 ± 6.69	
	CB			17.84 ± 2.33		46.2 ± 6.30	
	CC			19.01 ± 2.54		50.99 ± 7.23	

Abbreviations: CON – soy protein concentrate control; NB – nori (*Pyropia seriata*) biomass; NC – nori (*Pyropia seriata*) protein concentrate; CB – *Chlorella vulgaris* biomass; CC – *Chlorella vulgaris* protein concentrate; GLP-1, glucagon-like peptide-1; SEM, standard error of the mean.

4. Discussion

With the emergence of alternative/complementary sources of protein in our daily diet, it is important to understand the metabolic health effects of these protein sources. In this study, we attempted to compare the postprandial metabolic effects of the biomasses of macroalgae and microalgae, which are both naturally high in protein, and their respective protein-rich concentrates in comparison with soy protein concentrate, which was used as a positive control. To the best of our knowledge, this acute, randomized, controlled crossover trial was one of the first of its kind to investigate the feasibility and acceptability of consuming protein-rich algal concentrates within a real-life dietary context. Additionally, comparing these protein concentrates to their whole algal biomasses in terms of their effects on postprandial sensory attributes, satiety, and glucose and lipid metabolism, also enabled us to understand whether food processing methods to produce such ingredients may have an impact on sensory attributes and acute metabolic health. Our findings revealed that all algal samples, including the protein concentrates, were deemed acceptable in meal palatability as compared to the soy protein concentrate control, with the *Chlorella* protein concentrate and nori biomass aftertastes being even more preferred over soy. In terms of the overall palatability, there was no significant difference between the various test meals. All of the above indicates that the algal samples were at least equally palatable, if not marginally better than the control, in terms of palatability, highlighting its potential to be readily incorporated into meals. The practicality of incorporating these samples into a noodle soup dish highlights a feasible method of preparation and consumption, particularly within the Asian dietary and cultural context.

Similar effects between various treatments were also observed in postprandial glucose homeostasis markers and satiety outcomes. The similarities in postprandial serum glucose and insulin trends among the algal protein concentrates, biomasses, and soy protein concentrate were likely to stem from the isocaloric nature of the study meals and their similarities in nutritional composition. In our methodology, we purposefully decided to standardize the serving size of biomasses and protein concentrates to 10 g to better reflect the real-life dietary context of having such ingredients with real foods at an acceptable dose. This inevitably led to subtle differences in two macronutrients, namely dietary fiber and protein, between the test meals (Table 1b), although these *per se* had limited differences in terms of postprandial metabolism, particularly for satiety and glucose metabolism. We had included soy protein concentrate as a positive control given that soy is recognized as a high-quality protein source with documented benefits in glycemic control (Huang et al., 2019), providing a relevant benchmark for comparison with novel algal protein concentrates investigated in our study. Overall, our study findings suggest that in healthy Chinese males, both whole algae and protein-rich algal concentrates impacted glucose homeostasis and satiety comparably while retaining similar sensory attributes to soy protein concentrate.

The comparable satiating effects exhibited by algal biomasses and their protein-rich concentrates to soy suggests their potential utility to improve inter-meal satiety and reduce caloric intake in subsequent meals to a similar extent as soy protein (Nepocatych et al., 2019), although subsequent meal intake was not measured in this trial. Notably, ghrelin levels, which typically decrease upon meal consumption, initially increased at the 15 min time point for most samples before gradually decreasing from the 30 min time point onwards. Given that the half-life of ghrelin is between 10 and 30 min (Strassburg et al., 2008), it is not surprising to see a slight increase initially, production of which may have been stimulated further in a transient manner due to the appetizing properties (smell, sight) of the test meals. The release of ghrelin via activation of the vagal system has been observed after sham feeding (without actual food ingestion) in participants who were served a meal (Monteleone et al., 2010). This sham feeding study conducted by Monteleone and colleagues allowed participants to see, smell, and taste the food in a served meal but avoid consuming the food by instructing them to spit out each bite into a napkin rather than ingest it. Given that most of the postprandial studies to date do not often measure ghrelin at such an early time point such as in our study (i.e. 15 min following the start of meal intake), this trend we observed is rather unique. Furthermore, the monosodium glutamate in the noodle soup dish could have also stimulated appetite initially in the moments within the first few bites (Masic & Yeomans, 2014), which may have also stimulated ghrelin production; after which ghrelin secretion would have decreased once

the entire meal had been consumed.

Rapid declines in subjective hunger and prospective eating were observed alongside a sharp increase in fullness. Yet a gradual decrease in plasma ghrelin was seen from 0–60 min. This may be explained by rapid ghrelin level sensing by hypothalamic appetite-regulating neurons, which expeditiously signal satiety state despite a more gradual rate of change of ghrelin happening in plasma – which decays with a half-life between 10 and 30 min as previously mentioned (Schaeffer et al., 2013; Strassburg et al., 2008). This study, however, measured plasma ghrelin via the collection of peripheral blood samples and was not designed to detect changes in ghrelin by appetite-regulating neurons. These mechanisms of action of ghrelin would explain the seemingly poor correlation between the observed subjective appetite ratings and the gradual decline in plasma ghrelin levels.

Finally, the significant time × treatment effects and differences in incremental area under the curve ($iAUC_{0–180}$) between test meals for postprandial TG may have been due to the higher content of dietary fiber in the nori biomass (6.3 g per meal) and nori protein concentrate (4.0 g per meal) (Table 1b). Consistent with previous findings, incorporation of fiber into meals of varying composition tends to result in an attenuation of the postprandial TG response (Bozzetto et al., 2020; Cara et al., 1992; Maki et al., 2007), potentially through mechanisms such as delayed gastric emptying, lowered circulating bile acids, and altered lipid absorption (Bozzetto et al., 2018; Lee et al., 2020).

It is worth noting that the *Chlorella* biomass, despite having a similar fibre content (4 g), did not exhibit the same effects on postprandial TG as observed with nori. This suggests that the specific effects might be attributed to the unique composition of nori's cell walls, which are rich in sulphated glycans, such as carrageenan or agars, that are a common feature of *Porphyra spp.* (Jiménez-Escrig & Sánchez-Muniz, 2000; Lahaye, 1991; Xu et al., 2019). It has been shown by another research group that carrageenan retains bile salts permeation in an *in vitro* gastrointestinal membrane permeability assay (Sokolova et al., 2020), possibly preventing bile reabsorption and thus promoting excretion, which in turn reduces the availability of bile salts for fat emulsification, leading to decreased fat absorption and lower postprandial TG levels. The wider project team is currently exploring the possibility of undertaking detailed glycomics analyses, although this is beyond the remit of this present report.

However, this factor alone does not fully elucidate the difference of postprandial TG levels observed between nori biomass and protein concentrate beyond 90 min. The difference can be potentially elucidated by two mechanisms: First, the food processing that transforms nori biomass into a concentrate could selectively concentrate or diminish specific bioactive components. This alteration may impact the synergistic action of dietary fiber and other constituents on lipid metabolism, thereby modulating their collective effect on postprandial TG levels. Furthermore, the complexity of nori biomass as a food matrix is likely to affect the bioavailability and release kinetics of these bioactives. The inherent intact structure of nori biomass could slow digestion, thereby decelerating the release of beneficial compounds and ensuring a more prolonged mitigation of postprandial TG elevation. This suggests that the nori protein concentrate may not interact with postprandial TG metabolism as beneficially as when they are presented in the whole biomass matrix, which is an important consideration in harnessing its full spectrum of metabolic benefits for human health.

Furthermore, the effect may not be solely due to compositional differences in dietary fibre and other bioactives unique to nori biomass (e.g. phenolic content, eicosapentaenoic acids, minerals, etc. (Peñalver et al., 2024)) may be responsible for such effects. However, this would require exploration of detailed compositional analysis including using metabolomics approaches, not only on the food, but also after its consumption in humans, *in vivo*.

Additionally, there is evidence to suggest that the amount and type of protein in a meal may modulate postprandial triglyceridemia, perhaps through mechanisms such as delayed gastric emptying and attenuated fat absorption, although the protein levels in our study were not elevated (O'Reilly et al., 2011; Pal et al., 2010; Westphal et al., 2006).

Although this was an exploratory analysis, the reduction in postprandial serum TG by nori makes a case for subsequent research to extend the observation period beyond 3-hour post-ingestion to fully characterize the impact of these algae on postprandial lipidemia and longer-term lipid homeostasis. Furthermore, while the focus of our investigation was to evaluate the acute postprandial effects of our algal biomasses and concentrates, the measurements of the longer-term effects were not within the remit of the present study. Nonetheless there have been several independent longer-term trials undertaken with algal biomasses, for a minimum of 12 weeks. The findings included reductions in glucose, HbA1c, and HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) (Lee and Jeon, 2015; Mizoguchi et al., 2008; Panahi et al., 2012), as well as increased satiety in short-term randomized clinical trials (Mayer et al. 2014), though longer-term studies are lacking. In terms of lipids markers, decreased total cholesterol and LDL-cholesterol (Choi et al., 2015; Ebrahimi-Mameghani et al., 2017; Mizoguchi et al., 2008; Ramamoorthy and Premakumari, 1996; Ryu et al., 2014) following algae consumption has been observed following 4–8 weeks of algal biomass supplementation. Therefore, while we can only speculate that the protein concentrates used in our study may also have some beneficial effects to that obtained using similar algal biomasses, longer-term studies are warranted with appropriate dosing and duration of intervention, before any definitive conclusions can be made.

The strengths of our study include the randomized controlled crossover design, which minimizes inter-individual variability in postprandial metabolism, sensory impact, and satiety. By incorporating a realistic dose of algae or soy into a commonly consumed Asian noodle meal, we provided a pragmatic approach to algae consumption, enabling the design of future foods and meals at similar doses of these algae.

One of the limitations of our study was the absence of a no-supplement control group. Including such a group could have provided a clearer baseline ('negative control') for comparison and interpretation of the effects of test materials (NB, NC, CB, CC) and positive control (CON). However, we decided not to include this additional arm due to the challenges of maintaining protein balance across meals without supplementation in a crossover study design. Maintaining protein balance is an important consideration for acute studies measuring postprandial satiety and glucose homeostasis, both affected by amount of proteins, irrespective of protein quality. Nonetheless, previous studies have shown that soy supplementation was effective in relation to improving postprandial glycemia (Quek et al., 2016), and hence, using soy concentrate as a positive control was a better approach than having a no-supplement control. Furthermore, having five separate study sessions for each volunteer was already rather burdensome. Not including the no-supplement control group hence reduced the burden on volunteers, facilitating recruitment and retention. Additionally, given that this study was undertaken in an Asian population already familiar with algae, the generalizability of the findings to other populations may be limited and, hence, may require further investigation.

5. Conclusions

In summary, our study found whole algae and protein-rich algal protein concentrates at dietary acceptable doses to have similar effects to soy protein concentrate on postprandial metabolic, satiety, and sensory outcomes in healthy Chinese males. With comparable acceptability and metabolic effects to soy, algae and algal proteins present a sustainable and nutritious food ingredient option, particularly for individuals who are unable to consume soy or have greater preference for or access to algae. These findings underscore the potential of algae as a viable food ingredient in future food applications, promoting its role as a source of sustainable and nutritious alternative protein.

Ethical statement

Ethical approval for the involvement of human subjects in this study was granted by the National Healthcare Group (NHG) Domain-Specific Research Board (DSRB), Singapore (DSRB Reference No. 2022/00909; date of approval 17th February 2023) and registered under ClinicalTrials.gov (ID: NCT05765448).

CRediT authorship contribution statement

Jia Yee Wu: Writing – review & editing, Writing – original draft, Investigation. **Rachel Tso:** Writing – review & editing, Writing – original draft, Project administration, Investigation. **Yi Ning Yong:** Writing – review & editing, Writing – original draft, Investigation. **Susanna Poh Suan Lim:** Investigation. **Thomas Wheeler:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Arup Nag:** Writing – review & editing, Investigation, Conceptualization. **Lirong Cheng:** Writing – review & editing, Investigation, Conceptualization. **Md. Mahabubur Rahman Talukder:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Lee Huffman:** Writing – review & editing, Conceptualization. **Siew Young Quek:** Writing – review & editing, Conceptualization. **Melvin Khee Shing Leow:** Writing – review & editing, Supervision. **Sumanto Haldar:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2024.100436.

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