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Short Communication

Algal protein-based 3D-printed fish-analogs as a new approach for sustainable seafood

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ABSTRACT

Rising global demand for animal-products exceeds human-population growth. This unsustainable trend causes harmful ecological effects. Overfishing causes extinction of aquatic animals and a dangerous biodiversity loss harming aquatic ecosystems. Hence, replacing animal-based food, particularly beef and fish, with sustainable alternatives is an urgent vital global mission. Analogs of animal-based products include plant-based, tissue-culture-based and fermentation-based products. Fish analogs have mainly been based on plant-protein, fungi, tissue-culture, but to our knowledge, fish analogs made of algae, particularly macroalgae, as the major component and protein-source have not been reported. 3D-food-printing is a fast-developing technology, enabling formation of complex three-dimensional structures with various heterogeneous topologies and tailorable compositions. Herein, we report the co-extraction of proteins and polysaccharides from the red marine-macroalgae *Gracilaria cornea*, and using the extract in injection-based 3D-printing to form prototypes of salmon-fillet. Two bioinks were used: a red bioink dyed with microalgal-astaxanthin, for the muscle tissue, and a white bioink dyed with $CaCO_3$, for the intramuscular fatty-tissue. Algal proteins have excellent nutritional amino-acid composition, and the co-extraction with agar facilitates 3D-printing thanks to its pseudoplastic and gelling properties. This study highlights macroalgae as an exciting natural raw-material for fish analogs towards sustainable seafood production, thereby decreasing harm to ocean fisheries.

1. Introduction

Global warming, dwindling land and fresh water resources, rising pollution, and plummeting biodiversity necessitate pivoting to sustainable practices, particularly in agrifood (Kazir and Livney, 2021). Global demand for animal-based products (Falcon et al., 2022) rises more steeply than the world's population (United Nations, 2019), and the animal agrifood production will not be able to cope with this rising consumption. This unsustainable production results in harmful ecological and health effects and in animal suffering (The Good-Food-Institute, 2022). Overfishing leads to dwindling populations of fish and various other edible marine organisms dangerously diminishing biodiversity and harming the aquatic ecosystem (Zhong et al., 2023). Local environmental damage and spreading fish diseases (e.g. fish lice) caused by large-scale marine salmon farming constitute another concern (Torrissen et al., 2013). A major part of the solution is substituting animal-based food, particularly beef and fish, with sustainable environmentally-friendly alternatives (The Good-Food-Institute, 2022).

In this short communication we focus on fish substitutes. Fish analogs have mainly been based on plant- (Kazir and Livney, 2021), or fungal proteins (Andrew, 2023), or on fish tissue culture (Aarattuthodi et al., 2021), and some microalgal components have been included in certain products based on plant proteins to confer taste (Coleman et al., 2022; Siddiqui et al., 2024) or color (Kazir and Livney, 2021). Macroalgae have started appearing as components in fish analogs (Marwaha et al., 2022), e.g. in a shredded tuna or spread (BettaFish, 2022; Hooked Foods, 2019), in tuna-analog chunks made from tomato paste with kombu extract (Mimic Seafood, 2019), and in a "smoked salmon-slice" analog from macroalgal extracts and pea protein (Odontella, 2018). However, to the best of our knowledge, fish-fillet analogs made of algae, particularly macroalgae, as the major component and protein source have not yet been reported, neither was 3D-printing reportedly applied to make algae-based fish analogs. Red algae have high protein content and good nutritional quality, in terms of amino acid composition (Kazir et al., 2019). Gracilaria in particular has great potential in human nutrition, due to similar essential amino acid scores with respect to egg

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protein as a reference food protein (Terriente-Palacios and Castellari, 2022). Lipids in macroalgae, including gracilaria, are present at 1–5% of DW, and include omega-3 and omega-6 oils (Carpena et al., 2021; Freitas et al., 2021).

Mimicking the internal structure and texture of fish requires simulating their fibrous-gel structure. Major techniques used for structuring alternative proteins into analogous textures include hydrospinning, electrospinning, extrusion, and 3D-printing (Kazir and Livney, 2021). 3D-food-printing technologies are developing fast, enabling the creation of complex three-dimensional geometries with various topologies and tailorable compositions (Pitayachaval et al., 2018). While 3D food printing holds great potential for custom and sustainable food production, several limitations must be addressed for it to become a mainstream manufacturing technique: limitations regarding material properties ("inks" must be jettable: pseudoplastic-with low viscosity in the nozzle, high after deposition, yet with sufficient nutrient density; homogeneous-particle size much below nozzle diameter to avoid clogging; and no bubbles, which break the jet), scaling (fast, conveyor-based, printers need to be developed for high throughput, and good cost-effectiveness), and overall consumer acceptance (Lipton et al., 2015; Sun et al., 2015; Godoi et al., 2016).

Herein, we harnessed 3D-printing to form fish analogs based on macroalgae. We used the red seaweed *Gracilaria cornea,* also known as Irish moss or Ogonori (Anon, 2022), from which we co-extracted proteins and polysaccharides to achieve high nutritional quality and good printability. Due to its high global popularity, we selected salmon fillet as a model. We also utilized microalgal-astaxanthin (AX) as a salmon color pigment.

2. Materials and methods

2.1. Seaweed cultivation

The red seaweed Gracilaria cornea was cultivated in 700 L PVC tanks supplied with running seawater and aeration for several weeks using an outdoor cultivation site at Israel Oceanographic & Limnological Research (IOLR). These stocks were maintained year-round to provide seaweed biomass for the ongoing experiments with typical seawater temperatures (20–27 °C) and irradiance (100–700 μmol photons m^{-2} s^{-1}) (Tadmor Shalev et al., 2022). The stock seaweed biomass was 1.5 kg FW m^{-3} and harvesting took place after it grew to 3–4 kg FW m^{-3} usually in around 5 weeks. The seaweed was fertilized once a week with a mixture of 0.2 mM NaH_2PO_4 and 2.0 mM NH_4Cl. The cultivation protocols used in this study maintained optimal growth and physiological conditions of the seaweed before harvesting (Ashkenazi et al., 2019). The harvested biomass was cleansed in a 20 L tank filled with tap water. A batch centrifuge was used to remove surface water, and the "fresh weight" (FW) biomass of G. cornea was thus obtained.

2.2. Protein extraction protocols

Proteins and carbohydrates were co-extracted from the harvested biomass using pilot-scale processes. Four extraction protocols have been developed and tested (as detailed in the supporting information section). Table 1 describes the four protocols, and their advantages and disadvantages.

2.3. SDS-PAGE

Molecular weight distribution of the extracted proteins was analyzed using Tricine-SDS-PAGE, based on the method described by (Schagger, 2006). The protein bands were stained by Coomassie brilliant blue.

2.4. ζ-potential and particle size distribution of extracted algal proteins

Volume-weighted particle size distributions and ζ-potential

Table 1
The extraction protocols tested, and their advantages and disadvantages.

Protocol	Summary	Advantages	Disadvantages
1	Uses NaOH (1%) for alkaline treatment. Repeated homogenization and filtration steps. Involves spray drying and dialysis (2 kDa). Final product: spray dried powder.	Effective protein extraction: The use of NaOH and repeated homogenization enhances protein solubility, leading to effective protein extraction. Fine filtration: Dialysis (2 kDa) removes smaller unwanted molecules, improving purity. Spray drying: Ensures rapid drying and reduces thermal degradation of proteins. Good protein content. The protein content was estimated to be 54.5%	Low yield, insufficient for large-scale application. Optimization required. Complexity and time: Multiple filtration, dialysis, and drying steps make the protocol time-consuming and complex. Energy-intensive: The use of Ultra-Turrax® homogenizer at maximum speed for extended periods consumes significant energy and heats the solution. Costly: The requirement for a spray dryer and dialysis equipment may increase operational costs.
2	Algal biomass is homogenized and filtered. High ash content (~77%) is reduced by an additional NaOH wash. Final product: freeze-dried powder.	Simple Filtration Steps: Homogenization and filtering in water without added NaOH initially makes it simpler than Protocol 1. Ash reduction: A dedicated step to reduce the ash content ensures a cleaner extract, which facilitates carbohydrate extraction.	Ash-related issues: High initial ash content (~77%), considerable effort is required to purify the extract, which might reduce overall protein recovery. Low yield, especially after ash removal. optimization required. Additional NaOH Use: Requires a secondary NaOH treatment, increasing operational complexity.
3	Uses 0.5% NaOH solution for homogenization. Involves centrifugation, dialysis, and freeze-drying. Final product: freeze-dried green powder.	Protein enrichment: Alkaline extraction (0.5% NaOH) helps in dissolving proteins more efficiently. Dialysis: Further purification improves extract quality. The protein yield was highest among the studied extraction protocols (Table 2).	This greenish powder contained chlorophyll, as the main pigment, so additional steps were conducted to obtain a light-colored powder adjustable for any color required for the seafood analogs. Possible protein degradation: Extended exposure to NaOH, even at lower concentrations, might degrade some proteins, reducing functionality. Moderate Complexity: Multiple steps (homogenization, dialysis, and freeze-drying) make this protocol a bit more complex than the others.
4	Dry algal biomass in NaOH (0.5%) solution. Dialysis (1 kDa) and freeze-drying Final product: freeze-dried powder.	Efficient dialysis: Use of a 1 kDa membrane ensures removal of low molecular weight impurities, leading to a purer extract. Protein enrichment: Dialysis and NaOH	Extended time required: Long stirring times (up to 4 h) make the process slower than Protocols 2 and 3. Dry biomass requirement: Using dry biomass requires

(*continued on next page*)

Table 1 (*continued*)

Protocol	Summary	Advantages	Disadvantages
		treatment work effectively for protein extraction	an extra preparation step, adding complexity. Protein denaturation risk: NaOH use for long times may cause protein denaturation.

measurements were determined using a Zetasizer Nano instrument (Malvern Instruments Ltd., Worcestershire, UK). Dried extract samples were dissolved (1 mg/mL) in citrate phosphate buffer (at pH of 2.6, 4.3, 5.8 and 7.0) and stirred for 2 h at room temperature (RT) (1200 rpm). Measurements were performed in triplicates at RT.

2.5. Ash content determination

0.5 g of dry extract was oven-dried for 24 h at 100 °C and then burned at 525 °C for 3 h. Ash content was determined gravimetrically.

2.6. Pigments elimination

600 mL of ethanol were added to 8 g of Protocol-3 dry greenish algal extract, and the suspension was stirred overnight at RT and filtered. The filter cake was dissolved in 600 mL of ethanol and the suspension was stirred for 5 h at 50 °C. After cooling, the suspension was filtered and 700 mL of ethanol were added to the cake and stirred for 24 h at RT. After filtration, the cake was dissolved in 1 L of water at pH 8.5, dialyzed (1 kDa) against water and freeze-dried, yielding 3.5 g of dry ivory-colored extract.

2.7. Protein quantification by elemental analysis

For protein determination, total nitrogen in the samples was quantified using a CHNS elemental analyzer (Flash, 2000; Thermo Fisher Scientific). Acetanilide (C = 71.09%; H = 6.71%; N = 10.36%; S = 0%) was used as calibration standard and helium was the carrier gas.

2.8. Carbohydrate quantification

Total carbohydrate content in the purified samples was determined by the phenol-sulfuric acid method. 150 μL of concentrated sulfuric acid were added rapidly to 50 μL of sample or standard in a 96-well microplate, with 30 μL of 5% phenol. After 5min at 90 °C, the microplate was cooled to RT, and absorbance measured at 490 nm. Results were expressed as glucose-equivalence.

2.9. Preparation of bioinks

2.9.1. Full model

0.2 g extract (obtained using Protocol-3 after pigment removal) were suspended in 20 mL of water, stirred, and 50 mg of xanthan gum were added. Then, AX-oleoresin (Algatech Ltd.) dissolved in corn oil (600 μL of 1 mg/mL solution) was added. The mixture was homogenized (Polytron® PT 2100 Homogenizer, Kinematica) at 20000 rpm for 10 min and freeze-dried. 1 mL of water was added to the dry powder, and the mixture was blended, and heated at 90 °C for 20 min. After cooling down, 10 mg of AX-oleoresin were added, and the mixture was blended (calculated composition: 5.5% protein; 20% total solids), loaded into the 3D-printer syringe and centrifuged to remove large particles and air bubbles.

2.9.2. Two color model

2.9.2.1. Orange bioink. 0.2 g of extract (Protocol-3, 34.1% protein and 43.1% carbohydrates) and 50 mg of xanthan gum were suspended in 35 mL of water and stirred at RT for 30 min. Afterwards, 1 mL of 1 mg/mL AX-oleoresin in corn oil was added. The mixture was homogenized by Polytron at 20000 rpm for 5 min and freeze-dried. 1 mL of water was added to the dry powder, and the mixture was blended and heated at 90 °C for 20 min. After cooling, 5 mg of AX-oleoresin were added for final color adjustment, and the mixture was blended (calculated composition: 5.3% protein; 20.3% total solids) and loaded into the 3D-printer syringe.

2.9.2.2. White bioink. 0.2 g of extract (Protocol-3) and 50 mg of xanthan gum were suspended in 35 mL of water and stirred at RT for 30 min. Then, 1 mL of corn oil was added, and the mixture was homogenized by Polytron at 20000 rpm for 5 min and freeze-dried. 1.4 mL of water was added to the dry powder. The mixture was blended, heated at 90 °C for 20 min, and cooled to RT. 100 mg $CaCO_3$ powder was used as a natural white food coloring. (Calculated bioink composition: 2.5% protein; 34.5% Oil; 47.6% total solids).

2.10. 3D-printing

The models were printed using a 3D-printer (EnvisionTEC: 3D-Bioplotter Manufacturer Series), as described below.

2.10.1. Full model

The orange formulation was extruded through a 0.64 mm internal diameter (ID) nozzle at 6 mm/s and the applied pressure was 2.5 bar. The 3D-printing was done at 25 °C without any supporting frame.

2.10.2. Two color model

The formulations were extruded through a 0.64 mm ID nozzle at 6 mm/s. The applied pressures were 2.8 bar and 3 bar for the orange and white bioinks, respectively. The 3D-printing was done at 25 °C without any supporting frame.

3. Results and discussion

Herein we explored, for the first reported time, the possibility of preparing fish-fillet analog from a marine macroalga (seaweed), as the major component and protein source, and chose 3D-printing to form the desired shape, color pattern and structure. For this purpose, we co-extracted proteins and polysaccharides from the edible red seaweed genus, *Gracilaria*, known to have relatively high content of protein (depending on the species, season and growing conditions) of high nutritional quality. *Gracilaria* species are also rich in polysaccharides (predominantly agar), which we hypothesized would improve printability. Co-extraction of both major macromolecular fractions was also important for increasing efficiency of utilizing the raw material and reducing waste. To obtain salmon color we used AX-oleoresin from the green microalga *Haematococcus pluvialis*.

1 Protein and carbohydrate extraction

In a preliminary lab scale study, we co-extracted protein and carbohydrates from *Gracilaria* sp. according to a food grade protocol we had previously developed ("Protocol-5" in (Kazir et al., 2019)). Co-extraction decreases the amount of waste, and enables utilizing the functional properties of both the proteins and the carbohydrates. The dry extract contained 56% protein, and the rest mainly comprised carbohydrates. The main carbohydrates in gracilaria are agarose, a nonionic gel-forming polysaccharide, and agaropectin, an anionic polysaccharide, with carboxyl and sulphate side groups (Ranga et al.,

2022). We studied the interactions between proteins and anionic polysaccharides in the extract as a function of pH, by measuring particle size distribution and ζ potential.

Size distribution analysis of the *Gracilaria cornea* extract (Fig. 1A) revealed a bi-modal size distribution above pH ~ 5, with the smaller-diameter sub-population being the proteins and the larger-diameter sub-population being the polysaccharides. Below pH ~ 5, the two sub-populations merged into an intermediately-sized peak, indicating the formation of compact cohesive protein-polysaccharide electrostatic complexes. Accordingly, pH ≈ 5 is the pI of the extracted *Gracilaria cornea* protein mixture. At more acidic conditions, the proteins and the anionic polysaccharides (mainly agaropectin) were oppositely-charged, hence mutually-attractive, and above pH ~ 5 they were similarly-charged, hence mutually-repulsive. According to ζ-potential analysis (Fig. 1B), the *Gracilaria cornea* extract colloids exhibited a net-negative charge in the entire pH-range studied. Hence, there was much more carbohydrate-negative-charge, making the complexes negatively charged, hence soluble, even at pH = 2.5.

Molecular weight distribution of the extracted proteins was analyzed using SDS-PAGE (Fig. 2). Extracted algal proteins exhibited a wide range of molecular weights. The main bands had molecular weights of ~5 kDa, ~12 kDa, and ~18 kDa.

To produce a seafood-meat analog prototype we used, as a raw material, the red seaweed *Gracilaria cornea,* commonly used for extraction of agar, a valuable polysaccharide accounting for ~25% dw of the alga (Friedlander et al., 2001). To facilitate 3D-printability, we co-extracted the polysaccharide fraction along with the proteins, as agar confers shear-thinning and gelation properties. We designed and performed several food-applicable pilot-scale extraction protocols to obtain extract-powders. The main conditions and resulting protein yield and concentration factor of each protocol are described in Table 2.

To determine the protein content, the total nitrogen in each extract-sample was quantified using a CHNS elemental analyzer. The conversion factor found to provide an accurate quantification of the protein content for red algae was 4.59 (Lourenco et al., 2002). Using this conversion factor, the protein content in the extract of Protocol-1, was estimated to be 54.5% (Fig. 3). To determine the total carbohydrate content in the algal extract powder, the phenol-sulfuric acid method was applied. Protocol-1 extract contained 17.2% carbohydrate, and 4.9% ash (Fig. 3).

In contrast to Protocol-1, in Protocol-2 water was used as a medium for protein extraction from the wet algal biomass. A dry pink powder

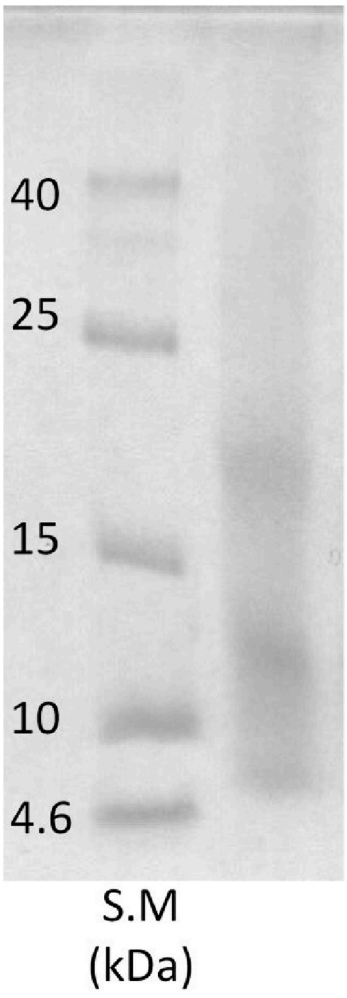

Fig. 2. SDS-PAGE analysis of gracilaria extract powder. S.M. represents size marker, in kDa.

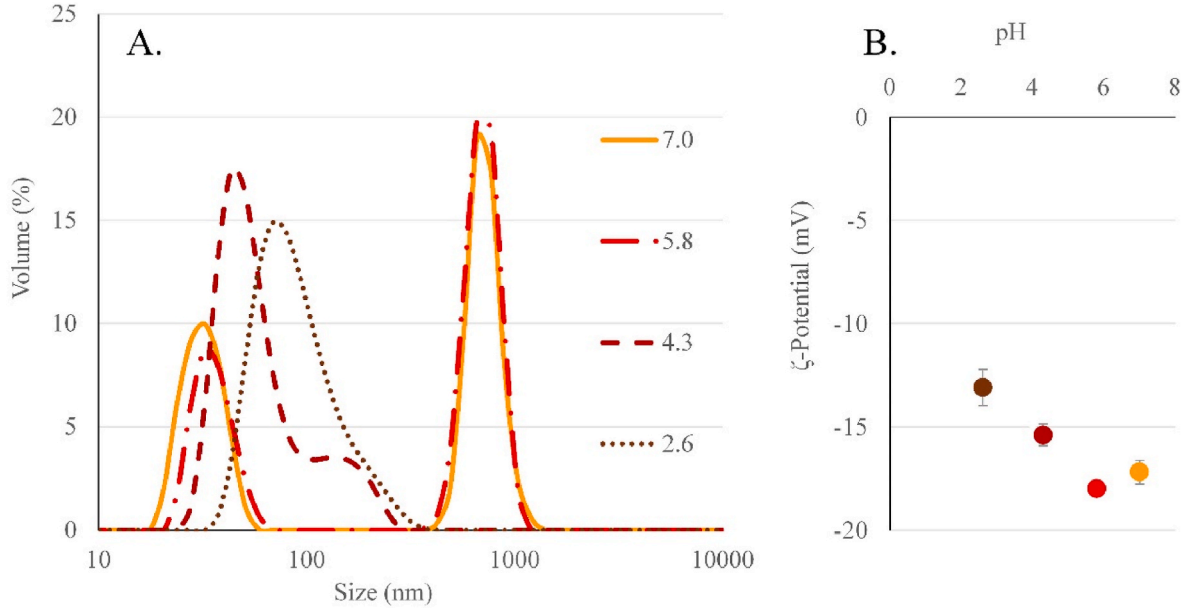

Fig. 1. (A.) Particle size distribution, and (B.) ζ-potential analysis of Gracilaria cornea extract.

Table 2
The main conditions and results of each extraction protocol.

Protocol	Algal matter	Algal mass to medium w/V	Extraction medium	Homogenization duration (min)	Base neutralization	Protein extraction yield	Protein concentration factor[a]
1	Wet	1:7	1% NaOH	11	HCl	6.6%	3.71
2	Wet	1:1	Water	20	–	7.4%	4.35
3	Wet	1:1	0.5% NaOH	15	–	13.1%	3.22
4	Dry	1:7	0.5% NaOH	10	HCl	5.7%	1.51

[a] (%protein in extract/%protein in algae) (dw basis).

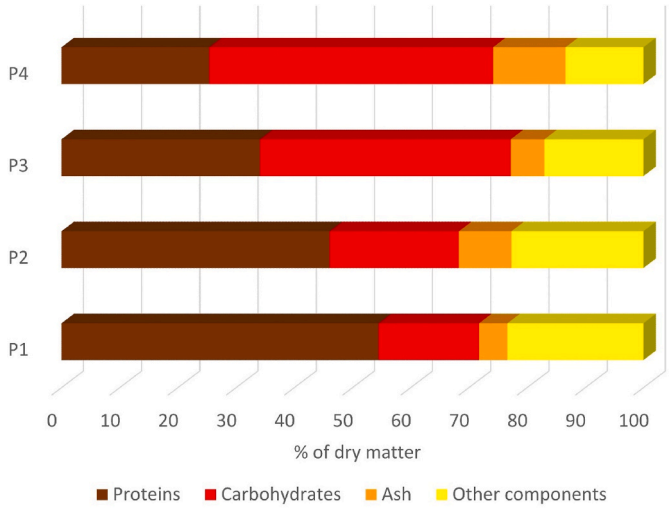

Fig. 3. Composition of the extracts obtained by the four different protocols. Protocol-2 results are after ash removal. Protocol-3 results are after pigments removal.

extract was obtained (Fig. S1 AB.), and its protein, carbohydrate and ash contents were determined. As ash content was very high (77%), steps were taken to remove the extra salts and the liquid was freeze-dried, yielding lilac-colored algal powder (Fig. S1 B.) with only 9% ash.

Protocol-3 mostly resembled Protocol-2 (Tables 1 and 2), except for the extraction medium, which was 0.5% aqueous sodium hydroxide. This protocol yielded 18 g of green powder (Fig. S1 AC.), comprising 40.6% protein and 25.9% carbohydrate. This greenish powder apparently contained chlorophyll, as the main pigment. Thus, additional steps were conducted to obtain a light-colored powder that is readily adjustable for any color required for the printable seafood analogs. Figure S1 C demonstrates the steps performed to remove the pigments, using ethanol. The content of protein and other components are shown in Fig. 3. After pigments removal, the protein content decreased to 34.1%, probably due to some loss during the washing steps.

Protocol-4 conditions resembled those of Protocol-1, except for using dry algal biomass as the starting material. A light-brown powder was obtained (Fig. S1 A.), and its composition is shown in Fig. 3. It comprised only 25.4% protein, the lowest of all tested protocols, indicating that extracting fresh biomass was advantageous over extracting dry biomass.

As expected, the results presented in Fig. 3 demonstrate that the protein extraction procedure significantly affected the composition of the extract. The protein content of the powders varied between 25% and 54% of the dry matter, while the carbohydrate percentage varied between 17% and 49%. The remaining non-protein, non-carbohydrate and non-mineral fractions in all extracts most probably comprised phenolic compounds, and other phytochemicals, including pigments, which might have been bound to proteins or to polysaccharides. Further optimization of the extraction protocol would be required for future production.

2. Three-dimensional printing of algal protein-based fish analogs

To produce seafood analogs, such as a salmon fillet, we applied 3D-printing of an algal protein-rich extract, partly harnessing the pseudoplastic properties and gelation ability of the natural agar component of the extract. The most common 3D-printing technique is based on syringe injection, wherein a formulated protein "bioink" solution is extruded through a moving syringe nozzle, and deposited on a surface, layer by layer, based on a computerized 3D-model. A digital 3D-model of the exterior appearance of a salmon fillet was purchased from 'Turbosquid.com'. The model was further developed, using DS SolidWorks® software, adding internal layers matching the exterior (Fig. 4a). This initial model was 88 mm × 33 mm x19 mm, but it may be enlarged to a real salmon fillet size. The products were 3D-printed using either one- (orange only), or two bio-inks (white and orange), differentiating between the white and the orange tissues of a salmon flesh. The training prototype was printed using pluronic bioink solution dyed with either red or white (TiO_2) food-coloring (Fig. 4b).

The bioink formulation must have good "printability", i.e. physicochemical properties enabling fast and effective shape formation and retention. These encompass high homogeneity, assuring constant flowability, preventing undesired clogging and line-discontinuities. Moreover, while flowing through the syringe nozzle, viscosity must be low, enabling high flow-rate at low pressure gradient. Then, a sharp rise in viscosity is required, upon exiting the nozzle and deposition at the desired place in the printed structure, to retain the shape. Also, adhesion to the previous layer, and further rigidification of the structure post-deposition, e.g. by gelation, are important. These functionalities (shear thinning, adhesion and gelation) are predominantly contributed by the polysaccharides, mainly the agar. We added some xanthan gum to enhance the pseudoplastic behavior. Fish-meat analogs are eaten raw, or after cooking. Therefore, ideally, a 'cookable' printed 3D-model should be durable through cooking processes. As our first attempt to print seafood-meat analog prototypes from extracted algal components, we chose to imitate the raw fish-meat analogs. Thus, we co-extracted the algal carbohydrates with the proteins, to utilize their functional properties in the formation and texturization of the 3D-printed salmon fillet. A different composition would be required for cookable-products.

First, we used the highest protein-content extract (Protocol-1) to formulate a bioink (Fig. S2). Since initially shape retention and gelation were insufficient, we added pure agar. We later added xanthan gum to further enhance shear-thinning, and AX-oleoresin in corn-oil as the salmon pigment. The formulation components were suspended in water and homogenized at high shear for a few min and freeze-dried. Drying is important for long-term storage, and enables reconstitution in water at a higher concentration. The dry orange-brown powder was reconstituted in water, heated at 90 °C for 15 min to unfold the proteins and dissolve the agar, then cooled down. We adjusted the printing parameters for this first bioink-formulation. The main controllable printing-process parameters include printing pressure, temperature, nozzle diameter, lateral speed, and lateral/vertical distances between adjacent lines. Various conditions were tested, but the formulation was too sticky and viscous, and the desired shape was unprintable.

We then evaluated printability of Protocol-3 extract (higher carbohydrate content). The extract was suspended in water and heated. AX-oleoresin in corn oil was added. Then, the bioink was cooled and stored until printing (Fig. S3A). The printing conditions are listed in

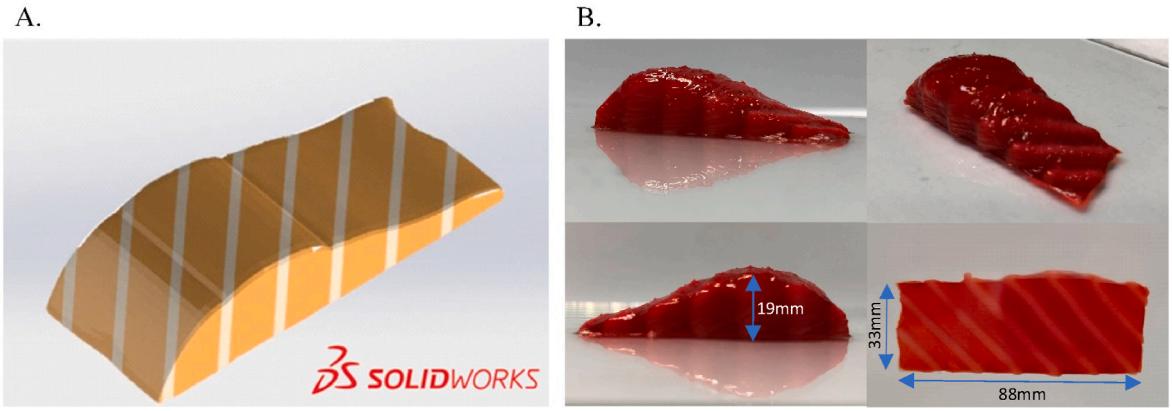

Fig. 4. A. The 3D digital-model used for printing the salmon fillet prototype. B. The training pluronic-acid based 2-color 3D-print, printed with the EnvisionTEC: 3D-Bioplotter.

Figure S3b. At 10 mm/s and 2 bar we managed to obtain a 3D-structure with 5 layers of mostly continuous lines. We then had to stop, as strands started tearing, apparently due to insufficient bioink elasticity. Thus, we modified the aqueous solution compositions. We prepared two other formulations in phosphate buffer: The first (Fig. S4A) comprised the Protocol-3 extract in pH 7 phosphate buffer. The second, contained also 0.5% of xanthan gum (Fig. S5A). However, printing of both bioinks under various pressures and printing speeds (Fig. S4B and S5B) did not yield the desired 3D-pattern. The first formulation was torn easily, and the second was very sticky with low printability. Therefore, we prepared another formulation by suspending 0.2 g of Protocol-3 extract (after pigment removal) in 20 mL of water. The suspension was stirred, and 50 mg of xanthan gum was added. Then, AX-oleoresin in corn oil emulsion was added (600 μL of 1 mg/mL solution). The mixture was homogenized for 10 min and freeze-dried. One mL of water was added to the powder, and the mixture was blended, and heated (20 min at 90 °C). After cooling to RT, the color obtained was not red enough, so 10 more mg of AX-oleoresin were added, and the mixture was blended, centrifuged to remove insoluble particles and air bubbles, and transferred to the 3D-printer syringe. Various printing parameters were tested, and the best parameters chosen are listed in Fig. 5A caption. While applying these parameters we succeeded in printing the complete 3D-prototype of a salmon fillet (Fig. 5A). The printed shape was stable for a long time and did not collapse as previous prints did.

We then printed a two-color 3D-model of a "smoked" salmon slice, using white and orange bioinks, representing the different tissues of salmon flesh (Fig. 5B). The orange bioink was prepared according to the monochromatic bioink formulation. The white bioink was prepared similarly, except for adding oil to form a white emulsion, and calcium carbonate as the white colorant. The parameters applied to print the orange and the white fragments are described in Fig. 5B caption. Obviously, optimization would be required for more realistic products, and future stages in this research will focus on developing and characterizing algal fish-fillet analogs simulating texture and other functional (e.g. cookability) and sensory properties of the real products.

4. Conclusions

We reported here the formation of fish-fillet analogs made of macroalgal extract as the main component and protein source. We used the red seaweed *Gracilaria cornea* as the raw material, and harnessed 3D-printing to form algae-based fish-fillet prototypes. Towards sustainability and waste minimization, we co-extracted proteins and carbohydrates, for both the high nutritional value of algal proteins, and the functional properties (shear thinning and gelation) of the agar. Four different extraction protocols were tested, and additional processes were developed to remove undesired pigments and ash. Microalgal AX was used as the salmon red-dye, and $CaCO_3$ as the white-dye. We formed a "salmon fillet" analog prototype, and a 2-colored "smoked" salmon-slice analog. This study highlights the potential of macroalgae as a natural and exciting raw material for fish analogs, towards sustainable seafood production and decreasing harm to ocean fisheries.

CRediT authorship contribution statement

Samaa Alasibi: Investigation, Validation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Meital Kazir:** Investigation, Validation, Formal analysis, Software, Visualization, Writing – review & editing. **Álvaro Israel:** Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. **Yoav D. Livney:** Conceptualization, Funding acquisition, Methodology, Resources, Data curation, Visualization, Writing – review & editing, Supervision, Project administration.

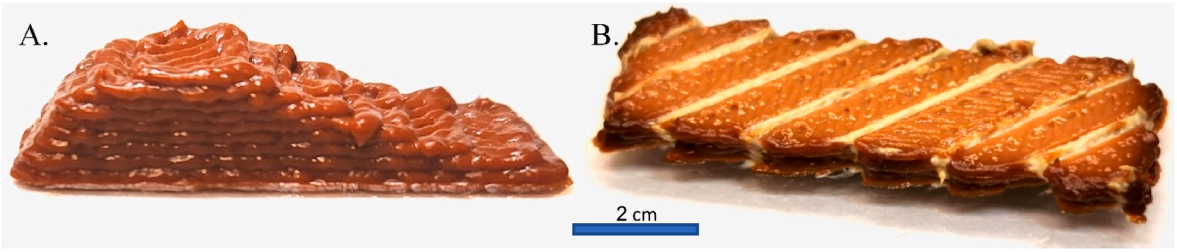

Fig. 5. A. 3D-printed monochromatic salmon-fillet prototype (Printing conditions: Needle = 0.64 mm, speed = 6 mm/s, Pressure = 2.5 bar; Temperature = 25 °C). B. 3D-printed two-color prototype of a "smoked" salmon slice. (Printing conditions: Both bioinks: Needle = 0.64 mm, speed = 6 mm/s, Temperature = 25 °C. Orange bioink: Pressure = 2.8 bar; White bioink: pressure = 3.0 bar).

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100905.

Data availability

Data will be made available on request.

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