UNIVERSITY OF GOTHENBURG

NOVEL CULTIVATION METHOD INCREASES CRUDE PROTEIN CONTENT AND IMPACTS SENSORY ATTRIBUTES OF RED SEAWEED *PALMARIA PALMATA*

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Abstract

The red seaweed *Palmaria palmata* could be a sustainable protein source with a low environmental footprint to feed a growing global population. However, its protein content should ideally be optimised to be on par with other protein sources and its sensory attributes as a food ingredient must appeal to consumers. This study aims to enhance the crude protein content of *P. palmata* by using herring production process water (HPPW) in the cultivation as nutrient enrichment. Moreover, the study establishes a sensory profile of cultivated *P. palmata* and investigates if HPPW changes its sensory attributes. It does so through two cultivation experiments and one sensory evaluation of the cultivated biomass. In the first experiment, a suitable dilution level of HPPW was established. This knowledge was used when scaling up in the second experiment where growth, crude protein content and chlorophyll *a* were compared between cultivation in HPPW and seawater controls. Biomass from the second experiment was then tested by a selected and trained panel in a sensory evaluation, using quantitative descriptive analysis (QDA). The results show that cultivation in HPPW significantly increased the crude protein content, chlorophyll *a* and growth of *P. palmata*. Additionally, the findings suggests that constant supply of nutrients, e.g. by having flow-through of water, is of great importance when growing *P. palmata* in land-based tanks. The sensory panel established that cultivated *P. palmata* has a strong taste of umami, salt and fish, a prominent ocean scent and a firm, sticky and chewy texture. These attributes were shared between the two evaluated treatments. However, the HPPW changed the appearance of *P. palmata* as colour, structure and glossiness significantly differed from seawater control. This study showcases that HPPW can have a positive impact on the nutritional characteristics and change the sensory attributes of *P. palmata*, establishing this red seaweed's role as an important sustainable protein source that contributes to circular food production.

1. Introduction

The way we produce food today puts substantial pressure on the environment. Food production requires vast amounts of freshwater, takes up almost half of all arable land, causes nitrogen pollution through fertilisation and contributes to over 25% of greenhouse gas emissions (Poore & Nemecek, 2018; Gephart et al., 2021). Simultaneously, the demand for nutrient-rich food is increasing with a growing, wealthier global population that is shifting to more resource-demanding dietary habits (Hertel, 2015). This calls for alternative food sources with a low environmental impact and high nutritional value, including high-quality proteins containing all essential amino acids (EAA).

Cultivating seaweeds is one promising solution as seaweeds grows fast without requiring freshwater, arable land or fertilisation. Not only are seaweeds resource efficient, but they also take up nutrients such as nitrogen and phosphorus, and greenhouse gases such as carbon dioxide. Seaweed cultivation could therefore contribute to mitigate eutrophication and lower greenhouse gas emissions. (Gephart et al., 2021)

The nutritional characteristics of seaweeds further strengthen its potential as a sustainable food source. Seaweeds are divided into three larger groups: brown (Phaeophyta), green (Clorophyta) and red (Rhodophyta). Natural variability in chemical composition exists between and within seaweed species with factors like seasonality and geographic area adding to the variance (Blikra et al., 2021). The protein content and quality are generally higher for red and green seaweeds than brown seaweeds (Mæhre et al., 2014). Moreover, the red and green seaweeds have up to 100 times lower levels of iodine and heavy metals than brown seaweeds; the risk of exceeding the recommended intake for humans is thus much lower for these species (Aakre et al., 2021). Although the lipid levels of seaweeds are low, the red seaweeds have been reported to have the most beneficial fatty acid composition (Mæhre et al., 2014).

Palmaria palmata, or dulse, is a red seaweed species found in sub-tidal areas in the North Atlantic with great potential for human consumption thanks to its nutritional properties and sensory attributes (Jönsson et al., 2023; Mæhre et al., 2014; Stévant et al., 2023). The protein content of *P. palmata* has been reported to range from 7-19 % dw (Stévant et al., 2023). Its composition of EAA is higher than many terrestrial counterparts with high levels of lysine as well as methionine which is the main limiting EAA in soy (Jönsson et al., 2023; Mæhre et al., 2014). *P. palmata* also has a beneficial composition of fatty acids, including long-chain omega-3 fatty acids (Mæhre et al., 2014). As for minerals, *P.palmata* is for example high in selenium and considerably lower in iodine and heavy metals than e.g. brown seaweeds (Mæhre et al., 2014; Jönsson et al., 2023).

However, there are still barriers in supply and demand to overcome for seaweed in general to succeed as a food resource in Europe. The European seaweed industry produced just over 20 thousand tonnes of seaweed in 2020 which is only 0.01% of the global market (FAO, 2022). The seaweed market destined for human consumption in Europe is also limited with seaweeds being an novel food in most European consumers' diets although it has historically been an important nutrient source (Blikra et al., 2021; Mendes et al., 2022). There is an growing openness among European consumers to incorporate seaweed into their diet with factors like taste and familiarity increasing consumer acceptance (Wendin & Undeland, 2020; Embling et al., 2022; Mellor et al., 2022). High sensory quality is another crucial aspect for a novel food

resource to win consumers' liking and lower scepticism towards new food (Lawless & Heymann, 2010; Blikra et al., 2021). *P. palmata* could help seaweeds receive public acceptance as it is considered specifically palatable with a sensory profile described as umami-rich with an intense scent of shellfish and a chewy texture (Jönsson et al., 2023).

As *P. palmata* and other seaweed species operate in a competitive food market their protein levels should ideally be increased to be comparable to those of well-established protein sources like soy (Mæhre et al., 2014). The protein content of seaweed can be enhanced by increasing the nitrogen availability for the seaweed, as nitrogen is an essential component of proteins (Roleda & Hurd, 2019). This can be utilised by growing seaweed growing in excretions from marine animals containing high concentrations of ammonium, NH_4^+, which generally is low in natural seawater (Roleda & Hurd, 2019). Grote (2016) assessed *P. palmata*'s potential as a biofilter in an integrated multi-trophic aquaculture system (IMTA) and found that the seaweed's protein content was significantly higher when cultivated in the ammonium-rich water from the fish farm. Another possibility is to grow seaweed in nutrient rich process water originating from food production, so-called side streams from different steps of food processing. Adding herring production process water (HPPW) when cultivating the green seaweed *Ulva fenestrata* resulted in over four times higher crude protein content than when grown in seawater control (Stedt et al., 2022). Utilising these excess nutrients from food production reduces effluents while allowing industries to diversify, cut costs and add circularity to the business model as an otherwise discarded by-product is turned into valuable protein-rich biomass in the form of seaweeds (Corey et al., 2013).

P. palmata's sensory attributes and the intensity of these attributes could change depending on how it is cultivated. *P. palmata* is mainly commercially cultivated in land-based tanks where environmental factors can be controlled (Schmedes & Nielsen, 2020), but previous studies examining the sensory characteristics of *P. palmata* have done so on wild-harvested biomass (Jönsson et al., 2023; Stévant et al., 2023). Adding nutrient-rich water when cultivating *P. palmata* could also impact the sensory profile. As an example, HPPW-treated *U. fenestrata* shared most of the sensory properties with control but differed significantly in colour, odour intensity and texture adhesiveness (Stedt et al., 2022).

The potential of HPPW as an additive cultivation media for *P. palmata* in a land-based cultivation setting has been explored in this study. Firstly, suitable dilution levels of HPPW, ranging from 0-320 µM NH_4^+, were investigated. The cultivation of *P. palmata* in HPPW was thereafter upscaled in a second experiment. The hypothesis for the cultivation experiments was that HPPW increases the growth, crude protein content and chlorophyll *a* of *P. palmata*. The cultivated biomass from the second cultivation experiment was used to establishing a sensory profile for land-based cultivated *P. palmata*. Through quantitative descriptive analysis (QDA), a selected and trained panel described, evaluated and quantified the sensory attributes of *P. palmata* under controlled conditions (Lawless & Heymann, 2010). The panel also established if and how HPPW impacts the appearance, odour, flavour and texture of *P. palmata*.

By performing manipulative experiments and sensory analysis, the aim of this thesis was to: (1) Investigate novel cultivation methods for increasing the crude protein content of *P. palmata* and (2) provide and compare sensory profiles of P. palmata from the different cultivation methods. The overarching aim is to contribute to the development of novel

sustainable protein sources through circular systems where otherwise discarded nutrients from side streams are valorised through seaweed cultivation.

2. Methodology

2.1 Herring production process water

HPPW was collected in a food-graded state (Sweden Pelagic AB, Ellös, Sweden) where whole herring is stored in indoor tubs (TUB) after landing, containing a 3% salt solution. The HPPW used in the cultivation experiments was collected at two different time points and filtered, 320 µM, to remove coarse particles. After collection and filtration, the HPPW was stored in a freezer at -60°C and thawed before use. NH_4^+ is the predominant nitrogen source of the HPPW (Table 1). The two different HPPW will hereafter be referred to as TUB1 and TUB2.

Table 1. Content of undiluted process water for TUB1 (collected 24/10-2022) and TUB2 (collected 23/1-2024). Dilution factor with seawater to get 40 µM NH_4^+, used in experiment 2, is provided.

Type	pH	Ammonium, NH_4^+ (µM)	Inorganic phosphorus, P (µM)	Dilution factor with SW to get 40 µM NH_4^+
TUB1	7.9	2067 ± 222	8745 ± 142	51
TUB2	8.2	2443 ± 281	Not analysed	59

2.2 Cultivation of *Palmaria palmata*

2.2.1 Material

The experiments used vegetative propagation of *P. palmata* collected from an indoor tank at Tjärnö Marine Laboratory (58°52′33.7″ N, 11° 08′44.9″ E). Between the two experiments, the seaweed was cultivated in half-strength Provasoli-enriched seawater PES (ratio 1:100) diluted with filtered (0.2 µM filter + UV-light application, salinity >30 PSU, 5°C) deep sea water (40 m) at 16:8h (L:D) light regime at an irradiance of 60 µmol m^{-2} s^{-1}. The addition of PES was stopped two weeks before the start of the second experiment.

2.2.2 Experiment 1

Nine treatments were compared in the first experiment: one seawater control and eight concentration levels of TUB1 diluted in seawater following exponentially increased levels of NH_4^+ (2.5, 5, 10, 20, 40, 80, 160 and 320 µM NH_4^+). Four replicates of each treatment were set up in tanks, in randomised order to avoid position bias, with 500 ml filtered (0.2 µM filter + UV-light application, salinity >30 PSU) deep sea water (40 m) and continuous aeration. Each tank included one blade of *P. palmata* (2 g l^{-1}) at the start of the experiment. Refill of process water and water exchange took place every third day. The experiment was set up in a temperature-controlled room at 11°C, 12:12h light regime with a light irradiance of 50 µmol m^{-2} s^{-1}. The experiment started on November 14th 2023 and ran for 14 days.

2.2.3 Experiment 2

The second experiment consisted of three treatments. Two treatments were set up in eight 25 l tanks, four replicates of each, with continuous aeration in a temperature-controlled room at 10°C, 16:8h light regime with a light irradiance of 40 µmol m^{-2} s^{-1}. The control treatment, received seawater only and the TUB2-treatment, received TUB2 at the concentration level of 40 uM NH_4^+ diluted in seawater (based on the results from experiment 1). Filtered (0.2 µM filter + UV-light application, salinity >30 PSU, water temperature 10°C) deep seawater (40

m) was used for the cultivation. Each tank was stocked with approximately 75 grams of biomass at the start of the experiment (3 g l^{-1}). Water was exchanged daily in all tanks to avoid nutrient depletion, while TUB2 was refilled every third day for TUB2-treatment. All the tanks were reshuffled in placement at every water exchange and cleaned when necessary. Additionally, a third treatment (flow-through control) was included where *P. palmata* was harvested from a greenhouse stock cultivation (90 l) at the end of the experiment (6 g l^{-1}). The flow-through control had a flow-through of filtered (0.2 µM filter + UV-light application, salinity >30 PSU, temperature 5°C) deep seawater (40 m) with continuous aeration and a light irradiance of 50 µmol m^{-2} s^{-1} at a 16:8 h light regime; a set-up designed to mimic cultivation in a commercial setting. The experiment ran for 13 days between the 8-21 March 2024.

2.2.4 Growth

Wet weight (ww) was used to determine the specific growth rates (SGR) in experiments 1 and 2. For experiment 1, all nine treatments were weighed on the first and last day of the experiment. For experiment 2, control and TUB2-treatment were weighed on the first and last day of the experiment. A lab-scale (Mettler PC440) was used for weighing after excess water had been removed with a salad spinner in a standardised way.

For experiment 2, photo scanning was used to determine the SGR of seaweed area for control and TUB2-treatment. Ten random individuals from each tank were hole-punched for identification and photographed at the start and end of the experiment. Photos were taken with a Canon 1100D (1/25, F22, ISO400) at a light table where the seaweed was placed under a glass slide to stay flat. The combined surface area of the individuals from their respective tanks was calculated. Four individuals from different tanks were unidentifiable at the end; the individuals were removed completely from the calculation. The free software program ImageJ was used to analyse the surface area.

SGR of ww and area was calculated according to the formula:

$$SGR = ((Ln(At) - Ln(A0)) / t) * 100$$

where At is the ww/area after t days of the experiment and A0 is the ww/area at the beginning of the experiment.

2.2.5 Total nitrogen and crude protein content

For experiment 1, randomised tissue samples were collected at the end of the experiment to estimate the nitrogen content between all of the treatments. For experiment 2, randomised tissue samples were collected at the start and end of the experiment to estimate the nitrogen content for all three treatments and to see if it increased from the start of the experiment. The tissue samples were frozen at -80 degrees, freeze-dried, turned into a fine powder using a mortar and put into aluminium tin capsules. The total nitrogen content was determined by combustion using an GSL elemental analyser coupled to an Isotope Ratio MS (EA- IRMS, 20 - 22, Sercon Ltd., Crewe UK) in experiment 1 and an organic elemental analyser coupled to a Delta V Advantage Isotope Ratio MS (Flash 2000, Thermo Scientific) in experiment 2. Based on the nitrogen content, the crude protein content was estimated using the conversion factor 5, a commonly used nitrogen-to-protein factor for seaweed (Angell et al., 2016).

2.2.6 Chlorophyll a

Randomised tissue samples from all three treatments in experiment 2 were collected for comparison at the end of the experiment to compare chlorophyll *a* (Chl-*a*) content between

the three treatments. The tissue samples were freeze-dried and ground into a fine powder. 20 mg of powder was extracted in a 90% acetone solution. The liquid extraction was placed in an ultrasonic bath for 10 minutes followed by 50 minutes on a shaking table. During this part, the samples were kept dark using aluminium foil. The samples were then put in 10 minutes of centrifugation at 4000 rpm. The resulting supernatant was transferred to cuvettes and using a spectrophotometer (Lambda XLS+) the absorbance was measured at the wavelengths 664 and 647 nm. The Chl-*a* content was estimated using the formula:

$$\text{Chl-}a = 11.93E_{664} - 1.93E_{647}$$

which is a formula specific to the spectrophotometric equation for seaweed extracted in 90% acetone solution.

2.3 Sensory analysis of *Palmaria palmata*

2.3.1 Material

P. palmata for sensory testing came from experiment 2, section 2.2.3. *P. palmata* from flow-through control was compared to TUB2-treatment. Biomass from the control treatment was excluded in the evaluation due to poor status at harvest time, resulting in no biomass from this treatment to run sensory analysis on. After harvest, the *P. palmata* was flash-frozen and stored in a -80°C freezer. It was milled while frozen to a paste using a meat grinder, 4.5-millimetre particle size. During the two weeks of sensory testing, the *P. palmata* was stored at -20°C and thawed one hour before serving. The serving samples were handled in a standardised way and served at room temperature during the training sessions and final evaluation. Each sample consisted of approximately 1 g of *P. palmata*, an amount based on the limited total amount of harvested biomass.

2.3.2 Experimental setup

Quantitative description analysis (QDA) was performed for the sensory evaluation and followed the ISO 8586 standard recommendations, developed to enhance the precision of measurement (Gustafsson, 2014). Panellists were selected through screening where their ability to distinguish odours, identify basic tastes and describe a food product's sensory characteristics was tested. Colour blindness was tested via the Ishihara test. The emphasis was on the participants' potential rather than performance in the selection process, as they improve through training (Lawless & Heymann, 2010). All of the selected panellists scored high in the test for odour and basic tests, showed a strong ability to put words on what they were sensing and had perfect colour vision. The application to perform this sensory evaluation was approved by the Swedish Ethics Review Act. All panellists received information about the sensory analysis, gave consent to participate and were free to discontinue their participation at any time.

The panel was trained to evaluate the appearance (A), odour (O), flavour/taste (F) and texture (T) of cultivated *P. palmata*, see section 2.3.1. Training of the panel (ISO 8586) took place during four sessions of 1.5 hours each. In the first session, the panellists developed the sensory attributes to be measured for *P. palmata*. This was done individually where they were asked to brainstorm words for each of the senses followed by a group discussion, guided by a panel leader, to reach a consensus on which attributes to use for evaluation (Lawless & Heymann, 2010; Meilgaard et al., 2016). The final attributes are found in Table 2. In the three following training sessions, the panel was calibrated to form a mutual understanding of how to interpret the chosen attributes, use the scale and perform the evaluation (Lawless & Heymann, 2010; Meilgaard et al., 2016).

An unstructured linear intensity 15 cm scale was used to determine the degree of intensity of an attribute (Meilgaard et al., 2016). The response was measured from the left endpoint to the mark made by the respondent. The scales used were either unipolar or bipolar depending on the attribute. The unipolar scales were assessed from not distinguishable to highly distinguishable whereas the bipolar scales combined two contrasting unipolar attributes decided by the panel.

The panel consisted of ten trained panellists in the final evaluation; seven females and three males aged between 20 and 51. The panel evaluated six samples, three replicates of the two treatments, to ensure reliability. The samples were served simultaneously in transparent plastic cups that were blind-labelled with three-digit codes. The participants used the software Fizz Collect (Version 2.70) for their assessments and evaluated one sample at a time with the serving order following a latin square design. The samples were evaluated in a white light setting. The evaluation area was designed in line with ISO standard to limit the influence between panel participants with a sensory booth for each panellist. The panellists were served water and neutral crackers as palette cleansers between the servings.

Table 2. Attributes, definition, reference and scale used in the sensory evaluation decided in consensus by the panel. The attributes have been freely translated from Swedish to English by the author of this paper.

Attribute	Definition	Reference	Scale (0-15 cm)
Appearance (A)			
A.Colour	Amount of red-purple colour	Red-brown (NCS 5540-Y90R) to red-purple (NCS 6030-R20B)	Red-brown to red-purple
A.Structure	Particle size	Prune puree to tapenade	Smooth to coarse
A.Gloss	Surface gloss	Uncooked lasagne sheet to black currant jelly	Matte to blank
Odour (O)			
O.Berries	Smell of red/blue berries	Dried cranberries	Not distinguishable to highly distinguishable
O.Ocean	Smell of ocean	Dried shellfish and seaweed	
O.Earthiness	Smell of moulded leaves	Moulded leaves	
O.Freshness	Smell of sliced cucumber	Cucumber	No freshness to high freshness
Flavour (F)			
F.Umami	Intensity of basic taste		Not distinguishable to highly distinguishable
F.Salt			
F.Sweet			
F.Sour			
F.Bitter			
F.Flower	Taste of fresh flowers	Elderflower juice, flowery tea	
F.Pepper	Intensity of pepperiness with a tingling sensation	Black pepper	
F.Fish	Taste of fresh fish and shellfish	Fish liver oil	
F.Grass	Taste of grass	Newly cut grass, timothy	
F.Intensity	Total intensity of flavours		Low to high intensity
Texture (T)			
T.Hardness	Hard and firm sensation	Soft bread to carrot	Soft to hard
T.Chewiness	Chewing resistance, elasticity	Pulp to peel of Granny Smith apple	Easy to chew to difficult to chew
T.Adhesiveness	Sticks to mouth and teeth	Canned corn	No adhesiveness to high adhesiveness

2.4 Statistical analysis

The statistical analysis was done in RStudios (Version 2023.12.1+402) except for the ANOVA's where SPSS (Version 29.0.2.0) was used. All data for the two cultivation experiments was visually checked with diagnostic plots for normality and homogeneity (density-, normality- and Q-Q plots) followed by the Shapiro-Wilks test. A Michaelis Menten model was used to fit the data from experiment 1 to estimate optimal concentration levels for SGR in ww and crude protein content. One-way ANOVA (p-value < 0.05) was used to analyse the difference in growth, crude protein content and Chl-*a* between treatments followed by Tukey's post hoc test for pairwise comparison.

Descriptive statistics were performed for all data from the sensory evaluation. A three-way ANOVA (p-value <0.05) was used to analyse how the sensory attributes differ between flow-through control and TUB2-treatment, with treatment and panellists as fixed factors and replicates as random factors. A principal component analysis (PCA) showing the clustering of scores based on panellist and treatment was done to assess panel performance and the commonalities between the treatments.

3. Results

3.1 Cultivation of *Palmaria palmata*

3.1.1 Experiment 1

The SGR in ww for the TUB1-treated *P. palmata* ranged from 0.2±0.3 % d^{-1} to 2.4±0.9 % d^{-1} compared to 0.1±0.2 % d^{-1} for control (mean ± sd, Figure 1A). The one-way ANOVA (Table 3) with the following pairwise comparison shows that the difference in SGR ww between control and the TUB1-treatments 10, 40, 80, and 320 µM NH_4^+ was statistically significant (<0.05).

P. palmata receiving TUB1 has a crude protein content ranging from 17.5±1.4 % dw to 24.5±3.2% dw, whereas control has a crude protein content of 13±0.7 % dw (mean ± sd, Figure 1B). This difference in crude protein content was statistically significant (<0.001) between the TUB1-treatments over 10 µM NH_4^+ and control, according to the one-way ANOVA and pairwise comparison (Table 3).

The fitted Michaelis Menten model curve for both SGR ww and crude protein content indicates that saturation occurs at a dilution level around 10 µM NH_4^+ (Figure 2A and 2B).

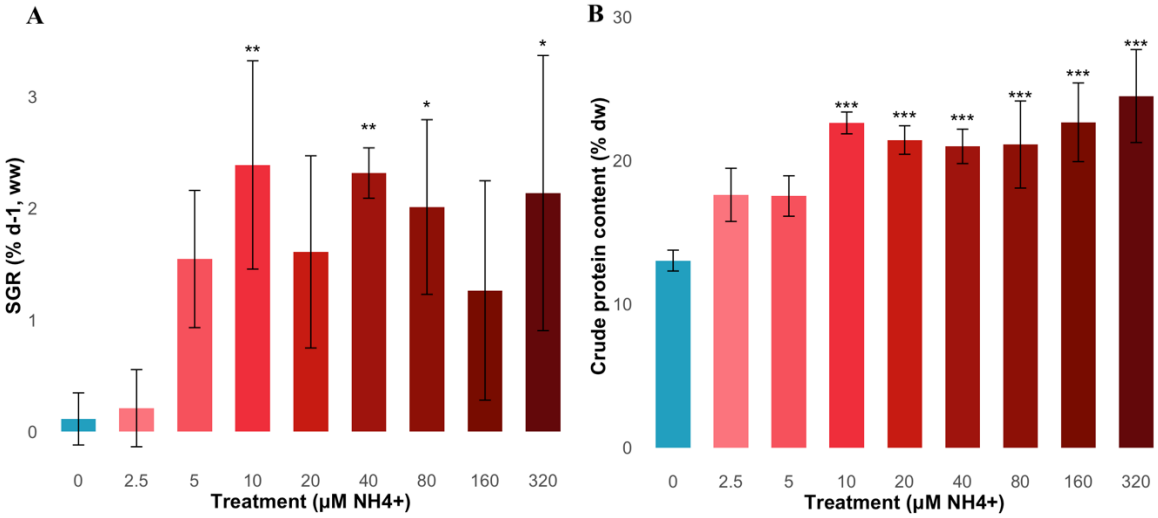

Figure 1. (A) SGR in ww (% d^{-1}) and (B) crude protein content (% dw) of *P. palmata* cultivated in control, 0, and eight different dilution levels of herring production process water (TUB1). Error bar indicates standard deviation of replicates. Asterisks indicates the eight treatments' significant difference to control based on the result from Tukey's post hoc test (***=<0.001, **=<0.01, *=<0.05).

Table 3. One-way ANOVA results of SGR (% d^{-1}, ww) and crude protein content (% dw) of *P. palmata* cultivated in control and eight different dilutions levels of herring production process water (TUB1). Asterisks indicates significant difference (***=<0.001, **=<0.01, *=<0.05).

Variable	Source of variation	df	MS	F-value	P-value
SGR (% d^{-1}, ww)	Treatment	8	2.8894	4.896	<0.001***
	Residual	27	0.591		
Crude protein content (% dw)	Treatment	8	49.30	12.27	<0.001***
	Residual	27	4.02		

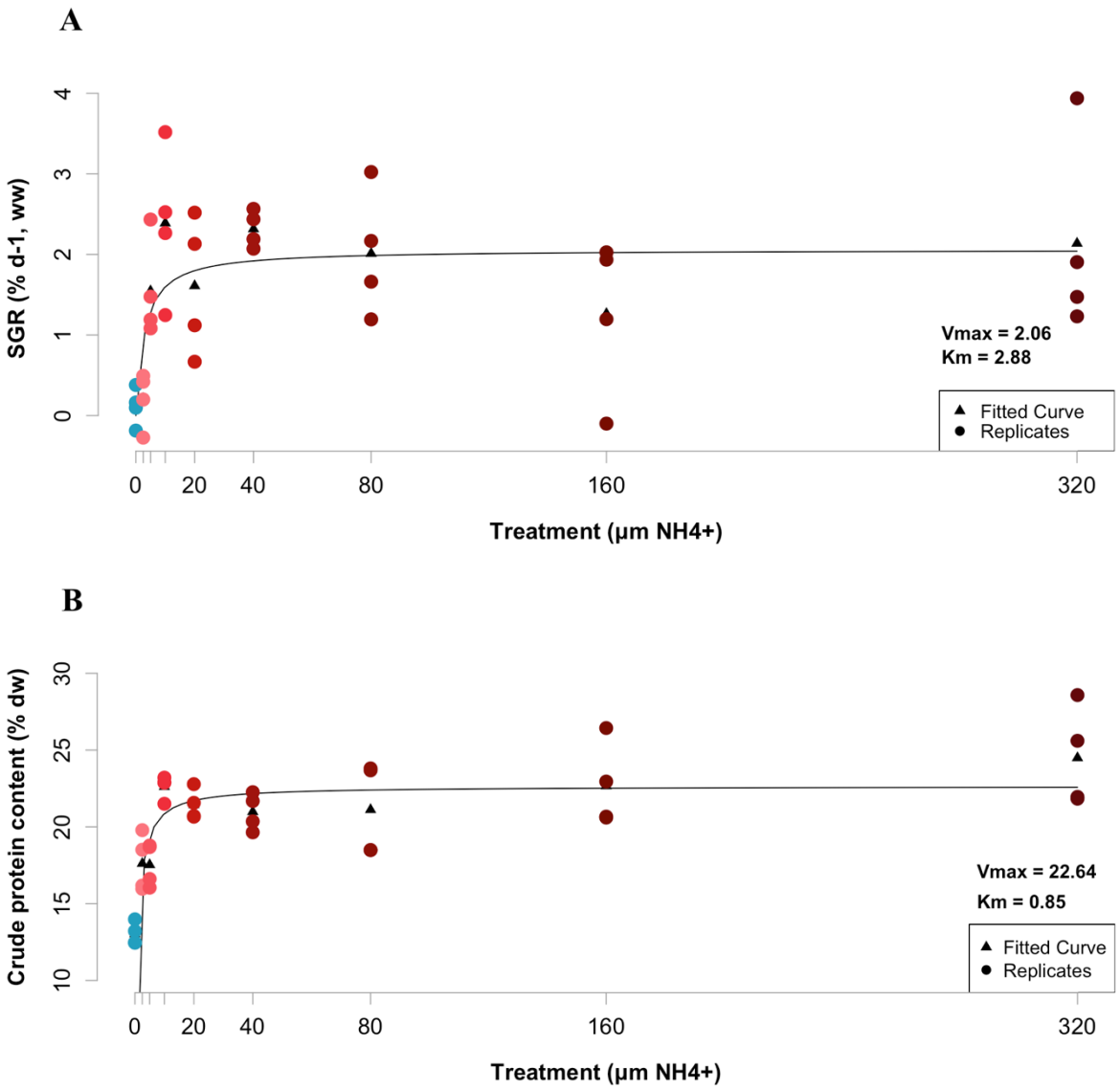

Figure 2. Fitted Michaelis Menten model for (A) SGR (% d^{-1}, ww) and (B) crude protein content (% dw) of *P. palmata* cultivated in seawater control and eight different dilution levels of herring production process water (TUB1). The curved line indicates the saturation point.

3.1.2 Experiment 2

P. palmata from TUB2-treatment had an SGR in ww of 0.39±0.04% d^{-1} whereas the control decreased in ww with -0.78±0.41% d^{-1} over the 13 days (mean±sd, Figure 3A). The SGR for area was 0.07±0.16% d^{-1} for TUB2-treatment and -0.25±0.3% d^{-1} for control (Figure 3B). The one-way ANOVA showed that there was a significant statistical difference (<0.01) in SGR for ww between control and TUB2-treatment (Table 4). No significant statistical difference between the two treatments was found for SGR for area.

TUB2-treatment had a crude protein content of 16.5±0.5 % dw, whereas it was 13.3±0.6 and 16.4±0.5 % dw for control and flow-through control (mean±sd, Figure 3C). At the start of the experiment, the biomass had a crude protein content of 13.7±0.6 % dw; an increase with 20.9 and 20.2 % dw for TUB2-treatment and flow-through control and a decrease with 2.4 % dw for control. The crude protein content of TUB2-treatment and flow-through control was significantly higher than control and the start biomass, according to the one-way ANOVA and post-hoc test (Table 4). No statistically significant difference was found between TUB2-treatment and flow-through control.

The Chl-*a* content for the three different treatments was 1±0.5, 2.6±0.6 and 2.7±0.4 µg/mg dw^{-1} (Figure 3D). The one-way ANOVA (Table 4) with associated post-hoc test shows that the Chl-*a* content for TUB2-treatment and flow-through control was significantly higher than control. No statistically significant difference was found between TUB2-treatment and flow-through control.

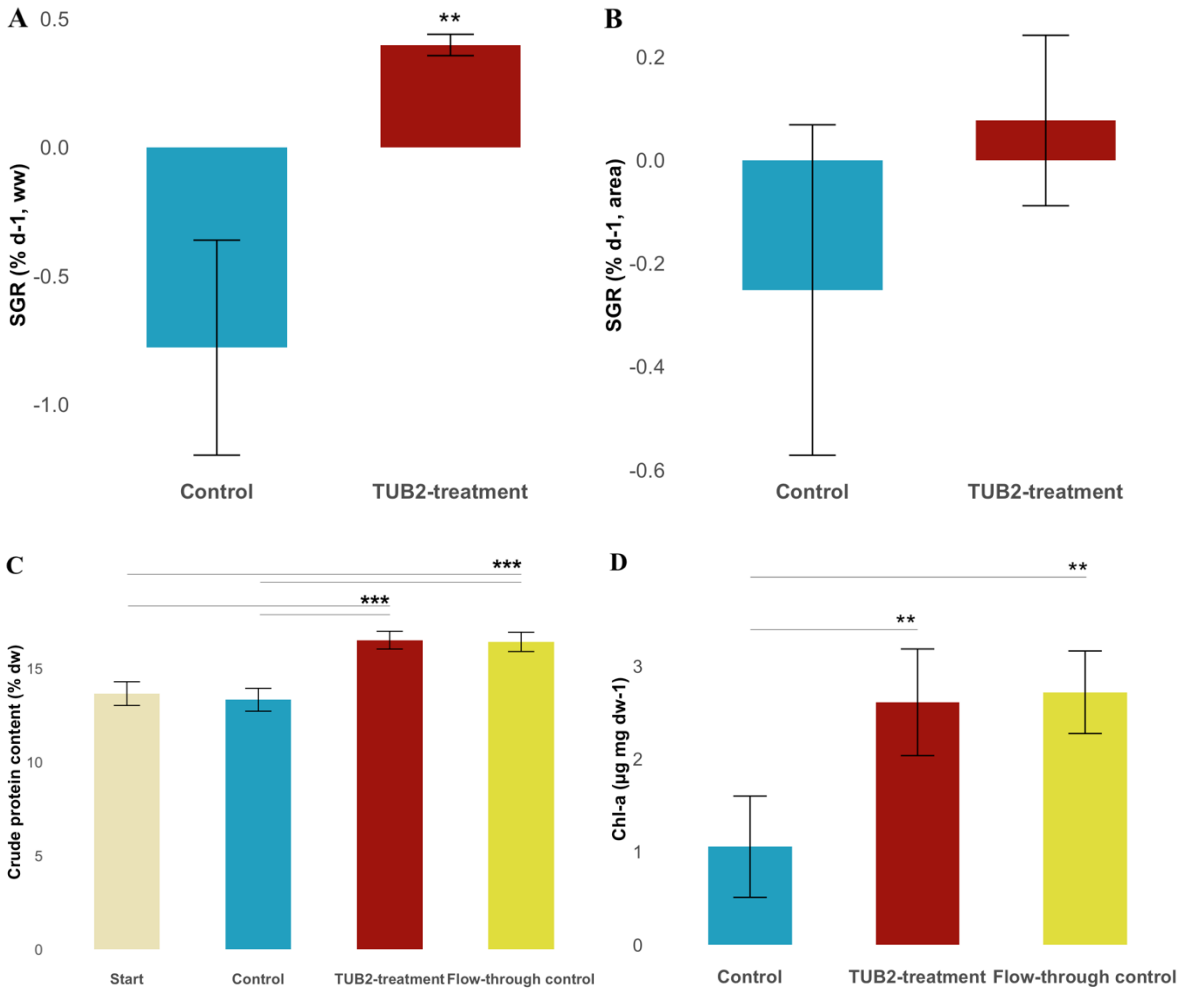

Figure 3. (A) SGR in ww (% d^{-1}) and (B) SGR in area (% d^{-1}) for control and TUB2-treatment of cultivated *P. palmata*. (C) crude protein content (% dw) at the start of the experiment and for the three treatments. (D) Chl-*a* (µg/mg dw^{-1}) for all three treatments of *P. palmata*. Error bar indicates standard deviation of replicates. Asterisks indicates significant difference between treatments based on the result from Tukey's post hoc test (***=<0.001, **=0.01, *=0.05).

Table 4. One-way ANOVA of SGR (% d^{-1}) in ww and area, crude protein content (% dw) and Chl-*a* (µg/mg dw^{-1}) of *P. palmata* cultivated in control, herring production process water (TUB2) and flow-through control. Asterisks indicates significant difference (***=<0.001, **=0.01, *=0.05).

Variable	Source of variation	df	MS	F-value	p-value
SGR (% d^{-1}, ww)	Treatment	1	2.7783	31.4	0.001**
	Residual	6	0.0885		
SGR (% d^{-1}, area)	Treatment	1	0.21591	3.325	0.118
	Residual	6	0.06493		
Crude protein content (% dw)	Treatment	3	11.848	37.64	<0.001***
	Residual	12	0.315		
Chl-*a* (µg/mg dw^{-1})	Treatment	2	3.461	12.61	0.002**
	Residual	9	0.275		

3.2 Sensory analysis of *Palmaria palmata*

The results of the sensory evaluation show that the two treatments, TUB2-treatment and flow-through control, share the majority of sensory attributes (Figure 4). The panel identified the dominant flavours of *P.palmata* as umami, salt and fish, with a high flavour intensity for both treatments. The taste of grass and pepper was somewhat lower but more distinct than flower, bitter and sour that were on the lower end of the scale (Table 5). Ocean was the most prominent odour, followed by freshness whereas the scents of berries and earthiness were considered low. *P. palmata* scored high on all the texture attributes; it has a firm sensation, a high chewiness and a strong adhesive tendency.

Appearance is the attribute group where the two treatments significantly differ (<0.05) from one another, which is shown in the three-way ANOVA (Table 5). TUB2-treatment was considered more red-purple than flow-through control which was perceived to be more red-brown. Flow-through control was found to have a coarser structure than TUB2-treatment. Moreover, the perceived glossiness for TUB-2 treatment was higher than flow-through control. A statistically significant difference (<0.05) between the treatments was also found for the flavour attributes flower, pepper and umami (Table 5).

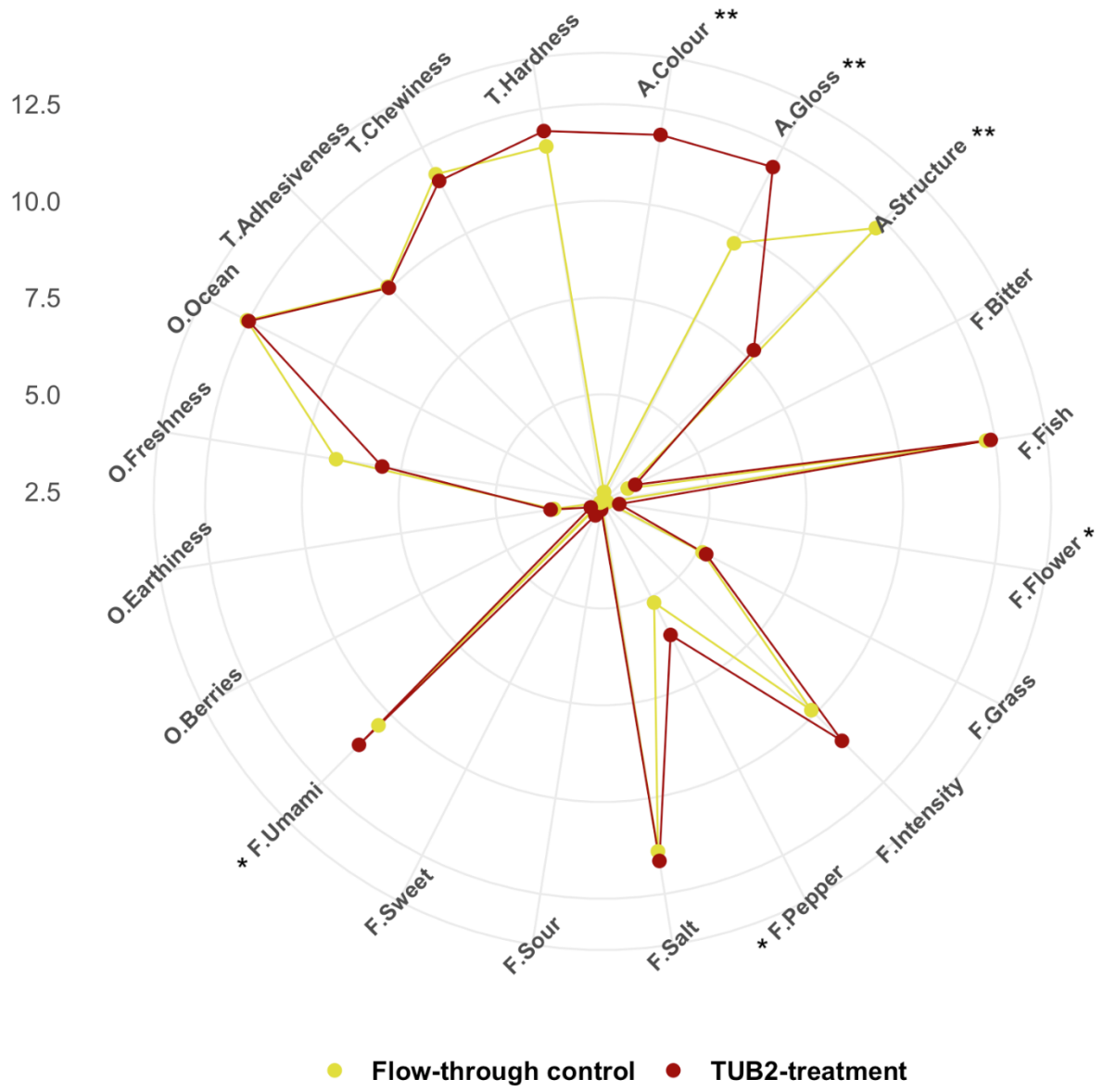

Figure 4. Sensory profile, showing the mean value on an unstructured linear intensity 15 cm scale, of *P. palmata* cultivated in two different treatments, flow-through control and TUB2-treatment. Asterisks indicates significant difference (***=<0.001, **=<0.01, *=<0.05).

Table 5. Three-way ANOVA and the mean ± sd score (unstructured linear intensity 15 cm scale) of the sensory attributes of *P. palmata* cultivated in two different treatments, flow-through control and TUB2-treatment. Treatment and panellist are treated as fixed factors whereas replicates as random factor. Asterisks indicates significant difference between the treatments (***=<0.001, **=0.01, *=0.05). The full ANOVA results are found in Table 6, Appendix 2.

	Flow-through control	TUB2-treatment	Treatment			
Attribute	**Mean ± sd**	**Mean ± sd**	**df**	**MS**	**F-value**	**p-value**
Apperance						
A.Colour	2.5 ± 1.9	11.8 ± 2.5	1	1309.5	330.8	0.003**
A.Gloss	9.7 ± 2.2	11.9 ± 1.3	1	73.3	279.5	0.004**
A.Structure	12.2 ± 1.6	7.8 ± 3.1	1	298.4	1644.3	0.001**
Odour						
O.Berries	2.3 ± 1	2.6 ± 1	1	1.1	7.3	0.1
O.Ocean	12.5 ± 1.4	12.5 ± 1.2	1	0.0	0.0	0.9
O.Earthiness	3.5 ± 2.2	3.6 ± 1.8	1	0.1	0.1	0.8
O.Freshness	9.2 ± 2.9	8 ± 2.8	1	21.8	9.6	0.1
Flavour						
F.Umami	10.4 ± 3.1	11.1 ± 2.5	1	7.6	43.2	0.02*
F.Salt	11.4 ± 2.5	11.6 ± 1.4	1	0.9	0.5	0.6
F.Sweet	2.2 ± 1.4	2.6 ± 1.3	1	2.5	1.2	0.4
F.Sour	2.4 ± 1.2	2.5 ± 1.7	1	0.1	0.1	0.8
F.Bitter	3 ± 1.9	3.2 ± 2.0	1	0.7	0.9	0.4
F.Flower	2.4 ± 1.1	2.7 ± 1.2	1	1.5	18.9	0.05*
F.Pepper	5.2 ± 2.9	6.1 ± 2.2	1	13.2	32.4	0.03*
F.Fish	12.3 ± 1.4	12.4 ± 1.5	1	0.2	0.3	0.7
F.Grass	5.1 ± 2.3	5.2 ± 2.3	1	0.2	0.1	0.8
F.Intensity	9.9 ± 2.1	11 ± 2.1	1	18.8	18.3	0.1
Texture						
T.Hardness	11.5 ± 1.7	11.9 ± 1.5	1	2.4	0.6	0.5
T.Chewiness	11.7 ± 1.6	11.5 ± 2	1	0.6	0.8	0.5
T.Adhesiveness	10.1 ± 3.1	10 ± 3.3	1	0.0	0.0	0.9

The PCA supports the finding that the two treatments overlap for the majority of the attributes (Figure 5A). Moreover, the PCA over panellist performance visualise that there is disagreement between the panellists for some attributes (Figure 5B). This disagreement is also displayed in the three-way ANOVA (Table 6, Appendix 2) where interaction between panellist and treatment occurs.

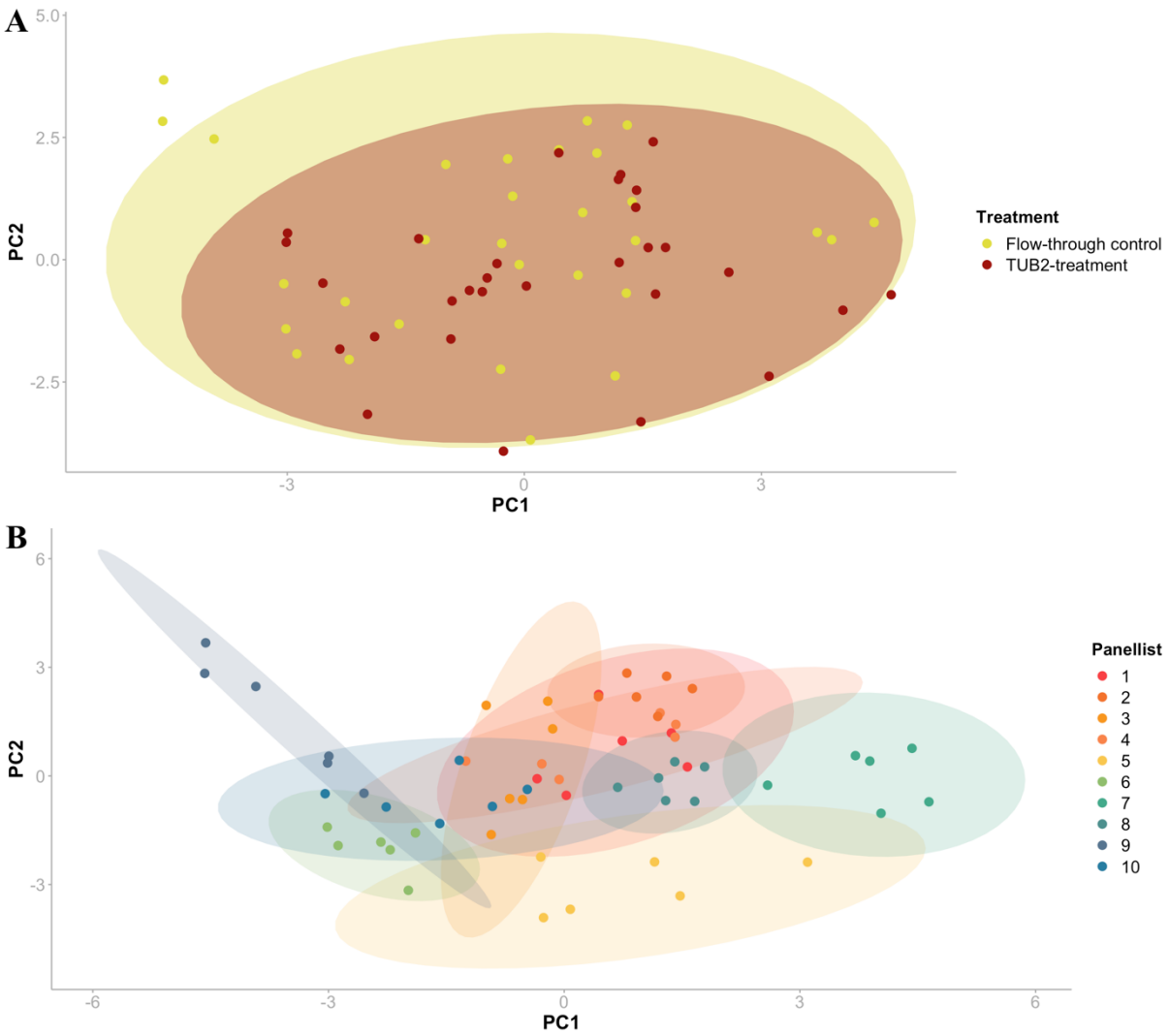

Figure 5. PCA with distribution of attribution scores clustered by (A) treatment and (B) panellist, showing attribute similarities between treatments and panellist performance.

4. Discussion

The findings from this study highlight the potential of using HPPW as cultivation media for *P. palmata* to enhance desired nutritional and physiological characteristics while retaining its sensory properties. Cultivation in nutrient-rich water from fish production increases the growth, crude protein content and Chl-*a* of *P. palmata* which is in agreement with what Grote (2016) found for *P. palmata* and Stedt et al. (2022) for *U.fenestrata*.

The first cultivation experiment indicates that significant differences in crude protein content and growth between TUB-treated *P. palmata* and control occur already at low dilution levels of HPPW, based on NH_4^+. A dilution level of 40 µM NH_4^+ was chosen for experiment 2 based on these findings. 40 µM NH_4^+ was the concentration level at which the range of the replicates was the narrowest for both growth and crude protein content and therefore considered most stable. Previous studies have also shown that *P. palmata* tolerate even higher NH_4^+ concentrations (Corey et al., 2013; Grote, 2016), which supported the decision to use the dilution level 40 µM NH_4^+ over 10 µM NH_4^+.

The second experiment's findings strengthen the hypothesis that HPPW impacts the nutritional and physical characteristics of *P. palmata*. The crude protein content significantly increased during the experiment for the TUB-treated *P. palmata* and when compared to control the crude protein content, growth in ww and Chl-*a* was significantly higher. The difference in crude protein content between these treatments is promising, but using a crude estimate is limiting as the protein level could be over- or underestimated and information on protein composition is not provided (Angell et al., 2016).

The described relationship in crude protein content and Chl-*a* between TUB-treated *P. palmata* and control was not found when TUB-treatment was compared with flow-through control. Moreover, all treatments in both experiments had remarkably low SGR rates and the crude protein content levels for TUB-treated *P. palmata* were lower in the second experiment than in the first. This points towards a non-ideal experimental setup that e.g., did not meet the seaweed's nutrient demand. As described by Roleda & Hurd (2019), nitrogen followed by phosphorus is commonly the main limiting element for seaweed growth with various abiotic and biotic factors influencing the uptake rate. Two weeks before the second experiment, when the biomass was cultivated in flow-through tanks with similar environmental conditions as described in section 2.2.1, the same biomass's SGR was 4% daily; a stark contrast to the <1% SGR in experiment 2. The *P. palmata* which received TUB had relatively higher SGR, increased levels of crude protein content and was in better condition at the end of the experiments than control which relied on nutrients from seawater only. The number of water exchanges was increased in the second experiment to counteract nutrient depletion, yet the low SGR rates persisted and the crude protein content was lower. The hypothesis of insufficient water exchange is supported by the additional flow-through control in experiment 2 which stayed in good condition throughout the experiment and with similar levels of Chl-*a* and crude protein content as the TUB-treatment. Nevertheless, these observations should not be interpreted as TUB not working but rather marks the importance of continuous water flow when cultivating *P. palmata*. Further trials with flow-through setup are needed to fully understand the impact of HPPW on *P. palmata*.

The sensory profile from the QDA portrays cultivated *P. palmata* as a red seaweed with an intense taste of umami, salt and fish, a distinct ocean scent and a firm texture that is chewy and with a tendency to stick to surfaces. The sensory profile provided by Jönsson et al. (2023) of wild-harvested *P. palmata* has many common denominators with this study's profile of cultivated *P. palmata*. One example is the recurring connection of attributes to marine- and land-based elements like fish, grass and pepper. As pointed out by Blikra et al. (2021) and Jönsson et al. (2023), these attributes are well-known food concepts for consumers and could be used by retailers and food developers to guide consumers in how to cook with this novel ingredient and harness the increasing interest in seaweed as food highlighted by Wendin & Undeland (2020).

Appearance is the attribute group where the panel found the largest difference between the two treatments. The significant difference in colour, gloss and structure indicates that cultivating *P. palmata* in HPPW impacts the seaweed's appearance. The identified colour difference is concordant with the study by Stedt et al. (2022) where *U.fenestrata* cultivated in a similar type of HPPW had a different colour than control. Observed colour change related to the addition of HPPW or similar nutrient-rich water has been connected to the Chl-*a* content of the seaweed (Aryee et al., 2018; Stedt et al., 2022). Optimisation of the pigmentation is relevant for making the seaweed more visibly appealing as food and providing multiple health benefits (Lee et al., 2017; Aryee et al., 2018; Kumar et al., 2023). The Chl-*a* analysis alone in this study does not explain the colour difference of the two treatments. However, the potential difference in e.g., water soluble phycobiliproteins could be a plausible explanation. The red and blue colour of phycobiliproteins have been reported to intensify when *P. palmata* is cultivated in water enriched with nutrients (Idowu et al., 2023). Further studies are encouraged to investigate whether this assumption holds true.

The panel compared two treatments that differed in their experimental design. The potential effect that other environmental factors, besides the addition of TUB, have had on the sensory attributes of *P. palmata* can therefore not be excluded based on the results of this study. Still, the significant difference in appearance highlights the potential of adjusting environmental factors in land-based seaweed cultivation to alter the sensory attributes of *P. palmata*. This finding is important for food product development, as desired attributes could be enhanced to be more appealing to consumers and drive the demand for seaweeds. More studies with consumer tests are encouraged to explore what food products containing *P. palmata* would be accepted by consumers. The subjective opinions from a consumer test could then be used as a complement to the objective responses gathered in this study's sensory evaluation (Meilgaard et al., 2016).

The panel indicated that the TUB-treated *P. palmata* was more umami-rich than flow-through control. This is promising as it could be an important attribute for food application as umami is a sought-after basic taste in cooking (Mouritsen et al., 2019). The taste of umami is strongly connected to the amount of glutamic amino acid (Stévant et al., 2023), suggesting that TUB increases this particular amino acid. However, this can only be confirmed through amino acid analysis showing the limitation of the crude estimate of protein used in this thesis. Moreover, the reported difference for the sensory attribute umami has to be viewed in the light of the panel's performance (Figure 5) and the same goes for the taste difference found for flower and pepper. Cross-over interaction was present for these attributes (Table 6, Appendix 2) meaning a disagreement between the panellists in how the treatments differ from each other is

present. This signals that the panel is not calibrated enough and should have been further trained before the final evaluation as this would likely have resulted in an increased agreement between the panellists (Næs et al., 2011; Sipos et al., 2021). One factor preventing more training was the low amount of biomass available during the sensory evaluation, which naturally impacted the amount of training and portion size. Nevertheless, the panel in unison identified significant differences in the appearance attribute group which shows that they are operating on a high level. Some variability and time constraints are also expected in sensory evaluation as the measurement tool comprises humans with different pre-understandings, naturally prone to human error and with limited time at their hands (Meilgaard et al., 2016; Sipos et al., 2021).

5. Conclusion

Overall, the findings of this study strengthen the notion that *P. palmata* has a potential as a sustainable future protein source. Cultivating *P. palmata* in HPPW increases its crude protein content which makes it a competitive, as well as sustainably produced, protein source. More research on the interplay between *P. palmata* and HPPW is encouraged by these findings. The findings also suggest that flow-through of water is crucial when cultivating *P. palmata*, something that should be taken into consideration in following trials. The sensory profile of cultivated *P. palmata* provided in this study facilitate the exploration of seaweed-based food that can drive the demand for this sustainable food resource. Lastly, changes in the cultivation process of *P. palmata*, like adding HPPW, impacts the sensory attributes which is useful knowledge when developing new food products.

Food production is in dire need of change to meet the nutritional needs of the global population in an environmental-friendly way. This study contributes to the knowledge of how to utilise marine resources in an effective and sustainable way (Gephart et al., 2021). Transforming HPPW from a discarded by-product to a nutrient-rich cultivation media for seaweed cultivation minimises waste which supports sustainable and circular food production (Corey et al., 2013). In combination with the previous reported nutritional characteristics of *P. palmata* by Mæhre (et al., 2014), the novel cultivation method and sensory profile presented in this study emphasises the important role *P. palmata* can play in meeting future protein demand.

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Appendices

Appendix I: Popular scientific summary

The world population is growing which intensifies the search for sustainable protein sources. Most food today is produced on land resulting in excessive use of fresh water, lack of arable land, eutrophication and release of greenhouse gas emissions. Because of this, using the ocean to grow food has gained recognition as a solution. Cultivating seaweeds is particularly interesting as seaweeds are a nutrient-rich crop that can grow fast without causing many of the problems associated with food production on land. The red seaweed *Palmaria palmata* contains many of the nutrients that are important for humans. It is also seen as a promising food ingredient due to its strong flavour of umami, shellfish scent and unique magenta colour.

This study investigates the potential of a new cultivation method to optimise the protein content of *Palmaria palmata* while keeping the sensory attributes that makes it promising as food. Water is used in almost every step when producing food and this so-called process water is commonly treated as waste by the food industry. However, since some process waters are rich in nutrients it could have potential as fertiliser to boost the protein of *Palmaria palmata*. In this study, process water from the herring industry was explored.

Two cultivation experiments and one sensory evaluation were done in this study. The first experiment established suitable concentration levels of herring production process water to grow *Palmaria palmata*. This knowledge was used in the second experiment when the cultivation was scaled up to provide biomass for the sensory evaluation. Growth, crude protein content and chlorophyll *a* were measured to compare the treatments. The sensory evaluation consisted of a selected and trained panel that under controlled conditions described what cultivated *Palmaria palmata* tastes, smells, feels and looks like. The panel also established if these attributes change when herring production process water has been used.

The herring production process water significantly increased the crude protein content, growth and chlorophyll *a* of *Palmaria palmata*. The cultivation experiments also showcased the importance of continuous water flow when cultivating *Palmaria palmata* in land-based tanks. Further studies are strongly encouraged to fully understand the potential of herring production process water when growing *Palmaria palmata*. The panel in the sensory evaluation described cultivated *Palmaria palmata* as a red seaweed with an intense taste rich in umami, salt and fish with a distinct ocean scent and a robust texture. Most of the attributes stayed the same despite differences in cultivation methods. However, appearance is where differences were found as *Palmaria palmata* grown in herring production process water was perceived more red-purple. Describing cultivated *Palmaria palmata*'s sensory attributes and how they differ between cultivation methods are relevant when developing new seaweed-based food products that consumers want to eat. Altogether, the findings of this study indicate that *Palmaria palmata* show potential to be one of the sustainable protein sources that can help meet the nutritional needs of a growing world population.

Appendix II: Three-way ANOVA of sensory attributes

Table 6. Three-way ANOVA of the sensory attributes of *P. palmata* cultivated in two different treatments, flow-through control and TUB2, with all sources of variations included. Treatment and panellist are treated as fixed factors whereas replicates as random factor. e = SPSS cannot compute the error degrees of freedom using Satterthwaite's method.

Attribute	Treatment				Panellist				Replicate				Treatment x Panellist				Treatment x replicate				Panellist x replicate			
	df	MS	F-value	p-value	df	MS	F-value	p-value	df	MS	F-value	p-value	df	MS	F-value	p-value	df	MS	F-value	p-value	df	MS	F-value	p-value
A.Colour	1	1309.5	330.8	0.003	9	8.5	2.2	0.078	2	6.4	1.6	0.4	9	5.9	1.5	0.2	2	4	1	0.4	18	3.9	1	0.5
A.Gloss	1	73.3	279.5	0.004	9	15.5	20.4	<0.001	2	3.5	17.5	0.4	9	1.9	2.3	0.1	2	0.3	0.3	0.7	18	0.8	0.9	0.6
A.Structure	1	298.4	1644.3	0.001	9	27.5	20.5	<0.001	2	0.8	9.9	0.9	9	5.5	3.8	0.01	2	0.2	0.1	0.9	18	1.3	0.9	0.6
O.Berries	1	1.1	7.3	0.1	9	3.4	7.3	<0.001	2	0.4	1.1	0.4	9	1.3	4.9	0.002	2	0.2	0.6	0.6	18	0.5	1.7	0.1
O.Ocean	1	0.0	0.0	0.9	9	8.9	31.0	<0.001	2	1.4	1.4	0.4	9	0.5	2.8	0.03	2	0.9	4.5	0	18	0.3	1.5	0.2
O.Earthiness	1	0.1	0.1	0.8	9	21.2	31.9	<0.001	2	0.1	0.1	0.9	9	2.0	2.6	0.04	2	1.3	1.7	0.2	18	0.7	0.8	0.6
O.Freshness	1	21.8	9.6	0.1	9	36.2	76.7	<0.001	2	0.2	3.1	1.0	9	10.4	3.9	0.01	2	2.3	0.9	0.4	18	0.5	0.2	1
F.Umami	1	7.6	43.2	0.02	9	41.7	34.2	<0.001	2	2.9	3.7	0.1	9	4.8	7.9	<0.001	2	0.2	0.3	0.8	18	1.2	2	0.1
F.Salt	1	0.9	0.5	0.6	9	15.3	12.4	<0.001	2	0.7	0.4	0.7	9	4.9	3.7	0.01	2	2	1.5	0.2	18	1.2	0.9	0.6
F.Sweet	1	2.5	1.2	0.4	9	7	7.5	<0.001	2	0.1	0	1.0	9	1.2	2.1	0.1	2	2	3.5	0.1	18	0.9	1.6	0.2
F.Sour	1	0.1	0.1	0.8	9	9.6	9.3	<0.001	2	1.5	0.9	0.5	9	1.4	2.9	0.03	2	1.2	2.3	0.1	18	1	2	0.1
F.Bitter	1	0.7	0.9	0.4	9	18.9	14.6	<0.001	2	1.2	1.1	0.5	9	0.7	0.7	0.7	2	0.8	0.8	0.5	18	1.3	1.3	0.3
F.Flower	1	1.5	18.9	0.05	9	5.1	12.2	<0.001	2	e	e	e	9	0.4	0.5	0.9	2	0.1	0.1	0.9	18	0.4	0.5	0.9
F.Pepper	1	13.2	32.4	0.03	9	24.5	14.1	<0.001	2	e	e	e	9	7.4	2.6	0.04	2	0.4	0.1	0.9	18	1.7	0.6	0.9
F.Fish	1	0.2	0.3	0.7	9	7.8	9.1	<0.001	2	1.4	1.7	0.4	9	1.4	1.7	0.2	2	0.8	1	0.4	18	0.9	1.1	0.5
F.Grass	1	0.2	0.1	0.8	9	23.4	10.7	<0.001	2	0.7	0.4	0.7	9	1.8	0.8	0.6	2	2	0.9	0.4	18	2.2	1	0.5
F.Intensity	1	18.8	18.3	0.1	9	20.5	27.4	<0.001	2	2.8	2.4	0.3	9	3.9	6.5	<0.001	2	1	1.7	0.2	18	0.7	1.2	0.3
T.Hardness	1	2.4	0.6	0.5	9	8.1	7.4	<0.001	2	2.3	0.6	0.6	9	1.6	1.0	0.5	2	4.3	2.6	0.1	18	1.1	0.7	0.8
T.Chewiness	1	0.6	0.8	0.5	9	13.2	7.7	<0.001	2	1.6	0.8	0.5	9	3.7	13.2	<0.001	2	0.7	2.4	0.1	18	1.7	6	<0.001
T.Adhesiveness	1	0.0	0.0	0.9	9	55.5	67.9	<0.001	2	1.7	0.6	0.6	9	5.4	4.1	0.01	2	3.4	2.6	0.1	18	0.8	0.6	0.8