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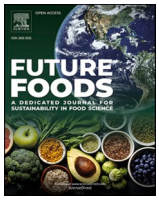

Sensory quality of emulsions prepared with the seaweed Ulva spp. or a derived protein ingredient

João P. Trigo [a,*], Karin Wendin [b,c], Sophie Steinhagen [d], Karin Larsson [a], Ingrid Undeland [a]

[a] Department of Life Sciences – Food and Nutrition Science, Chalmers University of Technology, SE 412 96 Gothenburg, Sweden
[b] Department of Food and Meal Science, Kristianstad University, Kristianstad, SE 291 88, Sweden
[c] Department of Food Science, University of Copenhagen, DK 1958 Frederiksberg C, Denmark
[d] Tjärnö Marine Laboratory, Department of Marine Sciences, University of Gothenburg, SE 452 96 Strömstad, Sweden

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ABSTRACT

The sensory quality of seaweed, whether as a whole biomass or as a protein ingredient, plays a crucial role in its successful commercialization. This study explored the effect of different *Ulva* species, biomass washing, and pH-shift-based protein extraction on the sensory quality of emulsions with 0, 5, and 10 % oil. A trained panel assessed the sensory profile, complemented by analyses of volatile compounds, total ash, and amino acids. Saltiness emerged as the primary distinction between emulsions with unwashed *U. linza* and *U. fenestrata*, due to higher ash in the former. Washing *U. fenestrata* retained sensory qualities despite reduced ash and increased content of the lipid oxidation-marker pentanal. Protein extraction up-concentrated total amino acids 2.9-fold, and yielded emulsions with reduced particle sensation and grassy flavor, while bitterness, sourness, dark color, pentanal, hexanal, and 2-ethylfuran increased. Increased oil content of emulsions lowered their grassy odor which correlated with reduced hexanal content. Overall, these findings can contribute to the development of food products containing seaweed or protein ingredients thereof that match consumer preferences.

1. Introduction

Seaweed can offer macro and micronutrients together with new sensory experiences, particularly in Western society where it has not been traditionally consumed (Jacobsen et al., 2023; Vellinga et al., 2022), although a recent consumer survey in Sweden showed an overall positive attitude towards seaweed as food (Wendin & Undeland, 2020). Moreover, seaweed is a sustainable food commodity since its cultivation has a near-zero carbon footprint, does not require arable land, irrigation, or pesticides/insecticides, and can counteract eutrophication (Gephart et al., 2021). However, since the taste of seaweed differs from what consumers are accustomed to, it is crucial to systematically investigate its sensory properties - either as a whole biomass or an ingredient - and ultimately match them to the preferences of Western consumers.

Among the different seaweeds cultivated in Europe, *Ulva* stands out as the most cultivated green genus (Araújo et al., 2021). It can exhibit an odor resembling lemon and fresh grass, distinguishing it from red and brown seaweeds, the latter of which are described as less salty and more crispy than *Ulva* spp. (Jönsson et al., 2023). Before processing seaweed for various applications, including food, it is common to wash it in fresh water to remove epiphytes and impurities e.g., sand (Poeloengasih et al., 2019). This treatment has been shown to induce compositional changes in *Ulva* spp., particularly due to the leaching of minerals to the washing waters (Harrysson et al., 2021; Poeloengasih et al., 2019). However, as far as we know, it remains unknown how washing influences the sensory quality of *Ulva*.

Besides being a source of whole food, seaweed has also been regarded as a potential source of food protein to complement terrestrial protein currently being limited by land and water supply (Celente et al., 2023). While it holds promise, most seaweeds, including *Ulva* spp., have a relatively low to medium protein content (4–35 % on a dry weight, dw, basis) when compared to e.g., beef (65 % protein on a dw basis), which calls for up-concentration processes as already done for pulses such as soybean (Holdt & Kraan, 2011; Rajpurohit & Li, 2023; USDA, 2018). Moreover, proteins in crude seaweed are moderately digestible due to e.g., the abundance of dietary fiber. We have recently shown that both the protein content and digestibility can be improved by extraction using the pH-shift method i.e., wet fractionation (Juul et al., 2022; Trigo et al., 2021). To date, no studies have examined the sensory quality of protein extracts recovered from seaweed, but their lower levels of dietary fiber

* Corresponding author.
E-mail address: trigo@chalmers.se (J.P. Trigo).

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(Trigo et al., 2023) and ash (Harrysson et al., 2018; Trigo et al., 2023) imply that their sensory properties would differ from the original biomass. Protein extraction could also induce a risk for lipid oxidation due to tissue disruption and extreme pH-shifts (Zhang et al., 2022).

Studies adding seaweed to bakery, meat, and dairy products often examine sensory quality as a function of seaweed incorporation level (Gullón et al., 2021; Quitral et al., 2022; Ścieszka & Klewicka, 2019). However, there is currently a limited understanding of how different food components *per se* interfere with the sensory quality and profile of seaweed-containing products. In the case of lipids, they influence both flavor and odor perception by affecting the viscosity of the food and modulating the partition of hydrophobic volatile compounds between the food and the air phases (Bayarri et al., 2006). For instance, increasing lipid levels above 50 % in a meat product reduced the diversity and concentration of furans and pyrazines - two compound volatile classes providing the characteristic roasted meat odor (Zhang et al., 2023).

This work aimed to investigate how the sensory quality of emulsions made with *Ulva* spp. was affected by species, biomass washing, protein extraction, and different emulsion oil levels. The hypotheses were the following: (i) biomass washing would decrease the perceived saltiness and allow other sensory attributes to become more pronounced and noticeable; (ii) protein extraction would affect mouthfeel, saltiness, and odor/flavor due to the removal of insoluble fibers, ash, and formation of lipid-oxidation products; (iii) increasing oil levels would influence the distinct organoleptic properties of *Ulva* spp. due to higher retention of hydrophobic volatile compounds in the emulsion oil-phase. The edible tested species were *Ulva fenestrata* Postels & Ruprecht and *Ulva linza* Linnaeus, both ubiquitous on the Swedish West Coast. The sensory quality of emulsions with 0, 5, and 10 % rapeseed oil was evaluated by a selected and trained panel and complemented with quantification of volatile compounds. Additionally, the total amino acids and ash content of the biomasses, as well as the protein extract, were determined to calculate protein up-concentration and explore potential links between ash content and saltiness.

2. Materials and methods

2.1. Seaweed biomasses and post-harvest biomass washing

The species *U. fenestrata* and *U. linza* were cultivated in tanks (90 L) in a seaweed greenhouse at the Tjärnö Marine Laboratory in a 16:8 h (L:D) light cycle and at an irradiance of 140 μmol photons m^{-2} s^{-1}. The light source was an INDY66 LED 60 W 4000 K 6000 lm. To ensure the seaweeds were suitable for the sensory tests, they continuously received filtered (5 μm) and UV-treated natural seawater without additional medium or chemicals in a flow-through system (flow = 10–14 L h^{-1}). Water motion was provided by permanent aeration. The salinity and temperature fluctuated depending on the prevailing weather and seasonal conditions. Seaweeds were harvested in July 2020. The molecular identification of both *Ulva* strains followed the description in Toth et al. (2020) and sequences of the tufa marker gene are publicly available in GenBank (*U. fenestrata*: MN240309-11; *U. linza*: OP267728). After harvesting, a portion of *U. fenestrata* was washed in cold tap water (~12 °C) for 2 min in a water-to-seaweed ratio of 1:20 after which excessive water was drained off using a salad spinner. The biomasses were then oven-dried at 40 °C and milled into a powder using a miller (Retsch ZM 200, Germany) with a 0.5 mm sieve and stored at -80 °C until further use. The *U. fenestrata* biomass used for protein extraction was cultured under the abovementioned cultivation method and harvested in individual batches during 9 months. This was needed due to the high amounts of biomass required to obtain enough quantities of protein extract for sensory analysis. After harvesting each batch, the fresh biomass was stored at -80 °C until further use.

2.2. Production of seaweed protein extracts

The production of protein extracts from *U. fenestrata* using the pH-shift method followed the protocol reported by Trigo et al. (2023). Before protein extraction, each batch was thawed and ground (Model C-E22N, la Minerva, Italy) with a 4 mm hole plate, followed by mixing it with cold distilled water in a 1:6 (w/w) ratio. Homogenization was done for 4 min at 8000 rpm (Silverson LM5, USA), followed by an osmotic shock incubation step for 1 h at 8 °C with stirring. After the incubation step, the pH of the homogenate was adjusted to 12 with NaOH (2 M) and left to incubate for 20 min while stirring. This was followed by centrifugation at 8500 × g for 20 min at 8 °C (Sorvall LYNX 6000, Thermo Scientific, USA). The resulting supernatant containing the solubilized proteins was decanted through a sieve (~0.5 mm) and its pH was adjusted to 2.0 with HCl (2 M) and incubated for 20 min with stirring. Subsequently, the supernatant was frozen overnight at − 20 °C and, on the following day, it was thawed in cold water before centrifugation at 8500 × g for 20 min at 8 °C. The resulting pellet, here referred to as *protein extract,* was freeze-dried. Lastly, the protein extracts produced from all batches were combined and stored at -80 °C before analysis.

2.3. Preparation of emulsions

Emulsions with 0 %, 5 %, and 10 % oil (w/w) were prepared with *U. fenestrata* (unwashed or washed), *U. linza* (unwashed), protein extract from unwashed *U. fenestrata,* or soy protein isolate (Engelhardt AB, Sweden). Although, by definition, the sample with 0 % oil is not an emulsion but rather a suspension or solution, it is henceforth referred to as an emulsion to simplify the terminology. The preparation of the emulsions started by manually mixing 2.5 g of dried test sample with 2.0 g of dried soy lecithin (Sosa Ingredients, Spain) in a 500 mL dispersing vessel (Kinematica GS40, Switzerland). This was followed by adding water and rapeseed oil (Martin & Servera, Sweden) to a final water+oil volume of 150 mL with oil contents of 0, 5, and 10 %. The solutions were then homogenized at 11 000 rpm for four minutes (Kinematica Polytron PT 2500 E homogenizer). During the first minute of homogenization, 2.3 g of xanthan gum (Guzmán Gastronomia SL, Spain) was gradually added to facilitate full dispersion. The emulsion production was performed at room temperature and a total of 15 different emulsions were produced, which were then coded with three-digit codes.

2.4. Sensory analysis

The emulsion samples were evaluated using a trained analytical sensory panel at Kristianstad University using Quantitative Descriptive Analysis. The six panelists were selected and trained according to ISO 8586:2012 and ISO 6658:2017. The panel generated attributes to be used in the descriptive analysis (Table 1) by training with a selection of the emulsion samples from the test design (Fig. 1). The samples used for attribute generation were selected to represent the extremes among the samples in order to include all aspects of the test design. Moreover, the panelists formulated a definition for each attribute and underwent training on utilizing the line scale, requiring consensus in evaluating the training samples. This evaluation encompassed both attribute formulation and understanding how to apply the scale. The line scale spanned from 0 to 100 mm and was anchored at 10 and 90 mm. The anchor at 10 mm was marked “a little”, whereas the anchor at 90 mm was marked “much”. The Quantitative Descriptive Analysis (QDA) was performed in a sensory laboratory equipped according to ISO 8589:2010 and using EyeQuestion (version 4.11.68, logic8) software for data collection. The evaluation was performed in two 2-hour sessions during two consecutive days. Each panelist evaluated all samples in duplicate. The panelists were served a single sample at a time in a randomized order with individual orders for each panelist to prevent bias from any overlapping effects within the test design. Water and neutral wafers were used to rinse and clean the mouth and palate between samples.

Table 1
Sensory attributes and their definitions.

Attribute	Label	Scale anchors	Definition
Appearance (A)			
Green	A-green	Little // Much	Scale running from no green at all to intense green
Shiny	A-shiny	Little // Much	Surface shininess
Odour (O)			
Total	O-total	Little // Much	Total intensity of all odors
Fresh cut grass	O-fresh cut grass	Little // Much	Freshly cut grass
Nutty	O-nutty	Little // Much	Nutty odor with hints of mushroom
Taste (T)			
Salty	T-salty	Little // Much	Basic taste
Sourness	T-sour	Little // Much	Basic taste
Sweetness	T-sweet	Little // Much	Basic taste
Umami	T-umami	Little // Much	Basic taste
Bitterness	T-bitter	Little // Much	Basic taste
Flavour (F)			
Fishliveroil	F-fish liver oil	Little // Much	Fish liver oil
Grass	F-grass	Little // Much	Freshly cut grass
Nutty	F-nutty	Little // Much	Nutty flavor with hints of mushroom
Texture (Tex)			
Adherence	Tex-adherence	Little // Much	Adhesive to spoon
Oily	Tex-oily	Little // Much	Fatty feeling in the mouth
Particles	Tex-particles	Little // Much	Particles felt in mouth after swallowing

2.5. Total ash and amino acids of seaweed biomasses and the protein extract

Ash content was determined gravimetrically by combusting 50 mg of freeze-dried seaweed biomasses and protein extracts. The combustion consisted of gradual heating to 550 °C at a rate of 200 °C/h, then maintenance at 550 °C for 6 h, followed by cooling to 200 °C. Samples were analyzed in triplicate unless stated otherwise.

Total amino acids were quantified according to our earlier protocol (Trigo et al., 2021). Briefly, 50 mg of freeze-dried seaweed biomasses and the protein extract were hydrolyzed in 6 M HCl (4 mL) at 110 °C for 24 hours. The hydrolyzed samples were diluted with 0.2 M acetic acid and filtered (0.22 μm) before LC-MS analysis (Agilent 1100 HPLC + Agilent 6120 quadrupole). A Phenomenex column (C18 (2) 250 μm × 4.6 μm × 3 μm) was used and the obtained data was compared against a set of 17 amino acid standards (Thermo Scientific). Samples were analyzed in triplicate unless stated otherwise. Due to the acid hydrolysis, tryptophane and cysteine were degraded and therefore not quantified.

2.6. Analysis of volatile compounds in the produced emulsions

Volatile compounds were determined using headspace solid-phase microextraction (HS-SPME) coupled to GC–MS based on the method reported by Sajib & Undeland (2020) with some modifications. Briefly, 8 mL of each emulsion was transferred to a 20 mL SPME vial that was pre-incubated for 5 min at 30 °C with stirring (500 rpm). Then, volatiles were extracted for 20 min at 30 °C with stirring (500 rpm) using a 75 μm Carbonxen/polydimethylsiloxane (CAR-PDMS)-coated SPME fiber (Supelco, Bellefonte, USA). Thereafter, the fiber was automatically inserted into the GC injection port at a depth of 38 mm. The separation and detection were carried out in a GC–MS system (GC-2010 Plus and GCMS-TQ8030, Shimadzu). The GC inlet temperature was maintained at 280 °C and helium was the carrier gas at a constant flow rate of 1.5 mL/min. The volatiles were separated using a fused silica ZB-1701 (Phenomenex, 30 m×0.32 mm, 1.00 μm). The MS was operated in the electron ionization mode and data acquisition was performed in scan mode in the mass range of 30–500 *m/z* with a scan rate of 0.224 s/scan. Compounds were pre-identified based on the linear retention index of a C8-C20 alkanes mixture and the NIST 11 library (**Supplementary material: Table S1**). Their identity was further confirmed with external standards acquired at Sigma-Alrich.

2.7. Statistical analysis

Principal components analysis (PCA) was performed in RStudio (v2023.06.2) to assess data obtained from sensorial analysis. Initially, the data was standardized using the *scale* function and the PCA was executed using the *prcomp* function. The factoextra package (Kassambara & Mundt, 2020) was used to visualize the outcomes of the PCA and the *kmeans* function was utilized to cluster its results. Sensory data were analysed by calculating mean values and standard deviations. Further, to analyse significant differences between samples a two-way ANOVA (samples x assessors) followed by Tukey's post-hoc test was performed and a regression analysis was also performed to analyse impact from the design variables, in which these were independent and the measurements were dependent factors. To measure statistical differences in the compositional data, the one-way ANOVA was used together with Tukey's post hoc test for pairwise comparisons. The non-parametric Kruskal-Wallis test was used if data was not normally distributed. Statistical tests were carried out using SPSS Statistics (IBM SPSS, version 26), unless otherwise specified. Differences were considered significant at $p \leq 0.05$.

3. Results and discussion

3.1. Sensory analysis

Table 2 presents the average sensory scores for emulsions with 0, 5, and 10 % oil made with unwashed and washed *U. fenestrata*, unwashed *U. linza*, pH-shift protein extract from unwashed *U. fenestrata,* and soy protein isolate. A regression analysis of the scores of all samples revealed that sample type significantly influenced fresh cut grass and nutty odor, green appearance, particle texture as well as salty, sour, and bitter taste and grass flavor. Moreover, the oil content of the emulsions significantly affected total odor, green appearance, adherence, sweet taste, and grass and nutty flavor (Table 2).

The PCA analysis of the average scores explained 73.8 % of the total data variation (Fig. 2); it also identified three clusters - seaweed biomasses, protein extract from *U. fenestrata*, and soy protein isolate. The main differences between the first two clusters were attributed to fresh cut grass odor, green appearance, particle texture, bitter and sour taste, and grass flavor. More specifically, emulsions with seaweed biomasses were characterized by a lighter green color and increased particle sensation after swallowing. Conversely, the protein extracts exhibited higher bitterness and sourness as well as a less intense grassy flavor.

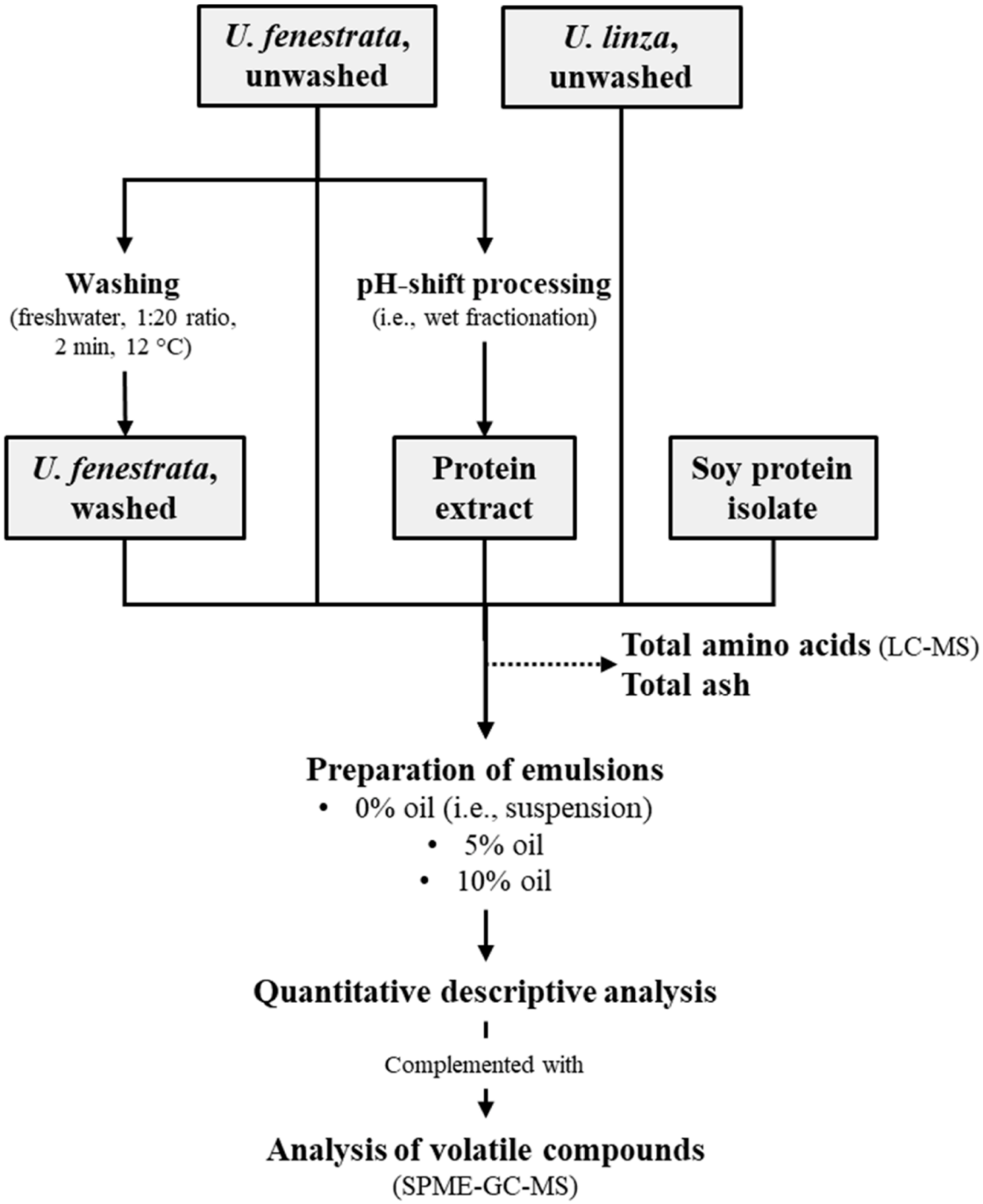

Fig. 1. Experimental design of the study.

Pictures of the three different biomasses and the protein extract are provided in **Supplementary material: Fig. S1**, where the latter sample exhibited green-brown tones compared to deeper green colors of the unwashed *U. fenestrata* and *U. linza*. The observed changes in mouthfeel agreed with the original hypothesis, which can be elucidated by the removal of non-soluble material such as fibers during protein extraction. The higher bitterness is likely a result of the co-extraction of phenolic compounds during pH-shift processing of *U. fenestrata*, a phenomenon we earlier documented in the form of a 40 % increase in total phenolic content, reaching around 0.1 % of the total dry weight, in the protein extract (Trigo et al., 2021). Among the different classes of phenolic compounds, the main ones present in *Ulva* spp. are phenolic acids, flavonoids, and tannins (Elmosallamy et al., 2021), all of which can be bitter-tasting (Karolkowski et al., 2023). Regarding sourness, which is closely related to acidity (Fischer & Noble, 1994), the average pH of emulsions containing unwashed *U. fenestrata* or the protein extract was 6.80±0.09 and 4.18±0.04, respectively. This pH difference likely led to the distinct sourness scores observed in protein extract-based emulsions, reflecting the low pH used for isoelectric protein precipitation. Adjusting the pH of protein extracts before their use is a simple strategy for mitigating this characteristic. Nevertheless, sourness characterizes fermented food products such as sour cream and many cheeses where crude seaweed protein extracts could be incorporated. The lower average scores in grassy flavor in the protein extracts compared to the unwashed

Table 2
Average sensory scores±standard deviation (on a scale from 1 to 100, N=6) from a quantitative descriptive analysis (QDA) of emulsions with increasing oil levels containing unwashed and washed *U. fenestrata*, unwashed *U. linza*, a pH-shift protein extract from unwashed *U. fenestrata*, or soy protein isolate.

Sensory descriptor	*U. fenestrata*, unwashed			*U. fenestrata*, washed			*U. linza*, unwashed			Protein extract from unwashed *Ulva fenestrata*			Soy protein isolate			*p*-value of regression	
	0 % oil	5 % oil	10 % oil	0 % oil	5 % oil	10 % oil	0 % oil	5 % oil	10 % oil	0 % oil	5 % oil	10 % oil	0 % oil	5 % oil	10 % oil	Oil	Sample type
O-total	62.4 ±11.4^{a}	56.7 ±19.6^{ab}	45.4 ±21.1^{b}	54.5 ±19.3^{d}	51.8 ±16.0^{d}	54.6 ±18.8^{d}	52.4 ±13.9^{g}	64.8 ±12.9^{h}	50.3 ±18.8^{g}	69.0 ±14.0^{k}	66.9 ±11.2^{kl}	55.3 ±13.9^{l}	29.2 ±11.2^{o}	22.1 ±7.0^{o}	25.9 ±13.8^{o}	0.05	n.s
O-fresh cut grass	58.4 ±20.6^{a}	49.4 ±16.7^{ab}	42.5 ±22.5^{b}	55.5 ±24.0^{d}	48.8 ±20.7^{d}	49.3 ±16.7^{d}	51.8 ±21.8^{gh}	58.9 ±18.3^{g}	45.2 ±16.7^{h}	36.8 ±31.1^{k}	34.4 ±25.6^{k}	36.9 ±32.1^{k}	8.4 ±5.3^{o}	8.1 ±7.0^{o}	7.7±7.1^{o}	n.s	<0.01
O-nutty	13.0 ±7.6^{ab}	13.2 ±8.5^{b}	20.9 ±10.9^{c}	17.4 ±10.0^{d}	17.8 ±8.8^{d}	20.2 ±9.5^{d}	14.6 ±6.6^{g}	13.7 ±10.3^{g}	21.3 ±9.5^{h}	10.6 ±10.0^{k}	12.6 ±14.0^{k}	16.6 ±11.6^{k}	39.9 ±25.3^{o}	45.4 ±26.0^{o}	37.4 ±24.9^{o}	n.s	0.04
A-green	80.4 ±9.2^{a}	57.4 ±16.6^{b}	45.3 ±17.2^{c}	78.0 ±12.5^{d}	62.1 ±17.7^{e}	51.2 ±17.0^{f}	77.6 ±9.8^{g}	66.6 ±14.2^{h}	55.2 ±17.0^{i}	21.5 ±13.8^{k}	26.3 ±18.0^{k}	26.2 ±16.5^{k}	5.8 ±4.3^{o}	5.8 ±4.9^{o}	6.3±4.1^{o}	<0.01	<0.01
A-shiny	64.0 ±16.4^{a}	60.8 ±18.8^{a}	60.4 ±19.2^{a}	64.5 ±16.4^{d}	59.8 ±17.7^{d}	60.8 ±19.6^{d}	65.5 ±15.3^{gh}	60.2 ±18.4^{hi}	58.2 ±19.6^{i}	57.5 ±21.5^{k}	54.9 ±24.4^{k}	53.0 ±22.5^{k}	63.8 ±22.2^{o}	56.4 ±23.8^{p}	60.5 ±23.8^{op}	n.s	n.s
Tex-adherance	22.3 ±4.5^{a}	32.1 ±9.7^{b}	34.5 ±9.9^{bc}	22.7 ±6.6^{d}	32.4 ±10.3^{d}	36.0 ±13.0^{e}	26.3 ±6.5^{g}	34.0 ±8.2^{hi}	37.7 ±13.0^{i}	27.6 ±11.4^{k}	37.1 ±11.6^{l}	37.8 ±7.3^{l}	17.3 ±4.5^{o}	26.3 ±7.3^{p}	28.8 ±5.6^{p}	<0.01	n.s
Tex-oily	61.0 ±23.4^{a}	61.0 ±20.7^{a}	60.0 ±16.5^{a}	53.5 ±21.8^{d}	58.1 ±16.1^{d}	56.2 ±16.8^{d}	55.7 ±24.5^{g}	65.5 ±11.0^{g}	60.3 ±16.8^{g}	64.9 ±20.8^{k}	61.2 ±20.5^{k}	58.3 ±17.4^{k}	39.0 ±25.1^{o}	39.9 ±29.2^{o}	48.2 ±26.1^{p}	n.s	n.s
Tex-particles	43.7 ±20.6^{a}	53.2 ±26.8^{b}	52.1 ±27.1^{bc}	53.4 ±23.5^{d}	54.3 ±22.9^{d}	57.6 ±21.7^{d}	37.9 ±22.6^{g}	36.4 ±20.0^{g}	38.9 ±21.7^{g}	11.0 ±2.7^{k}	10.6 ±5.6^{kl}	18.2 ±12.5^{l}	6.6 ±4.3^{o}	7.1 ±5.5^{o}	6.7±4.9^{o}	n.s	<0.01
T-salty	15.3 ±6.2^{ab}	13.2 ±8.8^{b}	23.2 ±6.0^{c}	14.5 ±6.8^{d}	17.4 ±5.2^{d}	18.8 ±5.0^{d}	45.1 ±21.7^{g}	38.8 ±18.1^{g}	42.9 ±5.0^{g}	22.9 ±10.3^{k}	23.5 ±12.1^{kl}	33.8 ±18.3^{l}	10.8 ±4.8^{o}	11.2 ±4.7^{o}	10.8 ±5.0^{o}	n.s	<0.01
T-sourness	22.4 ±12.9^{a}	19.1 ±10.1^{a}	23.7 ±15.6^{a}	24.5 ±15.8^{d}	23.5 ±13.1^{d}	20.9 ±9.8^{d}	33.1 ±21.4^{g}	26.6 ±13.7^{g}	29.5 ±9.8^{g}	43.4 ±26.5^{k}	39.6 ±26.9^{kl}	44.5 ±28.4^{k}	18.7 ±8.6^{o}	22.8 ±18.2^{o}	18.7 ±7.6^{o}	n.s	0.02
T-sweetness	16.4 ±8.7^{ab}	17.0 ±11.9^{b}	22.3 ±13.2^{c}	18.1 ±8.7^{d}	18.5 ±7.0^{d}	22.3 ±11.4^{d}	18.4 ±9.8^{g}	18.9 ±8.3^{g}	20.3 ±11.4^{g}	13.2 ±5.8^{k}	16.3 ±10.0^{kl}	21.0 ±13.6^{l}	20.1 ±11.0^{o}	19.7 ±8.7^{o}	23.0 ±12.7^{o}	0.02	n.s
T-umami	27.8 ±12.0^{a}	33.0 ±14.5^{a}	29.3 ±12.2^{a}	31.3 ±15.1^{d}	38.6 ±14.8^{d}	30.3 ±12.9^{d}	29.7 ±10.8^{g}	34.8 ±12.8^{gh}	36.9 ±12.9^{h}	25.2 ±14.1^{k}	23.2 ±11.2^{k}	36.3 ±17.7^{l}	30.2 ±14.0^{o}	40.4 ±18.2^{p}	34.9 ±18.2^{op}	n.s	n.s
T-bitterness	26.0 ±11.5^{a}	23.1 ±13.9^{a}	28.2 ±21.6^{a}	26.0 ±7.0^{d}	26.0 ±11.2^{d}	25.3 ±12.6^{d}	26.9 ±8.0^{g}	20.9 ±11.7^{g}	21.4 ±12.6^{g}	41.3 ±19.1^{k}	39.9 ±20.2^{k}	41.8 ±23.1^{k}	22.1 ±14.1^{o}	27.2 ±13.3^{o}	21.6 ±14.4^{o}	n.s	<0.01
F-fish liver oil	37.8 ±20.8^{ab}	42.2 ±17.2^{a}	30.5 ±14.9^{b}	39.5 ±24.1^{d}	30.5 ±13.0^{d}	35.1 ±20.5^{d}	36.3 ±21.8^{g}	31.1 ±16.4^{g}	30.4 ±20.5^{g}	39.0 ±11.7^{k}	40.8 ±13.7^{k}	45.4 ±22.5^{k}	11.3 ±8.6^{o}	12.5 ±12.3^{o}	11.8 ±8.6^{o}	n.s	n.s
F-grass	50.9 ±22.5^{a}	48.6 ±21.3^{ab}	39.5 ±17.9^{b}	52.8 ±23.1^{d}	43.0 ±19.8^{d}	40.6 ±17.1^{e}	48.5 ±24.3^{g}	44.0 ±15.0^{gh}	39.4 ±17.1^{h}	28.4 ±25.8^{k}	25.4 ±24.2^{k}	22.5 ±17.5^{k}	10.7 ±7.5^{o}	10.6 ±11.5^{o}	10.4 ±10.2^{o}	0.04	<0.01
F-nutty	14.8 ±9.7^{a}	18.9 ±16.0^{ab}	24.9 ±15.4^{b}	20.2 ±13.7^{d}	22.2 ±13.2^{d}	27.9 ±19.1^{d}	16.9 ±9.5^{g}	19.0 ±13.6^{g}	21.9 ±19.1^{g}	11.7 ±9.9^{k}	10.5 ±9.4^{k}	19.6 ±16.0^{k}	43.8 ±23.0^{o}	52.6 ±25.1^{p}	49.5 ±26.7^{op}	0.05	n.s

Values are given as mean±standard deviation. Different lowercase letters (a-p) mean significant differences between samples differing in oil content and within the same sample type and descriptor (Tukey's post hoc test, $p<0.05$).
n.s *not significant ($p>0.05$)*

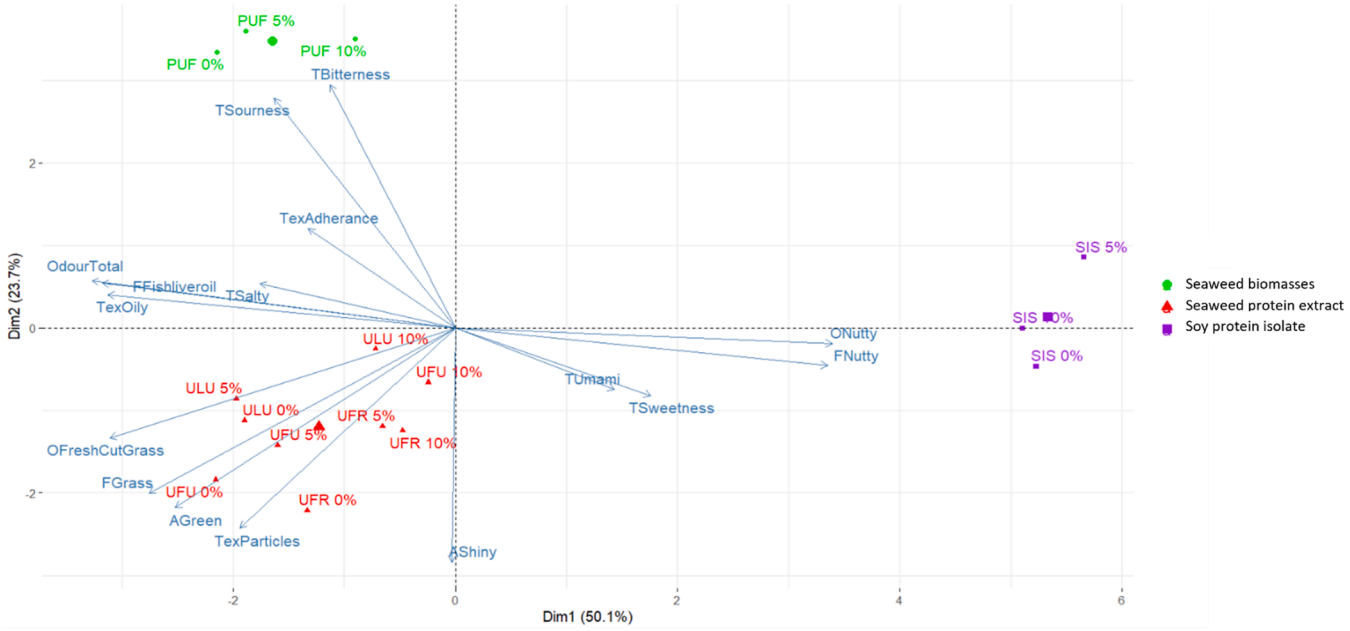

Fig. 2. Principal component analysis (PCA) of the average sensory scores of emulsions with 0, 5, and 10 % oil containing unwashed and washed *U. fenestrata* (UFU and UFR, respectively), unwashed *U. linza* (ULU), a pH-shift protein extract from unwashed *U. fenestrata* (PUF), or soy protein isolate (SIS).

U. fenestrata can enable the incorporation of *Ulva* protein extracts into a broader range of food formulations without overshadowing other flavors and odors. This result is further discussed in Section 3.3 alongside data from volatile analysis.

Several sensorial attributes were found to be important in distinguishing the *U. fenestrata* protein extract from soy protein isolate (Fig. 2). Specifically, nutty odor and flavor scores were higher in the soy protein isolate. On the other hand, total odor, bitter and sour taste, as well as fish liver oil flavor were more pronounced in the seaweed protein extract. The latter characteristic indicates that the potential food applications of protein extracts from *U. fenestrata* are narrower when compared to soy-based protein extracts, making them particularly suitable for products where a "marine" sensory profile is desired (Mouritsen et al., 2019). Such vehicles could be convenient crunchy snack products or powders to be used as a supplement or condiment (Figueroa et al., 2023). Hence, these extracts could serve as flavoring agents, while also providing dietary protein, polyunsaturated fatty acids (PUFA), bioactive polysaccharides, and essential elements (Harrysson et al., 2018; Trigo et al., 2023). Regarding bitterness, further research shall explore potential changes in bitterness in the presence of acids and salts from other food matrix components, as their concentration can either enhance or suppress the perceived bitterness (Breslin, 1996). As for sourness, emulsions containing the commercial soy protein isolate had an average pH of 7.17±0.19, which was approximately 3 pH units higher than the average pH observed in emulsions with seaweed protein extract. As discussed above, this pH difference was likely the main responsible for the differences in sourness scores and can be attributed to the industrial practice of neutralizing precipitated soy proteins prior to their use (Endres, 2001).

Emulsions with seaweed biomasses can be sub-categorized based on their oil content (Fig. 2). Those with a 10 % oil differed from the oil-free variants (i.e., suspensions) primarily in terms of a lighter green color. This sub-categorization is even more evident when performing the PCA without the emulsions containing soy protein isolate (**Supplementary material: Fig. S2**). The presence of oil droplets in emulsions with 5 or 10 % oil results in scattering and refraction of light, distinct from a continuous phase of either water or oil. Consequently, this scattering effect shapes how the emulsion interacts with light, ultimately influencing its appearance (Höhler et al., 2014). Other descriptors affected by increasing the oil level in the emulsions containing seaweed included a reduction in grassy flavor and odor (Fig. 2) accompanied by higher adhesiveness scores (Table 2). The changes in grass flavor and odor are discussed in Section 3.3, whereas the higher adherence texture scores are probably related to increased viscosity.

The washing of *U. fenestrata* retained scores for all sensory descriptors, including salty taste, contrary to the initial hypothesis. It is also worth mentioning that emulsions containing *U. linza* showed the highest scores in salty taste, thereby explaining why sample type had a significant effect on this sensory descriptor (Table 2).

3.2. Total ash content and amino acid analysis of Ulva biomass and protein ingredients

Ash determination was carried out to mainly relate it to saltiness since sodium is one of the most abundant minerals in *Ulva* spp. (Jönsson et al., 2023). Total ash in *U. linza* was 57 % higher than that in *U. fenestrata* (Table 3), thus explaining the relatively higher salty taste scores for *U. linza*. Additionally, the ash content in both species was above that reported elsewhere, particularly for *U. linza* where values ranged from 24 % to 36 % (dw), whereas for *U. fenestrata* they spanned from 16 % to 25 % (dw) (Gao et al., 2022; Hamouda et al., 2022; Harrysson et al., 2018; Steinhagen et al., 2022). Based on the cited studies for *U. intestinalis*, differences in ash content could be related to the effect of harvest location (Yellow and Mediterranean Seas vs. water collected from the North Sea in our study) and cultivation type (wild vs. tank-cultivated). Another potential effect relates to the morphology of *U. linza* since the biomass is composed of hollow compartments, making it difficult to drain seawater after harvesting.In contrast, *U. fenestrata*, with its leafy morphology, does not face this issue.

Washing *U. fenestrata* resulted in a biomass with 28 % less ash on a dw basis (Table 3). Despite this reduction, no clear differences in salty taste were found between unwashed and washed biomasses (Section 3.1). A study conducted on *Saccharina latissima* (Linnaeus) C.E.Lane, C. Mayes, Druehl & G.W.Saunders revealed that washing the kelp in freshwater at 45 °C for 2 min with a seaweed-to-water ratio of 1:4, reduced ash content by 52 %. The same study reported lower scores of salty taste in the washed biomass, when compared to *S. latissima* subjected to a similar washing process with seawater (Krook et al., 2023).

Table 3
Total ash and amino acids contents of the seaweed biomasses and protein ingredients used to prepare the emulsions (N=3, unless stated otherwise).

Sample	Total ash content (g/100 g dw)	Amino acids			
		Total content (g/100 g dw)	TEAA (g/100 g protein)	Limiting amino acid (s)***	N-to-protein conversion factor
U. fenestrata	27.2±0.6[a]	13.2±0.3[a]	40.3±0.3[ab]	Lysine	4.91
U. fenestrata, washed	19.6±0.3[b]	18.2±0.8[b]	39.4±0.9[a]	None	4.97
U. linza	42.6±<0.1[c]	9.5±0.1[a]	41.5±0.3[b]	None	4.79
Protein extract *U. fenestrata**	15.2**	41.1±0.1[c]	43.9±0.2[c]	None	n.a
Soy protein isolate	3.6±0.5[d]	78.5±2.7[d]	39.7±0.6[a]	None	n.a

Within each column, different letters (a-d) mean statistical differences between the samples (Kruskal-Wallis test, $p<0.05$); TEAA *total essential amino acids*; n.a *not analyzed*
*The total ash content, total amino acid content, TEAA, and limiting amino acid(s) of the original biomass were 24.8±1.1 %, 14.4±0.8 %, 40.6±0.6 %, and none, respectively; **N=1 due to limitation on sample amounts; ***Limiting amino acids according to the amino acid scoring pattern recommended for an adult by WHO/FAO/UNU (2007).

Thus, if one had selected higher water temperatures in our study, it could have facilitated more ash leaching, potentially contributing to lower saltiness scores. However, cold tap water is often enough to remove e.g., sand and epiphytes. In the present work, saltiness scores also remained similar between the protein extract and its original biomass albeit the former contained 38 % less ash. Additional factors contributing to saltiness may be associated with the up-concentration of peptides during biomass washing or pH-shift processing.. For instance, glutamate/glutamine-containing peptides have been identified to exhibit a pronounced salt taste enhancement effect in soy sauces (Le et al., 2022).

Total amino acids (TAA) were up-concentrated 2.85-fold after pH-shift processing (Table 3)and the TAA yield (i.e., the amount of TAA in the protein extract relative to the amount of TAA of the input material) was 1.62 %. The TAA up-concentration in this work falls within the mid-range of what is reported elsewhere for pH-shift-based protein extraction of *Ulva* spp. – up-concentration between 2.0 and 3.5-fold (Harrysson et al., 2018; Magnusson et al., 2019; Trigo et al., 2021). In contrast, TAA yield was lower than those reported in the earlier *Ulva* studies, where yields typically ranged from 8 to 20 %. One possible reason for this could be attributed to the non-optimized scale used to do the pH-shift processing. More specifically, this study utilized around 1.2 kg fw of starting material, a scale significantly larger than the 0.07–0.6 kg fw range employed in the aforementioned *Ulva* studies. As expected, subjecting *U. fenestrata* to tap water washing significantly increased TAA. This can be attributed to the leaching of ash, thereby increasing the relative contribution of TAA to the total dw. We observed a similar phenomenon when washing *S. latissima* (Trigo et al., 2023). Between Ulva species, *U. fenestrata* had a higher TAA content than *U. linza* ($p<0.05$). The contents for both species were slightly below those found in literature. For *U. fenestrata*, TAA content ranged from 16.5 to 29.5 % dw (Harrysson et al., 2018; Steinhagen et al., 2024; Trigo et al., 2021), while for *U. linza* it spanned from 12.5 to 20.0 % dw (Ganesan et al., 2014; Gao et al., 2022).

Concerning protein nutritional quality, the protein extract derived from *U. fenestrata* had the highest ratio of total essential amino acids (TEAA) to TAA, which was significantly higher ($p<0.05$) compared to the TEAA-level of the soy protein isolate (Table 3). Washing *U. fenestrata* retained the TEAA and no differences in this parameter were found between unwashed *U. fenestrata* and *U. linza*. According to the amino acid scoring pattern recommended for an adult by WHO/FAO/UNU (2007), all seaweed biomass and protein extracts, apart from unwashed *U. fenestrata*, exhibited no limiting amino acids (Table 3). Moreover, all seaweed biomasses were close to the N-to-protein conversion factor of 5 proposed by Angell et al. (2016).

3.3. Analysis of volatile compounds in the produced emulsions

Table 4 shows the content of 2-ethylfuran, pentanal, hexanal, and cyclohexyl isothiocyanate in emulsions containing seaweed or the pH-shift-based protein extract from *U. fenestrata*.

In terms of species-related effects, the contents of pentanal and hexanal, two common lipid oxidation products, were similar between *U. fenestrata* and *U. linza* ($p>0.05$). On the other hand, 2-ethylfuran, which can be generated by the reaction of unsaturated aldehydes and proteinaceous material (Adams et al., 2011), was only detected in *U. linza*. This furan is characterized by having chemical and malty aromas (Urlass et al., 2023) and it has been detected during storage of *Ulva lactuca* Linnaeus (López-Pérez et al., 2021) as well as in fresh *Undaria pinnatifida* (Harvey) Suringar (Ferraces-Casais et al., 2013). Moreover, 2-ethylfuran was likely odor-active in the emulsion with 0 % oil since its content exceeded by almost 2-fold its odor threshold in water (0.23 μg/100 mL as reported by Giri et al., 2010).

As shown in Table 4, washing the biomass resulted in emulsions with

Table 4
Content of selected volatile compounds present in emulsions containing seaweed and protein extracts thereof (N=3, unless otherwise specified).

Oil content (%)	Sample	Volatile compound (μg/100 mL emulsion)			
		2-ethylfuran	Pentanal	Hexanal	Cyclohexyl isothiocyanate
0	*U. fenestrata*	n.d	2.10±0.35[ab]	4.23±0.22[ab]	< LoQ
	U. fenestrata, washed	n.d	4.00±2.15[ab]	6.23±1.58[b]	< LoQ
	U. linza	0.39±0.03[a]	3.33±0.76[ab]	4.66±0.50[ab]	< LoQ
	Protein extract *U. fenestrata**	0.32	10.3	10.3	< LoQ
5	*U. fenestrata*	n.d	1.93±0.50[ab]	2.74±0.17[cd]	< LoQ
	U. fenestrata, washed	n.d	4.81±1.01[b]	2.63±0.37[bc]	< LoQ
	U. linza	0.07±0.02[b]	2.87±0.88[ab]	2.55±0.42[cd]	< LoQ
10	*U. fenestrata*	n.d	1.42±0.19[a]	1.97±0.03[d]	< LoQ
	U. fenestrata, washed	n.d	3.31±1.44[ab]	2.63±0.37[cd]	< LoQ
	U. linza	0.09±0.02[b]	1.95±0.31[ab]	1.84±0.35[d]	< LoQ

Within each column, different letters (a-d) mean statistical differences between the samples (Kruskal-Wallis test, $p<0.05$);*N=1 due to limitations in sample amounts; n.d *not detected*; LoQ *limit of quantification* (0.07 μg/100 mL)

increased content in pentanal (t=6.10, df=1, p=0.014); although an increase in hexanal content was also observed, it did not reach statistical significance (t=2.88, df=1, p=0.09). The higher pentanal content might be attributed to the leaching of ash into the washing waters (Section 3.2), hence increasing its relative content. Alternatively, the osmotic shock induced by washing with tap water could have facilitated interactions between lipoxygenase (LOX)- present in *Ulva* spp. (Kuo et al., 1996, 1997; Tsai et al., 2008) - and n-6 PUFAs, which account for about 12.9 % of the total fatty acids in *U. fenestrata* (Harrysson et al., 2018). The detected aldehydes, including pentanal, were thus likely a partial result of LOX-mediated lipid oxidation (Iglesias & Medina, 2008). Given the wide range in odor threshold reported elsewhere for pentanal (1.2 to 4.2 μg/100 mL water, Urlass et al., 2023), it is difficult to conclude whether its fermented notes, as described by Urlass et al. (2023), actively contributed to the odor of the 0 % oil emulsions.

Protein extraction from *U. fenestrata* increased pentanal and hexanal content 4.9 and 2.4-fold, respectively, and 2-ethylfuran was above the quantification limit (Table 4). Hexanal has a grassy odor and shares a similar formation pathway to pentanal; it can however also be produced via the degradation of preformed volatiles such as 2,4-decadienal or 2-octenal (Iglesias & Medina, 2008; Urlass et al., 2023). As introduced above, 2-ethylfuran can be generated when unsaturated aldehydes derived from e.g., LOX activity react with amino acids/peptides/proteins (Adams et al., 2011). Although we did not explore the relationship between LOX activity in the different sample categories and their concentration of the measured volatiles, it is reported that LOX isolated from *Ulva* spp. has an optimal pH of 7.5 while its activity decreases up to 50 and 80 % at pH 4 and pH 9, respectively (Kuo et al., 1996). Thus, we hypothesize that LOX-mediated lipid oxidation occurred during protein extraction, particularly during the 1 h osmotic shock step where the pH ranged from 6.1 to 6.9 – the exact pH value being dependent on the seaweed batch. We also expect non-enzymatic oxidation routes, involving the breakdown of hydrogen and lipid peroxides, to occur given the high levels of trace elements in *Ulva* spp. Moreover, chlorophyll in the presence of light can become excited and initiate lipid oxidation (Hu & Jacobsen, 2016). In pea protein extraction using the pH-shift method, hexanal was also found in significantly higher amounts in the protein isolate, whereas pentanal was not detectable either in the pea biomass or protein isolate (Sajib et al., 2023). Interestingly, Sajib et al. (2023) also reported a significant increase in 2-pentylfuran, a different furan from the one we found. Hexanal derives primarily from n-6 PUFA (Nogueira et al., 2019), whereas pentanal has been reported to develop to a greater extent in oxidized oils containing n-3 PUFA (Medina et al., 1999). Developments of hexanal and other saturated lipid oxidation-derived aldehydes such as octanal and heptanal during pH-shift processing have also been found when using fish biomass. However, their formation could be mitigated, for example, by cross-processing the fish with antioxidant-rich materials (Zhang et al., 2022).

Odor is a key component in flavor perception (Fanali et al., 2020). Therefore, one might expect a strong grassy/green flavor and odor in emulsions with protein extract since hexanal content was 43 times above its odor threshold in water (0.24 μg/100 mL as reported by Urlass et al., 2023) versus only 5.7–8.4 times in emulsions with unwashed *U. fenestrata*. However, this was not what was observed. Instead, the former emulsions had a lower intensity of grass notes, particularly in terms of flavor (Table 2). This suggests that 2-ethylfuran, which was above its odor threshold in the emulsion with protein extract, partially masked the perception of grassy odor/flavor and, (ii) the lower pH of the emulsions containing protein extract affected the adsorption capacity of proteins to seaweed volatiles with grassy notes and with odor thresholds below our method's detection limit, such as (Z, E)-3,5-octadien-2-one (Urlass et al., 2023; Yang et al., 2017). Regarding the first point, the scientific understanding of how the human brain translates volatile compounds into odor perception is still in its early stages. Nevertheless, it is known that for a specific odor receptor in the brain, a volatile can be an antagonist or agonist of another volatile (Xu et al., 2023). Thus, in our study, 2-ethylfuran might have acted as an antagonist of hexanal.

Increasing oil levels resulted in a significant reduction in the detection of hexanal (t=20.4, df=2, p<0.001), the differences being most evident between emulsions with 0 and 10 % oil (Table 4). This observation confirmed our initial hypothesis that oil would influence odor, mainly due to entrapment of e.g., hexanal within the emulsion oil phase, hence limiting its partition into the air phase. Ultimately, this likely contributed to the lower intensity of grassy odor in emulsions with 10 % oil compared to those with 0 % oil (Section 3.1).

To our knowledge, this is one of the first works on seaweed aiming at getting an in-depth understanding of how post-harvest techniques and downstream protein extraction influence sensory quality. Future trials shall evaluate how neutralizing the protein extract affects sourness and how bitterness could be controlled in the presence of acids and salts from other food matrix components. Furthermore, it would be relevant to relate saltiness to saltiness-providing ions such as sodium and potassium as well as to glutamate-containing peptides via peptidomics (Tanambell et al., 2024). Changes in mouthfeel caused by protein extraction could be complemented with tribology (Paul et al., 2022), while the occurrence of lipid oxidation due to washing and protein extraction could be confirmed by relating to key pro-oxidants in seaweed. To facilitate all these future studies, we consider that more efficient protein extraction methods for *U. fenestrata* are needed; the low total protein yield is currently one of the main hurdles to viable economic production of these protein extracts. On this matter, we have recently developed a new extraction method for *U. fenestrata* that delivers protein yields >300 % higher than conventional wet fractionation methods such as the one used in the present study (Trigo et al. under review).

Conclusion

This study aimed to study the sensory quality of emulsions made with *Ulva* spp. by focusing on effects from species, biomass washing, protein extraction, and increasing emulsion oil content. Saltiness was the main sensory difference between emulsions with unwashed *U. linza* and *U. fenestrata*, due to higher ash content in the former species. Washing of *U. fenestrata* retained sensory qualities, including salty taste, despite a decrease in ash content and an increase in pentanal content. The other dominating volatiles, hexanal and 2-ethylfuran, remained unchanged. Protein extraction resulted in emulsions with lower particle sensation and grassy flavor as well as enhanced darker green color, bitterness, and sourness. The three mentioned volatiles were present in higher concentrations in emulsions containing protein extract, indicating lipid oxidation development during protein extraction. Increasing the oil content of the emulsions affected the sensory quality of the seaweed biomasses, particularly by lowering grassy odor and hexanal content. Overall, this study offers new insights into the impact of seaweed species selection, post-harvest treatments, downstream processing, and oil content on sensory quality. Ultimately, these findings can contribute to the development of food products containing seaweed or protein ingredients thereof that match the preferences of Western consumers.

Ethical statement

According to the Swedish Ethics Review Act (SERA), it is not required ethical approval to conduct a sensory analysis in Sweden. Due to this regulation, no human ethics committee was consulted and/or formal documentation process is available. Moreover, SERA applies to research carried out in Sweden if the research includes the processing of sensitive personal data. This study includes questions about food perception which, according to the Data Protection Ordinance, are not classified as sensitive personal data. According to the General Data Protection Regulation, no responses to the questionnaire used in this study include information that can be traced to or used to identify any individual. All participants received written and oral information about

the sensory test and the ingredients of the included products and gave their informed consent to participate. Additionally, the participants could withdraw from the survey at any time without giving a reason and the products tested were safe for consumption.

CRediT authorship contribution statement

João P. Trigo: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Karin Wendin:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sophie Steinhagen:** Writing – review & editing, Resources, Funding acquisition. **Karin Larsson:** Writing – review & editing, Validation, Investigation. **Ingrid Undeland:** Writing – review & editing, Validation, Resources, Project administration, Methodology, 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

All data used was incorporated in this article.

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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.2024.100370.

References

Adams, A., Bouckaert, C., Van Lancker, F., De Meulenaer, B., De Kimpe, N., 2011. Amino acid catalysis of 2-alkylfuran formation from lipid oxidation-derived α,β-unsaturated aldehydes. J. Agric. Food Chem. 59 (20), 11058–11062. https://doi.org/10.1021/jf202448v.

Angell, A.R., Mata, L., de Nys, R., Paul, N.A., 2016. The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. J. Appl. Phycol. 28 (1), 511–524. https://doi.org/10.1007/s10811-015-0650-1.

Araújo, R., Vázquez Calderón, F., Sánchez López, J., Azevedo, I.C., Bruhn, A., Fluch, S., Garcia Tasende, M., Ghaderiardakani, F., Ilmjärv, T., Laurans, M., Mac Monagail, M., Mangini, S., Peteiro, C., Rebours, C., Stefansson, T., Ullmann, J., 2021. Current status of the algae production industry in Europe: an emerging sector of the blue bioeconomy. Front. Mar. Sci. 7 https://doi.org/10.3389/fmars.2020.626389.

Bayarri, S., Taylor, A.J., Hort, J., 2006. The role of fat in flavor perception: effect of partition and viscosity in model emulsions. J. Agric. Food Chem. 54 (23), 8862–8868. https://doi.org/10.1021/jf061537k.

Breslin, P.A.S., 1996. Interactions among salty, sour and bitter compounds. Trends. Food Sci. Technol. 7 (12), 390–399. https://doi.org/10.1016/S0924-2244(96)10039-X.

Celente, G., de, S., Sui, Y., Acharya, P., 2023. Seaweed as an alternative protein source: Prospective protein extraction technologies. Innov. Food Sci. Emerg. Technol. 86 (May), 103374 https://doi.org/10.1016/j.ifset.2023.103374.

Elmosallamy, A., Amer, T., Mohamed, S., Ali, Y., Hussein, S., 2021. Phytochemical constituents of *Ulva Lactuca* and supplementation to improve the Nile tilapia (*Oreochromis Niloticus*) haemato-biochemical status. Egypt. J. Chem. 0. https://doi.org/10.21608/ejchem.2021.60453.3296. –0.

Endres, J.G. (Ed.), 2001. Soy Protein products, characteristics, Nutritional Aspects and Utilization. Taylor & Francis.

Fanali, C., Utczas, M., Dugo, P., Mondello, L., 2020. Flavors and odors analysis. Chemical Analysis of Food. Elsevier, pp. 697–727. https://doi.org/10.1016/B978-0-12-813266-1.00016-4.

Ferraces-Casais, P., Lage-Yusty, M.A., Rodríguez-Bernaldo de Quirós, A., López-Hernández, J., 2013. Rapid identification of volatile compounds in fresh seaweed. Talanta 115, 798–800. https://doi.org/10.1016/j.talanta.2013.06.049.

Figueroa, V., Farfán, M., Aguilera, J.M., 2023. Seaweeds as novel foods and source of culinary flavors. Food Rev. Int. 39 (1), 1–26. https://doi.org/10.1080/87559129.2021.1892749.

Fischer, U., Noble, A.C., 1994. The effect of ethanol, catechin concentration, and pH on sourness and bitterness of wine. Am. J. Enol. Vitic. 45 (1), 6–10. https://doi.org/10.5344/ajev.1994.45.1.6.

Ganesan, K., Suresh Kumar, K., Subba Rao, P.V., Tsukui, Y., Bhaskar, N., Hosokawa, M., Miyashita, K., 2014. Studies on chemical composition of three species of *Enteromorpha*. Biomed. Prevent. Nutr. 4 (3), 365–369. https://doi.org/10.1016/j.bionut.2014.04.001.

Gao, G., Gao, L., Fu, Q., Li, X., Xu, J., 2022. Coculture of the Pacific white shrimp *Litopenaeus vannamei* and the macroalga *Ulva linza* enhances their growth rates and functional properties. J. Clean. Prod. 349 (October 2021), 131407 https://doi.org/10.1016/j.jclepro.2022.131407.

Gephart, J.A., Henriksson, P.J.G., Parker, R.W.R., Shepon, A., Gorospe, K.D., Bergman, K., Eshel, G., Golden, C.D., Halpern, B.S., Hornborg, S., Jonell, M., Metian, M., Mifflin, K., Newton, R., Tyedmers, P., Zhang, W., Ziegler, F., Troell, M., 2021. Environmental performance of blue foods. Nature 597 (7876), 360–365. https://doi.org/10.1038/s41586-021-03889-2.

Giri, A., Osako, K., Ohshima, T., 2010. Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. Food Chem. 120 (2), 621–631. https://doi.org/10.1016/j.foodchem.2009.10.036.

Gullón, P., Astray, G., Gullón, B., Franco, D., Campagnol, P.C.B., Lorenzo, J.M., 2021. Inclusion of seaweeds as healthy approach to formulate new low-salt meat products. Curr. Opin. Food Sci. 40, 20–25. https://doi.org/10.1016/j.cofs.2020.05.005.

Hamouda, M.M., Saad-Allah, K.M., Gad, D., 2022. Potential of seaweed extract on growth, physiological, cytological and biochemical parameters of wheat (*Triticum aestivum* L.) seedlings. J. Soil. Sci. Plant Nutr. 22 (2), 1818–1831. https://doi.org/10.1007/s42729-022-00774-3.

Harrysson, H., Hayes, M., Eimer, F., Carlsson, N.G., Toth, G.B., Undeland, I., 2018. Production of protein extracts from Swedish red, green, and brown seaweeds, *Porphyra umbilicalis* kützing, *Ulva lactuca* linnaeus, and *Saccharina latissima* (Linnaeus) J. V. Lamouroux using three different methods. J. Appl. Phycol. 30 (6), 3565–3580. https://doi.org/10.1007/s10811-018-1481-7.

Harrysson, H., Krook, J.L., Larsson, K., Tullberg, C., Oerbekke, A., Toth, G., Pavia, H., Undeland, I., 2021. Effect of storage conditions on lipid oxidation, nutrient loss and colour of dried seaweeds, *Porphyra umbilicalis* and *Ulva fenestrata*, subjected to different pretreatments. Algal. Res. 56, 102295 https://doi.org/10.1016/j.algal.2021.102295.

Höhler, R., Cohen-Addad, S., Durian, D.J., 2014. Multiple light scattering as a probe of foams and emulsions. Curr. Opin. Colloid. Interface Sci. 19 (3), 242–252. https://doi.org/10.1016/j.cocis.2014.04.005.

Holdt, S.L., Kraan, S., 2011. Bioactive compounds in seaweed: Functional food applications and legislation. J. Appl. Phycol. 23 (3), 543–597. https://doi.org/10.1007/s10811-010-9632-5.

Hu, M., Jacobsen, C. (Eds.), 2016. Oxidative Stability and Shelf Life of Foods Containing Oils and Fats. Elsevier. https://doi.org/10.1016/C2015-0-00077-6.

Iglesias, J., Medina, I., 2008. Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. J. Chromatogr. A 1192 (1), 9–16. https://doi.org/10.1016/j.chroma.2008.03.028.

Jacobsen, M., Bianchi, M., Trigo, J.P., Undeland, I., Hallström, E., Bryngelsson, S., 2023. Nutritional and toxicological characteristics of *Saccharina latissima, Ulva fenestrata, Ulva intestinalis*, and *Ulva rigida*: a review. Int. J. Food Prop. 26 (1), 2349–2378. https://doi.org/10.1080/10942912.2023.2246677.

Jönsson, M., Merkel, A., Fredriksson, C., Karlsson, E.N., Wendin, K., 2023. Nutritional, physicochemical, and sensory characterization of four common Northern European seaweed species intended for food. Algal. Res. 75, 103258 https://doi.org/10.1016/j.algal.2023.103258.

Juul, L., Steinhagen, S., Bruhn, A., Jensen, S.K., Undeland, I., Dalsgaard, T.K., 2022. Combining pressing and alkaline extraction to increase protein yield from *Ulva fenestrata* biomass. Food Bioprod. Process. 134, 80–85. https://doi.org/10.1016/j.fbp.2022.05.006.

Karolkowski, A., Belloir, C., Briand, L., Salles, C., 2023. Non-volatile compounds involved in bitterness and astringency of pulses: a review. Molecules 28 (8), 1–25. https://doi.org/10.3390/molecules28083298.

Kassambara, A., & Mundt, F. (2020). *Factoextra: extract and visualize the results of multivariate data analyses*.

Krook, J.L., Duinker, A., Larssen, W.E., Birkeland, I.M., Skeie, S., Horn, S.J., Stévant, P., 2023. Approaches for reducing the iodine content of the brown seaweed *Saccharina latissima*—effects on sensory properties. J. Appl. Phycol. https://doi.org/10.1007/s10811-023-02974-5, 0123456789.

Kuo, J.-M., Hwang, A., Hsu, H.H., Pan, B.S., 1996. Preliminary identification of lipoxygenase in algae (*Enteromorpha intestinalis*) for aroma formation. J. Agric. Food Chem. 44 (8), 2073–2077. https://doi.org/10.1021/jf950774c.

Kuo, J.-M., Hwang, A., Yeh, D.-B., 1997. Purification, substrate specificity, and products of a Ca 2+ -Stimulating lipoxygenase from sea algae (*Ulva lactuca*). J. Agric. Food Chem. 45 (6), 2055–2060. https://doi.org/10.1021/jf960892f.

Le, B., Yu, B., Amin, M.S., Liu, R., Zhang, N., Soladoye, O.P., Aluko, R.E., Zhang, Y., Fu, Y., 2022. Salt taste receptors and associated salty/salt taste-enhancing peptides:

A comprehensive review of structure and function. Trends. Food Sci. Technol. 129, 657–666. https://doi.org/10.1016/j.tifs.2022.11.014.
López-Pérez, O., del Olmo, A., Picon, A., Nuñez, M., 2021. Volatile compounds and odour characteristics of five edible seaweeds preserved by high pressure processing: Changes during refrigerated storage. Algal. Res. 53, 102137 https://doi.org/10.1016/j.algal.2020.102137.
Magnusson, M., Glasson, C.R.K., Vucko, M.J., Angell, A., Neoh, T.L., de Nys, R., 2019. Enrichment processes for the production of high-protein feed from the green seaweed *Ulva ohnoi*. Algal. Res. 41 (February), 101555 https://doi.org/10.1016/j.algal.2019.101555.
Medina, I., Satué-Gracia, M.T., Frankel, E.N., 1999. Static headspace gas chromatographic analyses to determine oxidation of fish muscle lipids during thermal processing. J. Am. Oil Chem. Soc. 76 (2), 231–236. https://doi.org/10.1007/s11746-999-0223-z.
Mouritsen, O.G., Rhatigan, P., Pérez-Lloréns, J.L., 2019. The rise of seaweed gastronomy: phycogastronomy. Botanica Marina 62 (3), 195–209. https://doi.org/10.1515/bot-2018-0041.
Nogueira, M.S., Scolaro, B., Milne, G.L., Castro, I.A., 2019. Oxidation products from omega-3 and omega-6 fatty acids during a simulated shelf life of edible oils. LWT 101, 113–122. https://doi.org/10.1016/j.lwt.2018.11.044.
Paul, V., Tripathi, A.D., Agarwal, A., Kumar, P., Rai, D.C., 2022. Tribology – novel oral processing tool for sensory evaluation of food. LWT 160, 113270. https://doi.org/10.1016/j.lwt.2022.113270.
Poeloengasih, C.D., Srianisah, M., Jatmiko, T.H., Prasetyo, D.J., 2019. Postharvest handling of the edible green seaweed *Ulva lactuca*: mineral content, microstructure, and appearance associated with rinsing water and drying methods. IOP Conf. Ser. Earth Environ. Sci. 253, 012006 https://doi.org/10.1088/1755-1315/253/1/012006.
Quitral, V., Sepúlveda, M., Gamero-Vega, G., Jiménez, P., 2022. Seaweeds in bakery and farinaceous foods: A mini-review. Int. J. Gastron. Food Sci. 28, 100403 https://doi.org/10.1016/j.ijgfs.2021.100403.
Rajpurohit, B., Li, Y., 2023. Overview on pulse proteins for future foods: ingredient development and novel applications. J. Future Foods 3 (4), 340–356. https://doi.org/10.1016/j.jfutfo.2023.03.005.
Sajib, M., Forghani, B., Kumar Vate, N., Abdollahi, M., 2023. Combined effects of isolation temperature and pH on functionality and beany flavor of pea protein isolates for meat analogue applications. Food Chem. 412, 135585 https://doi.org/10.1016/j.foodchem.2023.135585.
Sajib, M., Undeland, I., 2020. Towards valorization of herring filleting by-products to silage 2.0: Effect of temperature and time on lipid oxidation and non-enzymatic browning reactions. LWT 127, 109441. https://doi.org/10.1016/j.lwt.2020.109441.
Ścieszka, S., Klewicka, E., 2019. Algae in food: a general review. Crit. Rev. Food Sci. Nutr. 59 (21), 3538–3547. https://doi.org/10.1080/10408398.2018.1496319.
Steinhagen, S., Enge, S., Cervin, G., Larsson, K., Edlund, U., Schmidt, A.E.M., Wahlström, N., Kollander, B., Pavia, H., Undeland, I., Toth, G.B., 2022. Harvest time can affect the optimal yield and quality of sea lettuce (*Ulva fenestrata*) in a sustainable sea-based cultivation. Front. Mar. Sci. 9 https://doi.org/10.3389/fmars.2022.816890.
Steinhagen, S., Stedt, K., Trigo, J.P., Undeland, I., Pavia, H., 2024. A step towards closing the food-waste gap in novel protein sources: Post-harvest protein boost of the seaweed crop *Ulva* by herring production tub water. Future Foods 9, 100347. https://doi.org/10.1016/j.fufo.2024.100347.
Tanambell, H., Danielsen, M., Devold, T.G., Møller, A.H., Dalsgaard, T.K., 2024. *In vitro* protein digestibility of RuBisCO from alfalfa obtained from different processing histories: Insights from free N-terminal and mass spectrometry study. Food Chem. 434, 137301 https://doi.org/10.1016/j.foodchem.2023.137301.
Toth, G.B., Harrysson, H., Wahlström, N., Olsson, J., Oerbekke, A., Steinhagen, S., Kinnby, A., White, J., Albers, E., Edlund, U., Undeland, I., Pavia, H., 2020. Effects of irradiance, temperature, nutrients, and pCO2 on the growth and biochemical composition of cultivated *Ulva fenestrata*. J. Appl. Phycol. https://doi.org/10.1007/s10811-020-02155-8.
Trigo, J.P., Engström, N., Steinhagen, S., Juul, L., Harrysson, H., Toth, G.B., Pavia, H., Scheers, N., Undeland, I., 2021. *In vitro* digestibility and Caco-2 cell bioavailability of sea lettuce (*Ulva fenestrata*) proteins extracted using pH-shift processing. Food Chem. 356 (October 2020) https://doi.org/10.1016/j.foodchem.2021.129683.
Trigo, J.P., Stedt, K., Schmidt, A.E.M., Kollander, B., Edlund, U., Nylund, G., Pavia, H., Abdollahi, M., Undeland, I., 2023. Mild blanching prior to pH-shift processing of *Saccharina latissima* retains protein extraction yields and amino acid levels of extracts while minimizing iodine content. Food Chem. 404, 134576 https://doi.org/10.1016/j.foodchem.2022.134576.
Trigo, J.P., Steinhagen, S., Stedt, K., Krona, A., Verhagen, S., Pavia, H., Abdollahi, M., Undeland, I. A new method for protein extraction from *Ulva fenestrata* (sea lettuce) via surfactants and alkaline aqueous solutions. Unpublished results; under review.
Tsai, C.J., Li, W.F., Pan, B.S., 2008. Characterization and immobilization of marine algal 11-lipoxygenase from *Ulva fasciata*. J. Am. Oil Chem. Soc. 85 (8) https://doi.org/10.1007/s11746-008-1262-6.
Urlass, S., Wu, Y., Nguyen, T.T.L., Winberg, P., Turner, M.S., Smyth, H., 2023. Unravelling the aroma and flavour of algae for future food applications. Trends. Food Sci. Technol. 138, 370–381. https://doi.org/10.1016/j.tifs.2023.06.018.
USDA. (2018). *USDA national nutrient database for standard reference*. https://fdc.nal.usda.gov/fdc-app.html#/food-details/174030/nutrients.
Vellinga, R.E., Sam, M., Verhagen, H., Jakobsen, L.S., Ravn-Haren, G., Sugimoto, M., Torres, D., Katagiri, R., Thu, B.J., Granby, K., Hoekstra, J., Temme, E.H.M., 2022. Increasing seaweed consumption in the Netherlands and Portugal and the consequences for the intake of iodine, sodium, and exposure to chemical contaminants: a risk-benefit study. Front. Nutr. 8 (January), 1–14. https://doi.org/10.3389/fnut.2021.792923.
Wendin, K., Undeland, I., 2020. Seaweed as food – Attitudes and preferences among Swedish consumers. A pilot study. Int. J. Gastron. Food Sci. 22 (June), 100265 https://doi.org/10.1016/j.ijgfs.2020.100265.
Xu, L., Zou, D.-J., Firestein, S., 2023. Odor mixtures: A chord with silent notes. Front. Ecol. Evol. 11 https://doi.org/10.3389/fevo.2023.1135486.
Yang, Q.L., Lou, X.W., Wang, Y., Pan, D.D., Sun, Y.Y., Cao, J.X., 2017. Effect of pH on the interaction of volatile compounds with the myofibrillar proteins of duck meat. Poult. Sci. 96 (6), 1963–1969. https://doi.org/10.3382/ps/pew413.
Zhang, J., Abdollahi, M., Alminger, M., Undeland, I., 2022. Cross-processing herring and salmon co-products with agricultural and marine side-streams or seaweeds produces protein isolates more stable towards lipid oxidation. Food Chem. 382, 132314 https://doi.org/10.1016/j.foodchem.2022.132314.
Zhang, M., Li, M., Bai, F., Yao, W., You, L., Liu, D., 2023. Effect of fat to lean meat ratios on the formation of volatile compounds in mutton shashliks. Foods 12 (10), 1929. https://doi.org/10.3390/foods12101929.