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Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Alkaline pH increases protein extraction yield and solubility of the extracted protein from sugar kelp (*Saccharina latissima*)

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ARTICLE INFO

Article history:
Received 4 August 2022
Received in revised form 28 April 2023
Accepted 25 May 2023
Available online 29 May 2023

Keywords:
Seaweed
Macroalgae
Alternative food protein
Alkaline protein extraction
Protein solubility
pH-shift

ABSTRACT

Seaweed is gaining interest as a sustainable source of protein. An often-used method for protein extraction from seaweed is alkaline extraction. In this study, the robustness of alkaline protein extraction was tested against protein extraction at neutral pH for sugar kelp (*S. latissima*) harvested at different time points and at different cultivation sites. The harvest time and place significantly influenced the total nitrogen (N) content of the biomass, the N extraction yield as % of biomass N, and the protein solubility. However, there was no specific pattern regarding harvest season and biomass age. The N extraction yield as % of biomass dry matter content was more similar between biomasses. The N extraction yield (% of biomass N) varied from 1.59 % to 25.22 % (mean: 6.61 %) for the control extraction, whereas it was 5.32–52.96 % (mean: 16.03 %) for the alkaline extraction. Hence, the alkaline extraction increased the N yield, and gave a higher N concentration in the protein extracts and improved the solubility of the protein extracts across the different biomass batches compared to the control method at neutral pH. This states the robustness of the alkaline protein extraction for sugar kelp and suggests improved suitability in foods compared to the extraction at neutral pH.

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1. Introduction

Saccharina latissima (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders is the most produced seaweed species in Europe (Araújo et al. 2021), with commercial cultivation in several European countries, e.g., Denmark, Norway, Sweden and Faroe Islands (Bak et al. 2019, Boderskov et al. 2021). However, the production is limited compared to seaweed production in other parts of the world, mainly Asia (FAO, 2018), where there is a tradition for seaweed production and

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https://doi.org/10.1016/j.fbp.2023.05.008
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consumption. There is an increasing interest in seaweed production in Europe, as part of the movement towards a future with sustainable food production systems (Gephart et al. 2021). Among various applications, seaweed is gaining interest as a source of protein, due to the high biomass productivity, bioremediation potential and an amino acid profile containing all the essential amino acids (Marinho et al. 2015a, Bak et al. 2019). However, the protein needs to be extracted as the high content of fibers in the biomass impedes the digestibility of the protein (Horie et al. 1995, Dégen et al. 2007). Extraction/concentration of the protein has shown to increase protein digestibility of several seaweed species, e.g., *Ulva* spp. (Bikker et al. 2016, Trigo et al. 2021, Juul et al. 2022), *S. latissima* and *Palmaria palmata* (Krogdahl et al. 2021).

Protein extraction from *S. latissima* is often performed using alkaline extraction, solubilizing the proteins at high pH, and afterwards concentrating the protein through precipitation (Vilg and Undeland, 2017, Harrysson et al. 2018, Abdollahi et al. 2019). Biomass stabilization methods (e.g., sun-drying and freeze-drying) affect the extractability of the protein, the protein profile, and the protein functionality, such as the solubility of the final protein extracts (Abdollahi et al. 2019). The protein extract solubility in general increases with increasing pH and the biomass stability method mainly affects the solubility in the acidic pH range. Further, a freeze/thaw-step during acid precipitation increases the protein precipitation yield (Abdollahi et al. 2019). Regarding protein functionality, shifting the pH to alkaline conditions and back to neutral pH has shown to increase the solubility of alfalfa protein concentrate (Nissen et al. 2021), barley protein isolate (Silventoinen and Sozer, 2020), and pea protein (Jiang et al. 2017). Alkaline extraction, as opposed to screw press extraction, of *Ulva fenestrata* protein also showed to increase the protein solubility (Juul et al. 2021). This suggests that subjecting either the biomass during protein extraction or the resulting protein extract to an alkaline environment will increase the protein solubility of the final protein extract. The increased solubility is due to a partial unfolding of the tertiary protein structure known as the "molten globule" structure. This is caused by increased repulsion in the protein side chains during extreme pH conditions (Kristinsson and Hultin, 2003).

The aim of this study was to investigate the solubility of protein from *S. latissima* extracting the protein at neutral and alkaline pH, respectively. The protein extracted at alkaline pH was hypothesized to have a higher solubility in water at different pH values. Moreover, the alkaline extraction was hypothesized to give a higher protein extraction yield. The extractions were performed on a range of *S. latissima* biomasses harvested at different time points and at different cultivation sites to test whether biomass age and composition/quality affected the extraction yields and solubility of the protein extracts.

2. Methods

2.1. Chemicals

Deionized (18.2 MΩ) filtered water (0.22 µm) (MilliQ-water) was from a Milli-Q system, Millipore SAS (Molsheim, France). Sodium dodecyl sulfate (SDS), bovine serum albumin (BSA), and Folin-Ciocalteu (2 N) reagent was purchased from Serva (Heidelberg, Germany). $CuSO4{\cdot}5H2O$ was from Fluka (Switzerland) and HCl 37 %, NaOH, EDTA and Na-K-tartrate was purchased from Merck (Darmstadt, Germany).

2.2. Biomass

S. latissima was cultivated and harvested at three different cultivation sites, two in Denmark (Hjarnø and Grenaa) and one in Norway (Bergen). The biomass from Denmark was cultivated using a long-line cultivation system with lines suspended in the upper water column (1–4 m depth). The age and biomass quality varied as the biomass was deployed and harvested at different time points. Biomass harvested in June 2020 was fouled with bryozoans, and biomass harvested in November 2020 mainly with hydroids and bryozoans. The harvested biomass was drained and frozen at – 18 °C until processing. The biomass harvested in Norway was kindly donated by Nordisk Tang. Harvest sites and dates of the different biomass batches is found in Table 1 along with the age of the biomass (unknown for biomass harvested in Norway), and dry matter (DM) and nitrogen (N) content.

Prior to extraction and analysis, the frozen biomass was chopped roughly with a knife to ease processing and thawed at room temperature (RT). Run-off seawater was removed after thawing, and not included in the processing.

2.3. Biomass fermentation

A fraction of the biomass harvested in Norway was fermented by Nordisk Tang prior to protein extraction. The biomass was fermented with a solution of lactobacillus at

Table 1 – Biomass data. The sample name reflects the month and year of harvest and the location (H: Hjarnø, G: Grenå, B: Bergen, BF: Bergen fermented biomass). Age is defined as the number of days from deployment until harvest. Data is represented as mean ± SD (n = 3). DM: dry matter, N: Nitrogen.

Sample name	Harvest site	Deployment date	Harvest date	Age (days)	Dry matter (%)	N (% of DM)	Comments
Jun18H	Hjarnø	21–09–2017	07–06–2018	259	27.1 ± 1.4	0.50 ± 0.01	
Feb20H	Hjarnø	03–10–2019	27–02–2020	147	17.4 ± 0.9	4.90 ± 0.58	
Mar20H	Hjarnø	03–10–2019	26–03–2020	175	12.6 ± 0.3	3.34 ± 0.51	
Jun20H	Hjarnø	08–11–2018	03–06–2020	573	24.1 ± 0.7	0.52 ± 0.02	Epiphytes[ab]
Nov20H	Hjarnø	08–11–2018	26–11–2020	749	22.7 ± 0.2	2.27 ± 0.35	Epiphytes[a]
Jun19G	Grenå	14–11–2018	26–06–2019	224	20.2 ± 2.8	0.54 ± 0.00	
May17B	Bergen		22–05–2017		11.5 ± 0.8	1.56 ± 0.00	
May17BF	Bergen		22–05–2017		11.1 ± 0.7	2.91 ± 0.12	Fermented

[a] Bryozoans,
[b] Hydroids

28 °C until reaching a pH of 4 (24 h). The fermentation was carried out in open vessels. The fermented biomass was stored at – 18 °C until processing.

2.4. Protein extraction

Protein extraction was initiated by homogenizing the thawed biomass with MilliQ-water in a ratio of 1:38 (dry weight seaweed:water (w:w)). The biomass to water ratio was based on the DM of the biomass, as this differed between the biomass batches. High shear homogenization was carried out with a Silverson L5M mixer at 8000 rpm for 3 min on ice. The homogenate was incubated for one h (osmotic shock) on a magnetic stirrer at RT. For the alkaline extraction, the pH of the homogenate was increased to pH 12 using 1 M NaOH and incubated for 20 min at RT while stirring. A control extraction was performed leaving out the alkaline extraction step (Fig. 1). The native pH of the homogenate was pH 6.5. After the incubation steps to solubilize proteins from the biomass, the homogenate was centrifuged at 4800× *g* for 20 min at 4 °C to remove insoluble fibers. Soluble protein in the resulting supernatant fraction (supernatant 1, Fig. 1) was concentrated by acid precipitation, adjusting to pH 2 with 1 M HCl. The acidified supernatant was incubated for 20 min and afterwards frozen at – 18 °C over night to increase precipitation yield (Abdollahi et al. 2019). The day after, the acidified supernatant was thawed and centrifuged at 4800× *g* for 20 min at 4 °C to precipitate the protein. The resulting protein pellets were freeze dried. Extractions were performed in duplicates for each biomass batch.

Highest yielding pH levels for protein solubilization from biomass (pH 12) and protein precipitation (pH 2) was determined in preliminary experiments, confirming results from Vilg and Undeland (2017).

The nitrogen (N) yield was calculated as the amount of N in the final protein pellet as % of N in the biomass or as % of biomass DM:

$$N\ \ yield\ \ (\%\ of\ \ biomass\ \ N) = 100 \times \frac{N_{protein\ \ pellet}}{N_{biomass}} \quad (1)$$

$$N\ \ yield\ \ (\%\ of\ \ biomass\ \ DM) = 100 \times \frac{N_{protein\ \ pellet}}{DM_{biomass}} \quad (2)$$

2.5. Dry matter content

The DM content of the different biomass batches was determined using a moisture analyzer HR73 Halogen Moisture Analyzer (Mettler Toledo, USA). The measurement was performed in triplicate and 1 g of fresh weight (thawed) biomass was used for each run. Due to practicalities the DM was determined after storage at – 20 °C of the biomass, which could lead to water loss prior to the DM determination.

2.6. Total nitrogen content

The total N content was determined of the freeze-dried biomass and protein pellets with a DUMATHERM instrument (Gerhardt Analytical Systems, Königswinter, Germany) using 1.4 mg O_2/mg sample and an O_2 flowrate of 300 mL/min. EDTA was used as a standard. The analysis was performed in triplicate.

2.7. Protein determination by Lowry

Soluble protein in liquid fractions was measured by a modified Lowry assay (Markwell et al. 1978). A bovine serum albumin (BSA) standard curve (10–100 µg protein/mL) was used

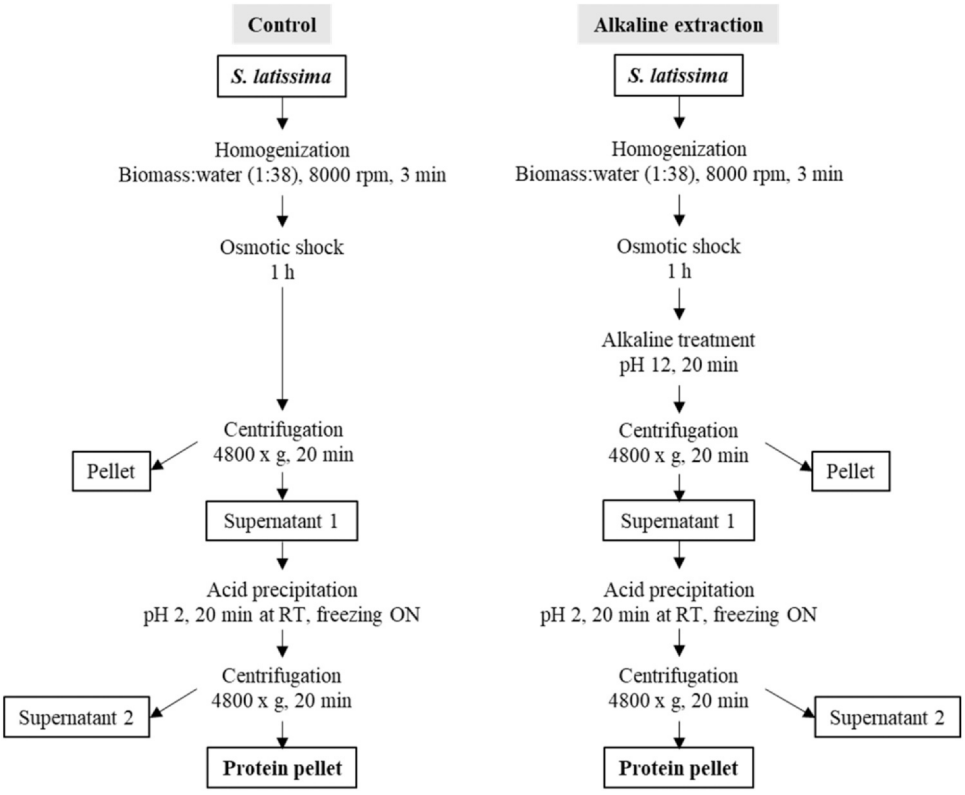

Fig. 1 – Flow-chart of protein extraction processes. The biomass:water ratio is based on the dry matter content of the biomass.

for quantification. 330 µL sample was mixed with one mL reagent C (consisting of 100 parts reagent A (2.0 % Na_2CO_3, 0.40 % NaOH, 0.16 % Na-K-tartrate and 1 % SDS) and one part reagent B (4 % $CuSO_4 \cdot 5\,H_2O$)) and left for 30 min at RT. 0.1 mL 1 N Folin-Ciocalteu reagent was added, and samples were incubated for 45 min in the dark. After incubation, 200 µL sample mix was added to a 96 micro-well plate and absorbance was measured at 750 nm. The analysis was performed in triplicate.

2.8. Protein solubility

The protein solubility of the freeze-dried protein pellets was determined by dispersing protein pellets in 30 mL MilliQ-water to a final concentration of 0.5 mg protein/mL. Protein dispersions were thoroughly mixed before aliquoting into 5 mL fractions in separate beakers. The pH of the separated fractions was either non-adjusted (pH 3) or adjusted to pH 5, 7, 9 or 11 using 1 M NaOH, respectively. The volume of added NaOH was noted. Samples were left for stirring for 30 min at RT prior to centrifugation at 6000× *g* for 10 min at RT. The protein solubility was then determined by measuring the protein content in the dispersions before and after centrifugation, quantifying protein using the modified Lowry assay (2.7). The analysis was performed once for each of the extraction duplicates.

2.9. Re-soluble nitrogen yield

The re-soluble nitrogen (N) yield was calculated as the soluble amount of N in the final protein pellet as % of N in the biomass:

$$Resoluble\ N\ yield = Protein\ solubility\,(\%) \times \left(100 \times \frac{N_{protein\ pellet}}{N_{biomass}}\right) \quad (3)$$

The relative amount of soluble N in the protein extract was assumed to be equal to relative amount of soluble protein (2.8).

2.10. Statistical analysis

The statistical analysis was carried out in the software R, version 4.0.3 (R Core Team, 2020). Data on yield was ln-transformed and tested for interaction between biomass samples and extraction method using ANOVA. Adequacy of models was tested by residual analysis. Multiple comparison was performed using the postHoc package (Labouriau, 2020) and *p*-values were adjusted for multiple testing by the method of controlling the false discovery rate (Benjamini and Hochberg, 1995). The N concentration of extracts between extraction methods was tested within each biomass batch with a t-test. Data that was not normally distributed was analyzed with the Kruskal-Wallis test using the postHoc package. The significance level was set to $p < 0.05$. Data is presented as mean ± SD.

3. Results and discussion

In general, the nitrogen (N) content in the *S. latissima* biomass was lowest when the biomass was harvested in June, regardless of biomass age and harvest location. The highest N content observed was in the biomass harvested in February. Fermentation further seemed to increase the N concentration from 1.56 ± 0.00 % to 2.91 ± 0.12 % of DM. The DM content also differed between biomass batches. The biomass from Bergen had the lowest DM (11–12 %) regardless of fermentation. Otherwise, the biomass DM was observed to differ from 12.6 ± 0.3 % (March 2020, Hjarnø) to 27.1 ± 1.4 % (June 2018, Hjarnø) (Table 1). The high DM contents could be due to water loss during freezing/thawing prior to the DM determination, leading to an increased DM content upon analysis.

As hypothesized, the alkaline extraction gave significantly ($p < 0.01$) higher N extraction yields than the control method across all biomass samples. The incubation step at pH 12 increased the solubilization of protein from the biomass, as has also been observed by Vilg and Undeland (2017). This led to an increased N yield. There were significant differences in N extraction yields between biomasses harvested at different times, but not within a specific pattern regarding harvest season and biomass age (Fig. 2). The highest N extraction yield as % of biomass N was observed for the Jun20H biomass (52.96 ± 8.35 %), even though it had a similar N content to the other biomass batches harvested in June (Jun18H and Jun19G) (Table 1). This suggests a different N composition between the biomass batches, possibly the Jun20H batch having a higher proportion of protein-N as opposed to non-

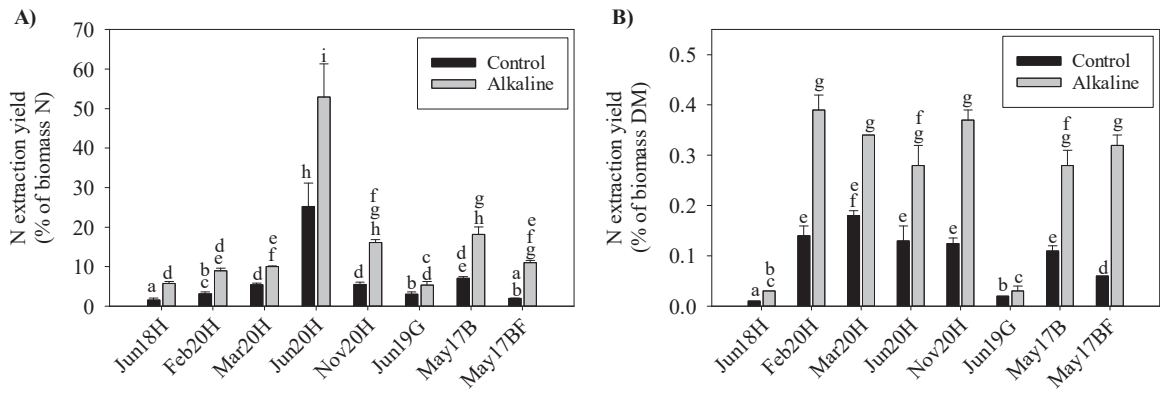

Fig. 2 – Nitrogen (N) extraction yield for different biomasses of *S. latissima*, using the control and the alkaline extraction, calculated as amount of N in protein extracts as a percentage of A) biomass N and B) biomass dry matter (DM). The sample names reflect the harvest time (month and year) of the biomass and the letter in the end signifies the cultivation site (H: Hjarnø, G: Grenå, B: Bergen, BF: Bergen fermented biomass) (Table 1). Data is represented as mean ± SD. Different notations among bars indicate significance of difference ($p < 0.05$).

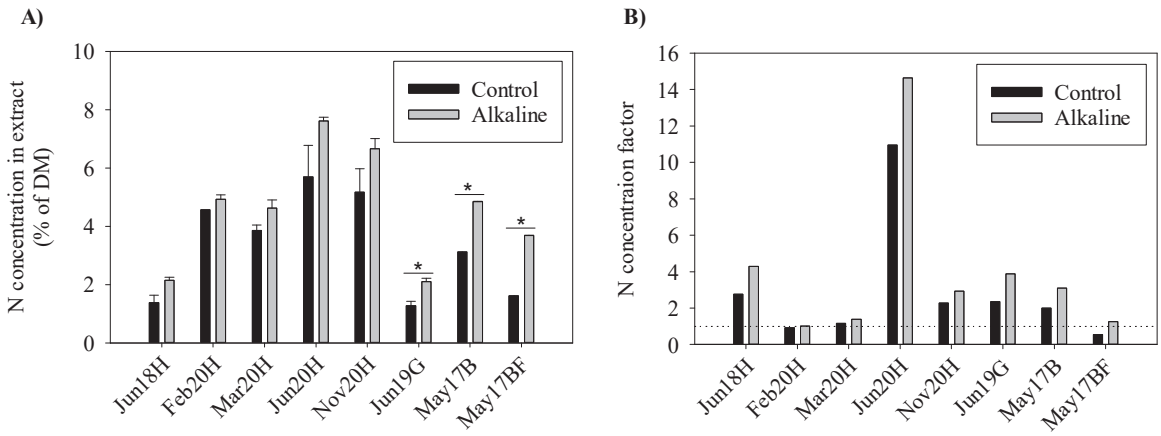

Fig. 3 – A) Nitrogen (N) concentration in extracts and B) the N concentration factor from biomass to extract. The dotted line is marking a concentration factor of one, which means the N concentration was increased from biomass to extract if the concentration factor is above the dotted line. In A) * marks a significant difference ($p < 0.05$) in N concentration between the treatments within the single biomass batches. Data in A) is visualized as mean ± SD, n = 2.

protein-N. The Jun20H sample was more than twice the age of the other June samples but was fouled with bryozoans upon harvest. Fouling has previously been shown to significantly increase the content of N in *S. latissima*, however, not altering the protein content significantly (Marinho et al. 2015a,b). For the control extraction, the N yield varied from 1.59 % to 25.22 % (mean: 6.61 %), whereas it varied from 5.32 % to 52.96 % (mean: 16.03 %) for the alkaline extraction. The N extraction yield differed to a higher extent between harvest times when the N extraction yield was calculated as % of biomass N as opposed to as % of biomass DM. This suggests a general variation in the amount of non-protein N between the biomass batches, as previously shown for biomass from Danish waters, characterized by a high seasonal variation in available N (Marinho and Holdt, 2017). Contrary, in an area such as the Faroe Islands where there is a low variation in N-availability, the N-to-protein conversion factor does not differ significantly with season (Bak et al. 2019). Another explanation for the differences in N yields as % of biomass N is that the protein profile, amount of free amino acids and protein extractability are different between batches. Looking into the N yield as % of biomass DM, only the samples from Jun18H and Jun19G significantly differed from the other batches. Fermentation did not change the N extraction yield for the alkaline condition, whereas the yield was significantly lower upon the control extraction, even though the N content of the biomass was almost twice the amount in the fermented biomass (Fig. 2). For comparison, Abdollahi et al. (2019) shows a protein yield as percentage of biomass protein of 19.3 % with the same biomass storage method and extraction method as in this current study. By freeze drying the biomass prior to protein extraction they increase the protein yield to 26.4 %. Vilg and Undeland (2017) show a protein yield of 16.01 %. It is important to note that the two mentioned studies calculate the protein yield, whereas this current study show the N yield. However, both studies fall within the range of the results in this current study if it is assumed that protein yield and N yield can be used as similar expressions – this will, however, be dependent on the N-to-protein factor, which can vary between biomasses. Further, as this study show, the biomass age and harvest time and place can influence the biochemical composition and extraction yield to a high degree.

The extracts from pH-shift processing had significantly ($p < 0.05$) higher N concentration than those from the control processing across biomass batches. Looking into the separate batches, the difference was significant for samples Jun19G, May17B and May17BF, whereas a tendency towards increased N concentration upon alkaline extraction was seen for the remaining samples (Fig. 3). This might be due to the improved extraction yield of the protein upon the alkaline condition. The concentration further differed significantly ($p < 0.001$) between extracts from the different biomass batches. However, looking into Fig. 3B it is visible that the N content was not concentrated from biomass to protein extract for all batches, such as for Feb20H, Mar20H and May17BF, which were the batches with the highest biomass N content. This may suggest a relatively high content of non-protein N, e.g., in form of chlorophyll, inorganic nitrogen or free amino acids (Angell et al. 2016). Non-protein N is not precipitated to the same extent as the protein bound N. The highest extract N concentration and N concentration factor was found for the Jun20H sample, which was the sample fouled with bryozoans.

The solubility of the extracted protein across biomass batches was significantly higher ($p < 0.02$) for extracts from pH-shift than from control, following the hypothesis. Alkaline treatment has also proved to increase the solubility of protein from other biomasses such as *Ulva fenestrata* (Juul et al. 2021) and alfalfa (Nissen et al. 2021). However, looking into the single biomass batches, the protein solubility was higher for the control extract than for the pH-shift extract in Mar20H, suggesting a different distribution of proteins compared to the other biomass samples. The solubility between treatments did not seem to vary much in samples Jun20H, May17B, and May17BF. Solubility across treatments was dependent on biomass batch ($p < 0.0001$) (Fig. 4).

The N extraction yield and the protein solubility is reflected together in the re-soluble N yield (Fig. 5). The pattern of the re-soluble N yield did not differ significantly from the pattern of the N extraction yield but showed to be between 43 % and 87 % of the N extraction yield depending on the biomass sample. The re-soluble N yield is an indication of the functional N yield and resembles the proportion of N that is bound in soluble protein.

The increased N yield and N concentration of extracts across the different biomass batches proves the robustness

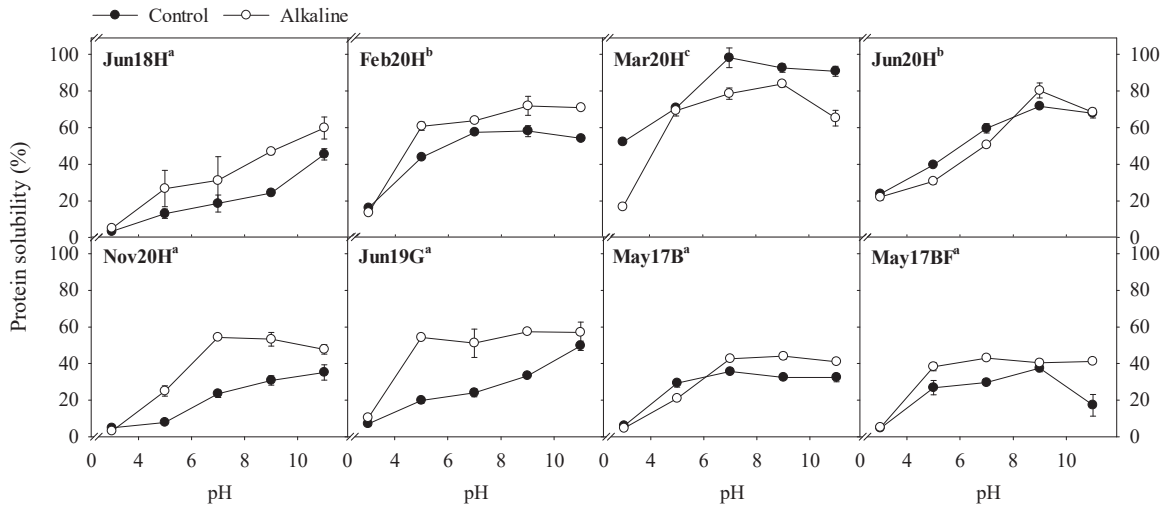

Fig. 4 – Protein solubility of extracts at different pH values ranging from pH 3 to pH 11. Different letters after batch name indicates significance of difference ($p < 0.05$) in protein solubility between biomass batches across treatments (control and pH-shift). Data is presented as mean ± SD, n = 2.

of the alkaline extraction compared to neutral pH. Further, thinking in a perspective of protein from *S. latissima* as a food ingredient, the ability of the alkaline extraction to increase the solubility of the extracted protein is an advantage. It is important, however, to keep in mind that alkaline extraction may lead to aminoacyl cross-linking as seen for other biomasses such as *Ulva fenestrata* (Juul et al. 2021). In the study by Juul et al. on *Ulva fenestrata*, the high pH condition induced lysinoalanine and lanthionine amino acid cross-links. The induced cross-links were however not expected to influence the nutritional quality of the protein significantly and no significant difference was seen for the *in vitro* protein digestibility compared to the other investigated extraction methods (mechanical screw press extraction and alkaline extraction at pH 8.5). Further, the alkaline conditions did not induce L/D-amino acid racemization (Juul et al. 2021). It would be beneficial to test whether the alkaline extraction

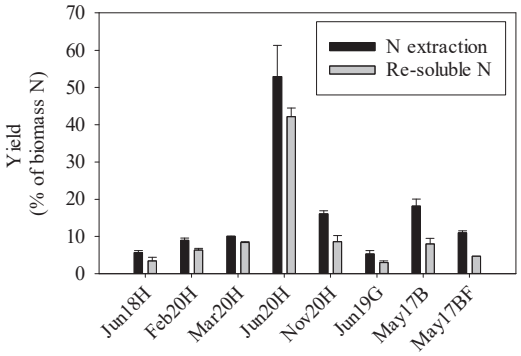

Fig. 5 – Yield of re-soluble N in extracts and N extraction yield (same as the N extraction yield from alkaline extraction in Fig. 2 A) as percentage of biomass N. The re-soluble N yield was calculated as the re-soluble fraction (Fig. 4) of the N extraction yield (Eq. 3). Only the highest yield for each sample as a function of extraction method and pH-dependent solubility is visualized in the figure. For all samples, the highest re-soluble N yield was obtained by the alkaline extraction method. Optimal pH for protein solubilization was pH 7 for May17BF, pH 9 for Feb20H, Mar20H, Jun20H, Nov20H, Jun19G, and May17B, and pH 11 for Jun18H.

induce such modifications during the extraction of *S. latissima* protein, as well as looking into the amino acid composition and ratio of essential amino acids. Unfortunately, it was not possible during the current study due to limited sample amount.

4. Conclusion

Protein was extracted from *Saccharina latissima* of different age, harvest time and cultivation sites. Alkaline extraction proved to increase the protein yield more than twice in terms of amount of extracted N compared to the neutral pH condition (pH 6.5). The alkaline extraction further resulted in a higher N concentration in the final protein extract as well as it in general increased the solubility of the extracted protein. Nitrogen extraction yield and protein solubility differed between the different biomass batches, but no specific pattern was observed as a function of biomass age, harvest time and cultivation site. The re-soluble N yield was between 43 % and 87 % of the total N extraction yield depending on the biomass sample. The N extraction yield as % of biomass N content differed from 1.59 % to 25.22 % (mean: 6.61 %, median: 4.26 %) for the control extraction, whereas it was 5.32–52.96 % (mean: 16.03 %, median: 10.53 %) for the alkaline extraction. The N extraction yield differed to a higher extent between harvest times when the N extraction yield was calculated as % of biomass N as opposed to as % of biomass DM.

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.

Acknowledgment

The study was supported by GUDP, j. no. 34009–19-1615.

References

Abdollahi, M., Axelsson, J., Carlsson, N.-G., Nylund, G.M., Albers, E., Undeland, I., 2019. Effect of stabilization method and freeze/thaw-aided precipitation on structural and functional

properties of proteins recovered from brown seaweed (saccharina latissima). Food Hydrocoll. 96, 140–150.
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.
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 (1247).
Bak, U.G., Nielsen, C.W., Marinho, G.S., Gregersen, Ó., Jónsdóttir, R., Holdt, S.L., 2019. The seasonal variation in nitrogen, amino acid, protein and nitrogen-to-protein conversion factors of commercially cultivated faroese saccharina latissima. Algal Res. 42, 101576.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc.: Ser. B (Methodol.) 57 (1), 289–300.
Bikker, P., van Krimpen, M.M., van Wikselaar, P., Houweling-Tan, B., Scaccia, N., van Hal, J.W., Huijgen, W.J., Cone, J.W., López-Contreras, A.M., 2016. Biorefinery of the green seaweed Ulva lactuca to produce animal feed, chemicals and biofuels. J. Appl. Phycol. 28 (6), 3511–3525.
Boderskov, T., Nielsen, M.M., Rasmussen, M.B., Balsby, T.J.S., Macleod, A., Holdt, S.L., Sloth, J.J., Bruhn, A., 2021. Effects of seeding method, timing and site selection on the production and quality of sugar kelp, saccharina latissima: a danish case study. Algal Res. 53, 102160.
Dégen, L., Halas, V., Babinszky, L., 2007. Effect of dietary fibre on protein and fat digestibility and its consequences on diet formulation for growing and fattening pigs: a review. Acta Agric. Scand., Sect. A — Anim. Sci. 57 (1), 1–9.
FAO, 2018. The global status of seaweed production, trade and utilization. Globefish Res. Program. 124.
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.
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.
Horie, Y., Sugase, K., Horie, K., 1995. Physiological differences of soluble and insoluble dietary fibre fractions of brown algae and mushrooms in pepsin activity in vitro and protein digestibility. Asia Pac. J. Clin. Nutr. 4 (2), 251–255.
Jiang, S., Ding, J., Andrade, J., Rababah, T.M., Almajwal, A., Abulmeaty, M.M., Feng, H., 2017. Modifying the physicochemical properties of pea protein by ph-shifting and ultrasound combined treatments. Ultrason. Sonochem. 38, 835–842.
Juul, L., Danielsen, M., Nebel, C., Steinhagen, S., Bruhn, A., Jensen, S.K., Undeland, I., Dalsgaard, T.K., 2021. Ulva fenestrata protein – comparison of three extraction methods with respect to protein yield and protein quality. Algal Res. 60, 102496.
Juul, L., Stødkilde, L., Ingerslev, A.K., Bruhn, A., Jensen, S.K., Dalsgaard, T.K., 2022. Digestibility of seaweed protein from ulva sp. And saccharina latissima in rats. Algal Res. 63, 102644.
Kristinsson, H.G., Hultin, H.O., 2003. Effect of low and high ph treatment on the functional properties of cod muscle proteins. J. Agric. Food Chem. 51 (17), 5103–5110.
Krogdahl, Å., Jaramillo-Torres, A., Ahlstrøm, Ø., Chikwati, E., Aasen, I.-M., Kortner, T.M., 2021. Protein value and health aspects of the seaweeds saccharina latissima and Palmaria palmata evaluated with mink as model for monogastric animals. Anim. Feed Sci. Technol. 276, 114902.
Labouriau, R. , 2020. Posthoc: Tools for post-hoc analysis. R-package version 0.1.1. ⟨https://CRAN.R-project.org/package=postHoc⟩.
Marinho, G.S. and S.L. Holdt , 2017. Nitrogen-to-protein conversion factor of seaweed varies with season. Book of abstracts sustain 2017. Technical University of Denmark.
Marinho, G.S., Holdt, S.L., Angelidaki, I., 2015a. Seasonal variations in the amino acid profile and protein nutritional value of saccharina latissima cultivated in a commercial imta system. J. Appl. Phycol. 27 (5), 1991–2000.
Marinho, G.S., Holdt, S.L., Birkeland, M.J., Angelidaki, I., 2015b. Commercial cultivation and bioremediation potential of sugar kelp, saccharina latissima, in danish waters. J. Appl. Phycol. 27 (5), 1963–1973.
Markwell, M.A.K., Haas, S.M., Bieber, L.L., Tolbert, N.E., 1978. A modification of the lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. 87 (1), 206–210.
Nissen, S.H., Schmidt, J.M., Gregersen, S., Hammershøj, M., Møller, A.H., Danielsen, M., Stødkilde, L., Nebel, C., Dalsgaard, T.K., 2021. Increased solubility and functional properties of precipitated alfalfa protein concentrate subjected to ph shift processes. Food Hydrocoll. 119, 106874.
R Core Team , 2020. R 4.0.3, the r project for statistical computing. ⟨https://www.r-project.org/⟩.
Silventoinen, P., Sozer, N., 2020. Impact of ultrasound treatment and ph-shifting on physicochemical properties of protein-enriched barley fraction and barley protein isolate. Foods 9 (8), 1055.
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, 129683.
Vilg, J.V., Undeland, I., 2017. pH-driven solubilization and isoelectric precipitation of proteins from the brown seaweed saccharina latissima-effects of osmotic shock, water volume and temperature. J. Appl. Phycol. 29 (1), 585–593.