

Optimizing protein extraction of *Ulva lactuca* and *Saccharina latissima*

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Question

What is the optimal pH and temperature for the extraction of proteins from the seaweed *Ulva lactuca* and *Saccharina latissima*?

Background

With an increasing world population and a demand for high quality proteins, seaweed is more often seen as a possible solution. Without the need for arable land, with a high yield per hectare and a good amino acid content, seaweed has a huge potential for the supply of high quality protein. Unfortunately the total protein content of most seaweed species is low and therefore an extraction and purification step of the protein is often required.

Methods

Sample preparation

Experiments with *Ascophyllum nodosum* showed no negative effect of freeze drying on the percentage of N extracted (Figure 1). Therefore freeze dried material was used for all experiments in this study and ground to a size of <0.3 mm.



1 Pre-treatment effect

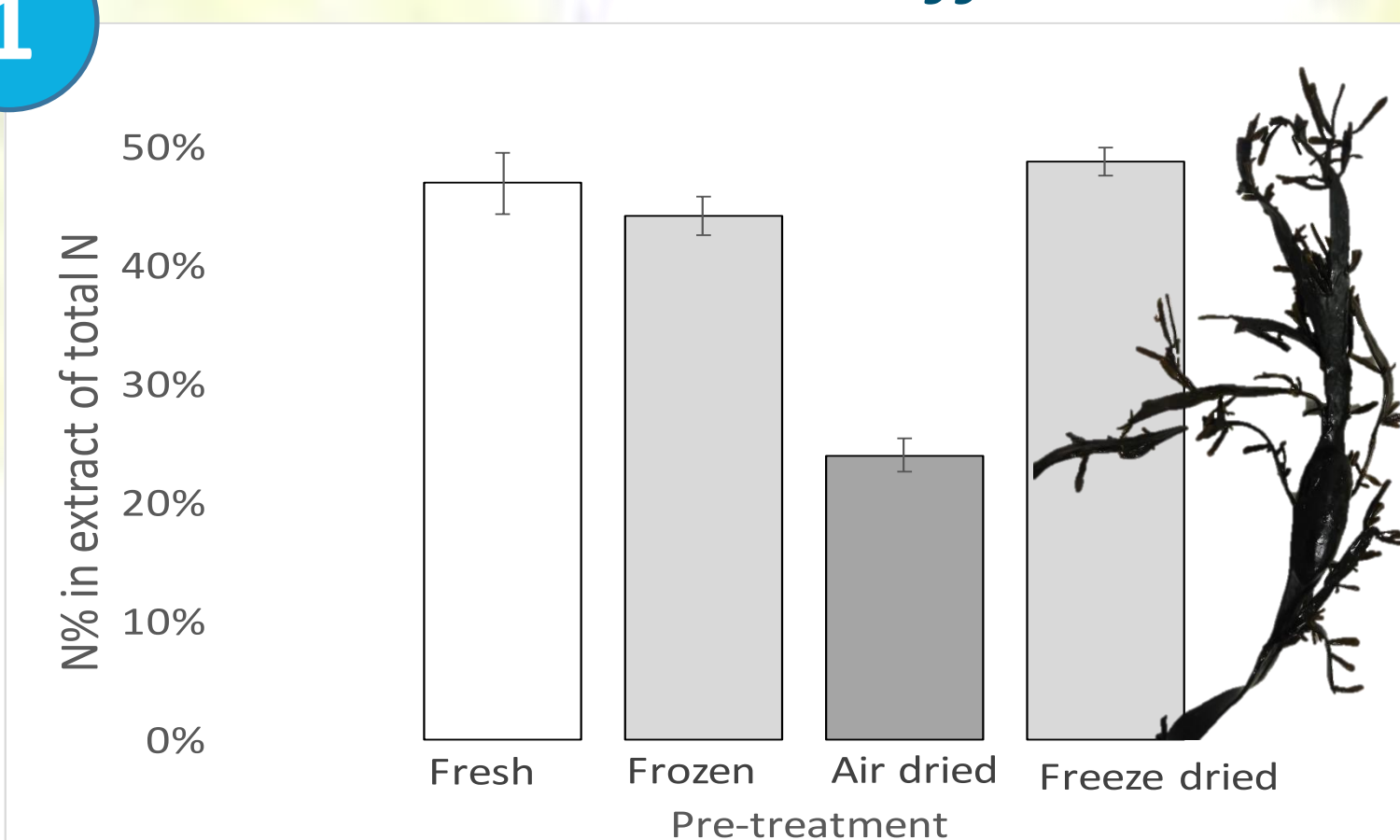


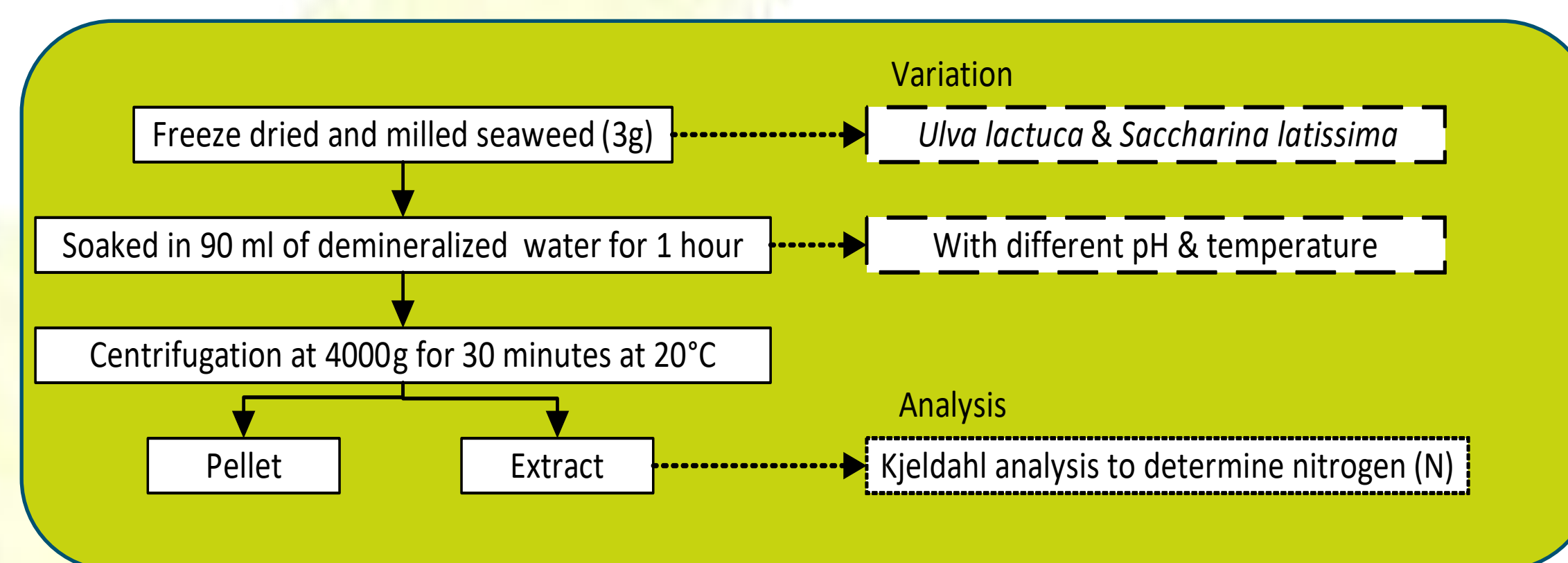
Figure 1. Fresh, frozen, freeze dried and air dried (60°C) *A. nodosum* was extracted for 1 hour, at 50°C, with pH 11 under constant shaking with an RPM of 120 and a dry biomass to water ratio of 1:30. Total nitrogen in the extract was quantified and is expressed as a percentage of the total nitrogen in the starting material in the extract. Error bars = SD and n=3.

Protein extraction

In this study a single step aqueous protein extraction was tested on *S. latissima* and *U. lactuca* as described in Box 1. To test pH effects the pH of the extracts was adjusted with 1M NaOH. To test temperature the seaweed was soaked in 0.1M NaOH at temperatures from 20°C to 50°C.

Methods

Box 1. Extraction scheme



Protein (nitrogen) determination

Total nitrogen content in the extract and starting material was measured with the Kjeldahl method.

In general multiplying measured nitrogen percentage (N) by 5-6.25 gives an accurate estimate of protein percentage.

The percentage of total nitrogen extracted was calculated with the following formula:

$$\% \text{ N in extract of total N} = 100\% \times \frac{\text{total N in extract}}{\text{total N in dry seaweed}}$$

Results

PH dependent extraction efficiency

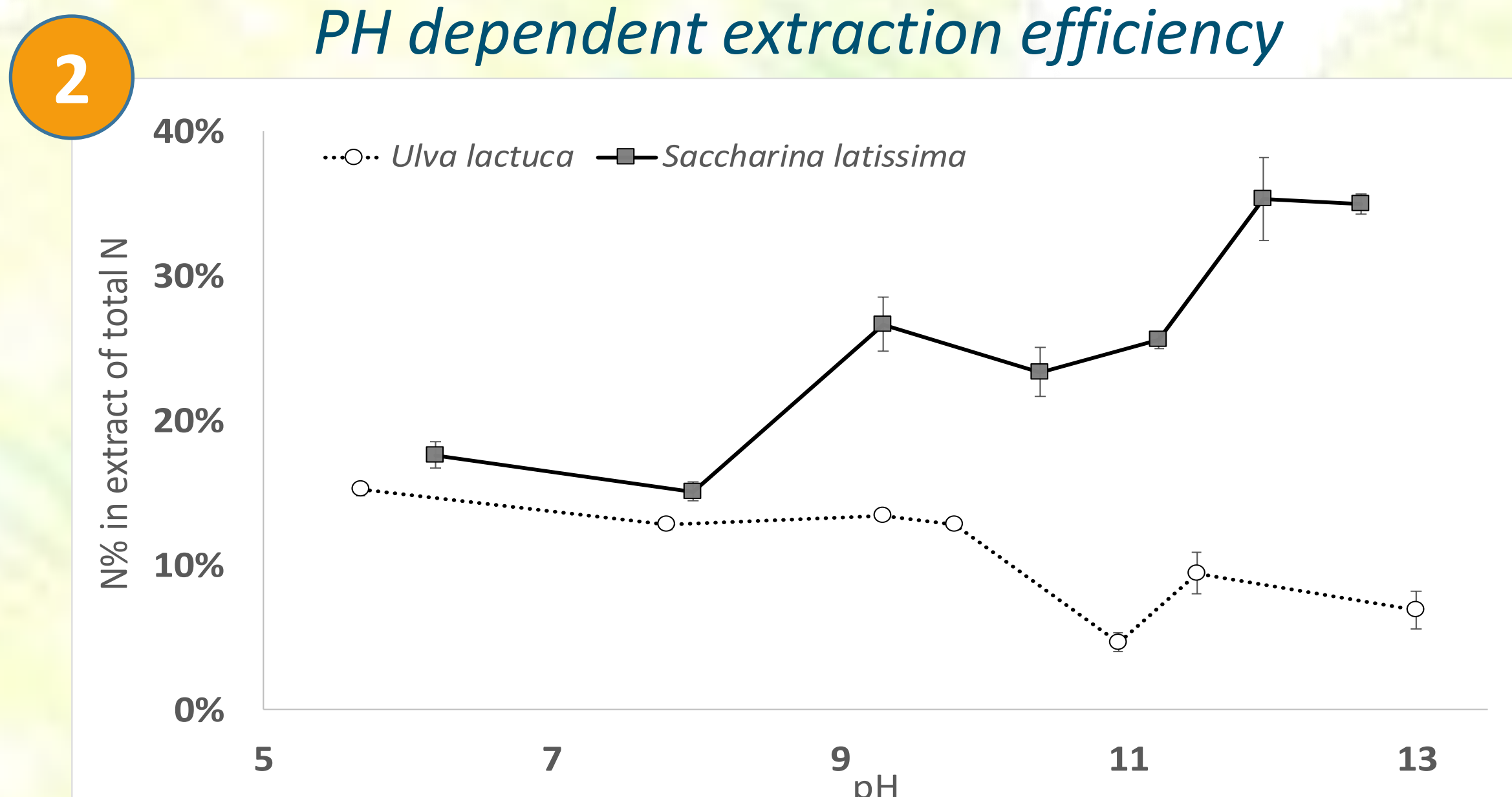


Figure 2. Freeze dried material was extracted for 1 hour, at 20°C, with varying pH adjusted with 1M NaOH to a total volume of 90ml (only the first extraction had no NaOH added), under constant shaking with an RPM of 140 and a dry biomass to water ratio of 1:30. Total nitrogen in the extract was quantified and is expressed as a percentage of the total nitrogen in the starting material in the extract. Error bars = SD and n=3.

3 Temperature dependent extraction efficiency

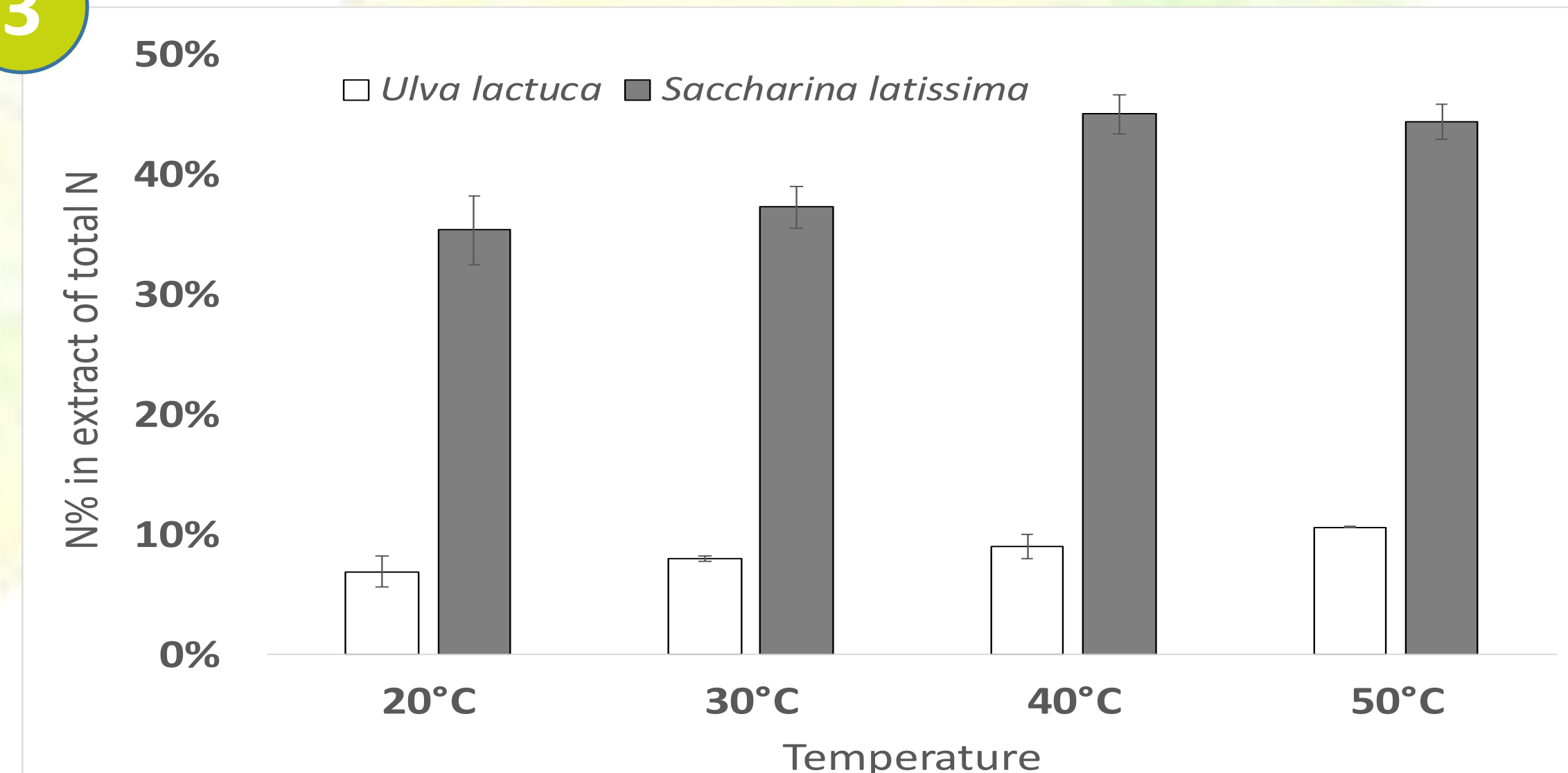


Figure 3. Freeze dried material was extracted for 1 hour in a 0.1M NaOH solution, at varying temperatures, under constant shaking with an RPM of 140 and a dry biomass to water ratio of 1:30. Total nitrogen in the extract was quantified and is expressed as a percentage of the total nitrogen in the starting material in the extract. Error bars = SD and n=3.

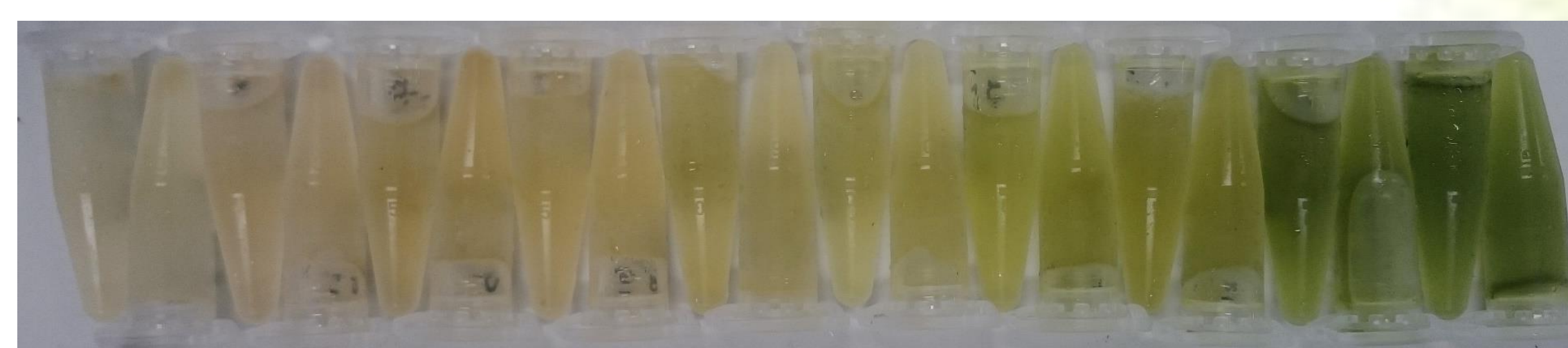


Figure 4. Coloring of *S. latissima* extracts after different treatments. From left to right pH and temperature increases. Most left extract was treated at 20°C and no pH increase, most right extract was treated at 50°C in a 0.1M NaOH solution.

Conclusions

- The extraction efficiency for *S. latissima* is optimal at a pH of 12 and 40°C. Extraction efficiency was increased by 156% from 17,6% to 45,0%.
- Increasing the pH has a negative effect on the extraction efficiency in freeze dried *U. lactuca*. Increasing the temperature up to 50°C had a slight positive effect.

Discussion

Nitrogen as protein

- Not all nitrogen will be protein and this is different per species. Including amino acid composition is preferred.

PH

- An increased pH resulted in a higher percentage of extracted protein for *S. latissima* (up to 12) but a lower percentage for *U. lactuca*.

Temperature

- For both species increasing the extraction temperature resulted in more extracted protein. For *S. latissima* increasing the temperature from 40°C to 50°C had no further effect.

Sample preparation

- The pre-treatment effect tested with the brown seaweed *A. nodosum* seemed a good indicator for the brown seaweed *S. latissima* but not for the green seaweed *U. lactuca*.
- Freeze drying may have a negative effect on the solubility of protein in *U. lactuca*.

Future research

- Comparing the amino acid composition of untreated seaweed, extract and pellet.
- Test the pre-treatment effect for *U. lactuca* and *S. latissima*.

Acknowledgements

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