Report on investigation of stability of iodine in seaweed biomass and seaweed extracts

Project: På sporet av ny mat – innhold og biotilgjenglighet av jod, uorganisk arsen, kadmium og kobber fra tare.

1. Background

Volatile nature of iodine is often considered a problem in iodine analysis, especially when working with acidic solutions. There is, however, not much evidence available on this topic and no systematic test on the stability of iodine in seaweed or seaweed extracts was found in the literature.

The purpose of this study was therefore to find if total amount of iodine is changed in 8-week period at different temperatures.

2. Plan of the experiment

Samples of *Saccharina latissima* (dry biomass sample or extract in solutions with different pH) were exposed to two different temperatures (20 °C and 60 °C) in addition to the "reference temperature" which was –20 °C, for 3, 5 or 8 weeks. In addition, two biomass subsamples were kept at room temperature for 0.5 year. All the samples were kept in the dark for the entire period of the experiment.

Labelling system: temperature-time-type of sample-duplicate

Temperature	Time	Type of sample	Duplicate
• F → freezer (–20 °C) • R → room T (approx. 20 °C) • H → high T (60 °C)	• 3W → 3 weeks • 5W → 5 weeks • 8W → 8 weeks • 6M → 6 months	• SB → solid biomass (approx. 1 g of dry sample) • EW → extraction in water • EAc → extraction in acid • EAl → extraction in alkaline	• a • b • B1/B2 (blanks)

Scheme:

bottle lable	sample\week	1	2	3	4	5	6	7	8
SB_1	F-SB_a								
SB_2	F-SB_b								
SB_3	R-3W-SB_a								
SB_4	R-3W-SB_b								
SB_5	H-3W-SB_a								
SB_6	H-3W-SB_b								
SB_7	R-5W-SB_a								
SB_8	R-5W-SB_b								
SB_9	H-5W-SB_a								
SB_10	H-5W-SB_b								
SB_11	R-8W-SB_a								
SB_12	R-8W-SB_b								
SB_13	H-8W-SB_a								
SB_14	H-8W-SB_b								
SB_15	R-6M-SB_a								→
SB_16	R-6M-SB_b								→

EW_B1	EW_blank_a
EW_B2	EW_blank_b
EW_1	F-EW_a
EW_2	F-EW_b
EW_3	R-3W-EW_a
EW_4	R-3W-EW_b
EW_5	H-3W-EW_a
EW_6	H-3W-EW_b
EW_7	R-5W-EW_a
EW_8	R-5W-EW_b
EW_9	H-5W-EW_a
EW_10	H-5W-EW_b
EW_11	R-8W-EW_a
EW_12	R-8W-EW_b
EW_13	H-8W-EW_a
EW_14	H-8W-EW_b

EAc_B1	EAc_blank_a
EAc_B2	EAc_blank_b
EAc_1	F-EAc_a
EAc_2	F-EAc_b
EAc_3	R-3W-EAc_a
EAc_4	R-3W-EAc_b
EAc_5	H-3W-EAc_a
EAc_6	H-3W-EAc_b
EAc_7	R-5W-EAc_a
EAc_8	R-5W-EAc_b
EAc_9	H-5W-EAc_a
EAc_10	H-5W-EAc_b
EAc_11	R-8W-EAc_a
EAc_12	R-8W-EAc_b
EAc_13	H-8W-EAc_a
EAc_14	H-8W-EAc_b

EAl_B1	EAl_blank_a
EAl_B2	EAl_blank_b
EAl_1	F-EAl_a
EAl_2	F-EAl_b
EAl_3	R-3W-EAl_a
EAl_4	R-3W-EAl_b
EAl_5	H-3W-EAl_a
EAl_6	H-3W-EAl_b
EAl_7	R-5W-EAl_a
EAl_8	R-5W-EAl_b
EAl_9	H-5W-EAl_a
EAl_10	H-5W-EAl_b
EAl_11	R-8W-EAl_a
EAl_12	R-8W-EAl_b
EAl_13	H-8W-EAl_a
EAl_14	H-8W-EAl_b

Start of the experiment: 14. 11. 2018
3 weeks: 5. 12. 2018
5 weeks: 19. 12. 2018
8 weeks: 9. 1. 2019
Longer period: 15. 5. 2019

3. Additional experiments

Due to the results of the main experiment (see Results section), two additional experiment was performed.

3.1 Water loss

Iodine content in extracts at 60 °C seemed to increase over time, so we assumed that some water might be lost during the experiment, despite the fact that tubes were closed. Since this was not expected, tubes were not weighed before and after the experiments and therefore another experiment was performed with water, but at the same temperature and time.

3.2 Stability of iodide and iodate in 2% HNO_3

Unexpected results were observed also for iodine content in acidic extracts. Therefore, stability of two iodine species – iodide (I^-) and iodate (IO_3^-) was further tested in 2% HNO_3.

4. Procedures

4.1 Stability of iodine in seaweed biomass and extracts

a) Biomass samples

Iodine in biomass samples was determined following the standard method for determination of iodine in animal feed by ICP-MS, DS/EN 17050:2017. To 0,3 mL of sample, 5 mL of Milli-Q water and 1 mL of 25% TMAH were added and tubes were placed to the oven for 3 h at 90 °C. After cooling, extracts were diluted with Milli-Q water to 50 mL. Before measurement, samples were further diluted 10000-fold with 0.5% TMAH. Te was added as an internal standard in concentration 1 ng Te/mL of measured solution. Iodine was measured by ICP-MS (Thermo iCapQ) and quantification was done based on external calibration curve, prepared in 0,5% TMAH in the range between 0.1 and 20 ng I/mL. Iodine was extracted from samples on January 16, 2019 and measured on January 18, 2019. In the samples that were stored at room temperature for 6 months, iodine was measured by Annette Landin on August 6, 2019.

b) Extracts

For stability of extract, first extraction solutions were prepared. Besides Milli-Q water also Milli-Q water of which pH was adjusted to 4 with HNO_3 for acidic extraction or to 10 with NH_3 for alkaline extraction. Then 6 mL of corresponding solution was added to 0.3 g of sample. Tubes were placed in the oven for 3 h at 90 °C. After cooling, samples were centrifuged (5 min, 7000 g). Supernatant was transferred into the 15 mL tubes, but since it was not possible to transfer clear supernatant only, 15 mL tubes were centrifuged again (5 min, 7000 g). After second centrifugation, clear supernatant was transferred into another 15 mL tube which were used for the experiment. Extractions were performed on November 14, 2018.

After the experiment, extracts were diluted in the same rate as they were for the biomass samples after extraction. To water extracts 25% TMAH was added in the amount that resulted in 0.5% TMAH in the solution and samples were further diluted with 0,5% TMAH. For acidic and alkaline extracts, pH was adjusted with 25% TMAH to 12.7–12.8, which is a pH of 0.5% TMAH. Samples were further diluted with 0.5% TMAH. Calibration curves were prepared in the range between 0.1 and 20 ng I/mL for each type of extracts individualy in the same media. Therefore, a solution with corresponding pH was prepared first, then pH was adjusted with TMAH, these solutions were further diluted in the same rate as samples and finally used to prepare calibration curves. Iodine was measured by ICP-MS (Thermo iCapQ) on January 24, 2019.

4.2 Test of water loss

Tubes were filled with 2 mL of Milli-Q water (approx. the amount of extract in the main experiment), weighed and left at –20 °C (reference), 20 °C and 60 °C. After each period, corresponding tubes were cooled to room temperature, weighed and stored in the freezer until the end of the experiment. Tubes were stored at the same place as the samples in previous experiment. Water loss obtained in the experiment was used to correct determined iodine content from the main experiment.

4.3 Stability of iodide and iodate in acid

Three replicates of blank samples (2% HNO_3) and two replicates of solution of each iodine species (10 ng I/mL in 2% HNO_3) were prepared for each tested period: 0 days (reference), 1, 2, 3, 7, 10 and 14 days. To each tube, 5 mL of corresponding solution was added. Solutions were stored in open tubes in fume hood for the stated period. After that period, tubes were closed and stored in the freezer until measurement. Tubes were weighed before the experiment and the relevant tubes after each period to evaluate water loss during the experiment, which was later considered in calculation of the results.

After the experiment, 0.9 mL of 25% TMAH was added, since it was previously tested that 5 mL of 2% HNO_3 + 0.9 mL of 25% TMAH gives pH around 12.8, which is the pH of 0.5% TMAH that is usually used for iodine determination by ICP-MS. Te (1000 ng/mL, 0.1 mL) was added to each tube as an internal standard. Samples were diluted to 10 mL with 0.5% TMAH.

Calibration curve was prepared in the range between 0.1 and 5 ng I/mL in the same matrix as samples (25 mL 2% HNO_3 + 4.5 mL 25% TMAH, diluted to 50 mL with 0.5% TMAH).

5. Results

5.1 Stability of iodine seaweed biomass and extracts

a) Biomass samples

Results of iodine content in seaweed dry biomass samples exposed to different temperatures for different time periods are shown in Figure 1. Iodine content remained at the same level regardless of time and temperature of storage. Statistics analysis were not performed, the error bars in Figure 1 represent standard deviation of 2-3 determinations.

a) Extracts

Results of iodine content in extracts are shown in Figure 2. The trend in all extracts was the same, regardless of pH. Calculated to content in the sample, we can say that iodine in water, acidic and alkaline media was extracted in the same rate as when using standard method for iodine determination. Iodine content in extracts, stored at room temperature did not change over 8 weeks. Statistics analysis were not performed, the error bars in Figure 2 represent standard deviation of 2-3 determinations. On the other hand, iodine content in extracts, stored at higher temperature (60 °C) increased over time. Since statistical analysis was not performed, we cannot say if there are statistically significant differences or not, but there certainly is a trend of increasing iodine content in these extracts over time. Although the tubes with extracts were closed, probably some of the liquid evaporated and thus iodine was concentrated in the solution. Since we did not expect evaporation from closed tubes, we did not weighed the tubes before and after experiment. Therefore, another experiment was performed to investigate this further (see section 5.2).

There is a common believe that iodine is unstable in acidic solutions. Analyte can be lost by formation of volatile iodine species in acid. However, the results from this study does not show any loss of iodine from acidic extracts even after 8 weeks at 60 °C. Iodine content as well as the overall trend in acidic extracts was the same as in water and alkaline extracts. We cannot argue that we did not lose iodine because the tubes were closed which would prevent evaporation/volatilization, since some liquid most probably evaporated and so could volatile iodine species.

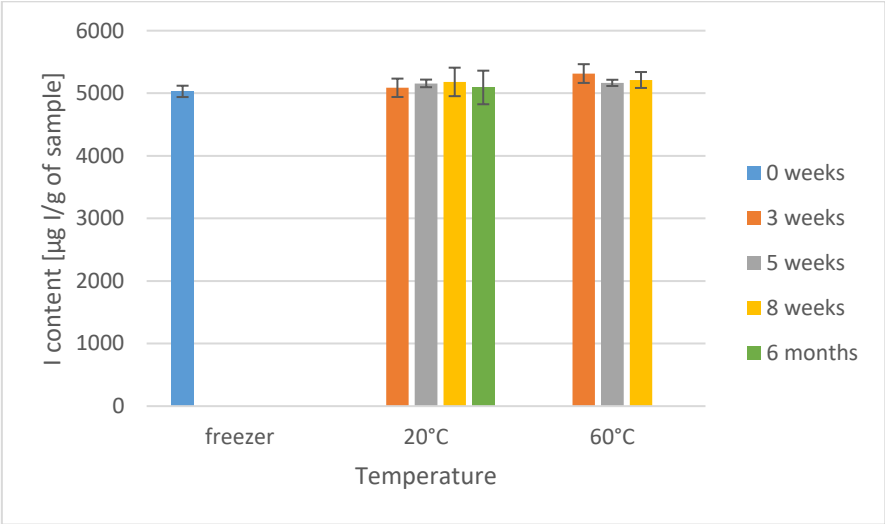

Figure 1: Iodine content in seaweed biomass samples exposed to different temperature for different periods. Reference sample was stored in freezer for the entire period of experiment (blue column).

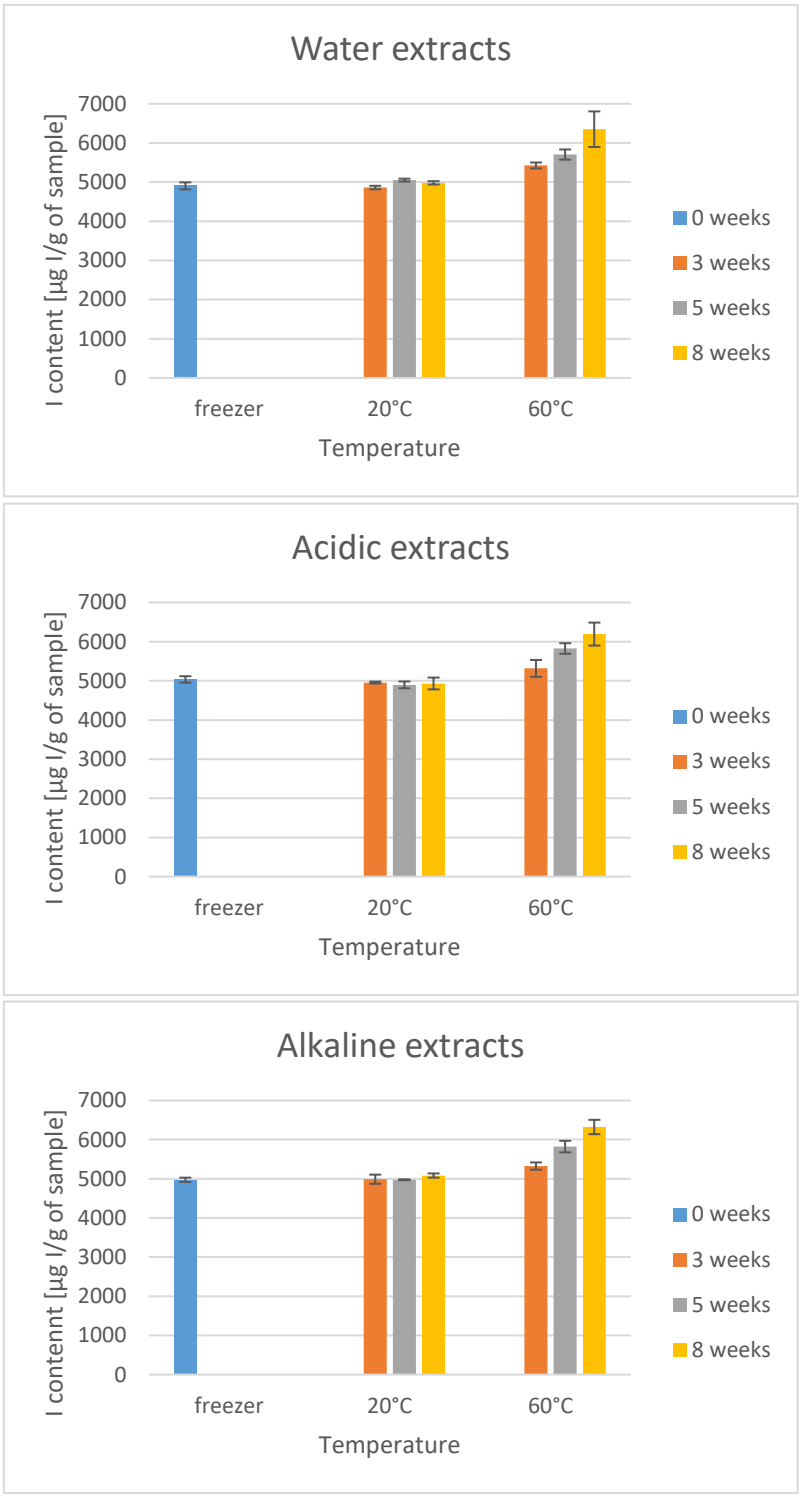

Figure 2: Iodine content in extracts of seaweed samples exposed to different temperature for different periods. Reference sample was stored in freezer for the entire period of experiment (blue column).

5.2 Water loss

As show on Figure 2, iodine content in extracts at 60 °C seemed to increased over time. However, In additional experiment we found that some water was actually lost even though the tubes were closed. When the results were recalculated considering the loss, iodine content remained stable also at 60 °C after 8 weeks (Figure 3).

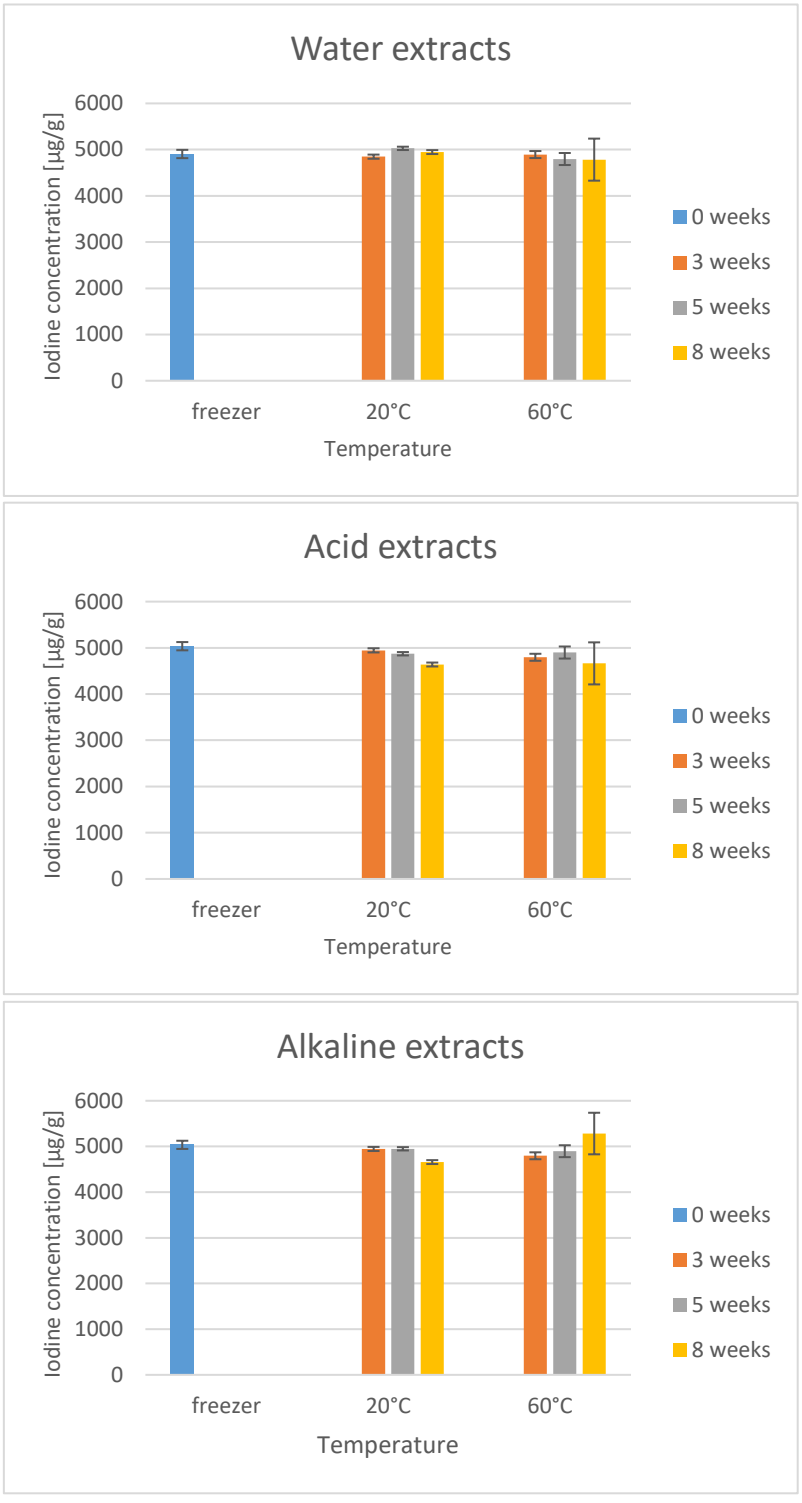

Figure 3: Iodine content in extracts of seaweed samples exposed to different temperature for different periods – recalculated results considering water loss. Reference sample was stored in freezer for the entire period of experiment (blue column).

5.1 Stability of iodide and iodate in acid

Iodine is usually considered volatile in acid solutions and therefore it was unexpected that iodine content in acidic extracts remained stable even at 60 °C for 8 weeks. Although the tubes were closed, water partly evaporated during 8 weeks, and so could iodine. Additional experiment was performed with iodide and iodate solution to evaluate loss due to volatilization. The results are presented in Figure --- as percent of the reference value, which was the one that was stored directly in the freezer at the beginning of the experiment.

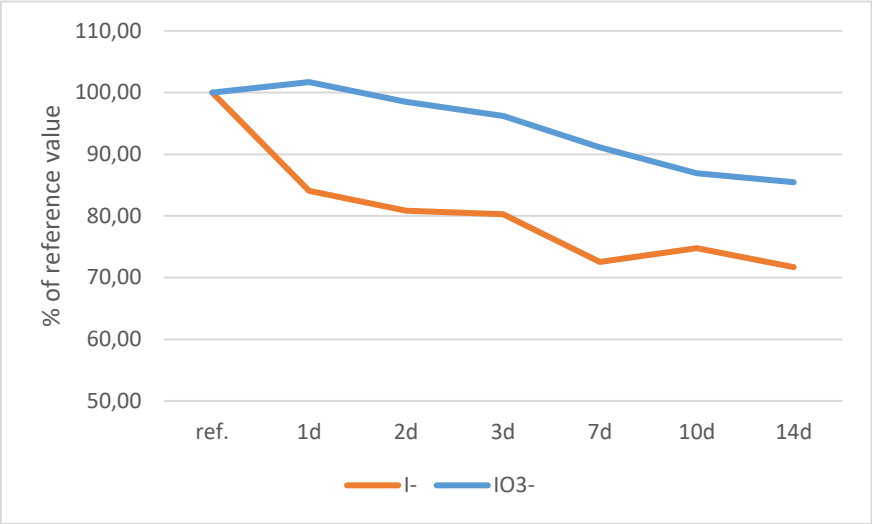

Figure 4: Loss of iodide and iodate in 2% HNO3 during 14 days.

Very small differences were observed for iodate in the first three days, while later the concentration in the tube decreased more. After 14 days, iodine concentration was approx. 15% lower than at the beginning when iodate was used. On the other hand, more than 15% of iodide was lost in the first day, but the concentration was then quite stable for the next two days (2 and 3). Another drop in iodine concentration was observed after 7 days (approx. 28% less than at the beginning), but it was similar after 10 and 14 days.

These results suggest that iodine is indeed volatile in acidic solution, more in the form of iodide than in the form of iodate, and acid digestion might not be a good option for sample preparation. However, no loss was observed from acidic seaweed extracts. It is possible that closed tubes prevented the loss. Another explanation would be that the majority of iodine in the seaweed extract was not in inorganic form.

6. Conclusions

According to the study, there is no need for storing the dry samples in which iodine is to be determined in fridge or even freezer, since temperature up to 60 °C does not affect iodine content in dry seaweed samples for at least 8 weeks. At room temperature (around 20 °C) samples can be stored for at least 6 months without changes in iodine content.

Extracts can be prepared at least 8 weeks before measurement and stored at room temperature regardless of the solution pH (between 4 and 10) without questioning the trueness of the total iodine results, since iodine content does not change under these conditions. However, if extracts are stored at higher temperatures (up to 60 °C), water loss should be evaluated by weight and considered in calculations.

If iodine is present in the acidic solution in inorganic form (I^- or IO_3^-) there is a risk to be lost due to volatilization. Use of alkaline extraction is therefore preferred over acidic digestion as sample preparation procedure.