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On the use of pulsed electric field technology as a pretreatment to reduce the content of potentially toxic elements in dried *Saccharina latissima*

Marthe Jordbrekk Blikra [*], Dagbjørn Skipnes, Torstein Skåra

Department of Processing Technology, Seafood Division, Nofima AS, P.O. Box 8034, NO-4068, Stavanger, Norway

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ABSTRACT

Seaweeds, like sugar kelp, are increasingly popular for food production, but their application is often limited by the content of iodine and other potentially toxic elements (PTEs). Boiling and blanching are efficient in reducing the iodine content (-38-94%), but are energy demanding processes and could therefore be too expensive for viable commercial applications. Pulsed electric field (PEF) processing is gaining interest for commercial processing of seaweeds, aiming to reduce the energy demand for the pre-treatment.

In this work, two conditional settings (energy levels: 2.7 and 14.4 kJ/kg) of PEF were evaluated as pretreatments prior to drying of sugar kelp, and compared to no pretreatment and freezing/thawing at −20/4 °C. Both PEF treatments reduced the iodine content significantly, by approximately 40%, compared to no pretreatment. Similarly, the content of mercury was reduced by approximately 19%. Freezing prior to drying did not significantly alter the content of PTEs in dried kelp. The energy input associated with PEF processing was <10% of the calculated input for traditional processing.

These findings are promising as the industry is looking into rapid, non-destructive processing methods for reducing the energy requirements associated with drying and preservation, while improving the safety of products.

1. Introduction

In Europe, seaweed is an interesting up-and-coming source of food with new flavors (Mouritsen, Williams, Bjerregaard, & Duelund, 2012), high fiber content, and high mineral content (Holdt & Kraan, 2011). The interest in natural and sustainable food is forecasting its increased utilization as a raw material for food production in Western countries (Blikra, Altintzoglou, et al., 2021), as long as adequate raw material can be supplied. Seaweed cultivation has been practiced for centuries in Asian countries, and western cultivation methodology and practice is also advancing (Buschmann et al., 2017; Stévant, Rebours, & Chapman, 2017). In Europe today, sugar kelp (*Saccharina latissima*) is the most cultivated specie, and a major interest in food creation using sugar kelp as an ingredient is seen amongst many small and medium sized enterprises (SMEs) in the Nordic countries. However, the content of potential toxic elements (PTEs), most notably iodine (200–7000 mg/kg dw; Blikra, Henjum, & Aakre, 2022), is limiting the advised maximum daily consumption of dried (but otherwise untreated) sugar kelp to 0.15 g (Blikra, Wang, James, & Skipnes, 2021). Application of suitable processing technologies for iodine reduction could be a solution for tackling this bottleneck.

Although iodine is a mineral requirement, both deficiency and excess pose health risks (e.g., Farebrother, Zimmermann, & Andersson, 2019; Laurberg, Pedersen, Knudsen, Ovesen, & Andersen, 2001). An advised consumption of 150 μg/day is recommended for adults with slightly higher values during pregnancy (175 μg/day) and lactation (225 μg/day; EFSA Panel on Dietetic Products and Nutrition and Allergies (NDA), 2014). A maximum daily consumption of 600 μg/day is recommended in Europe. No harmonized European limit is established for iodine, although a limit of 2000 mg/kg dw is proposed in France (CEVA, 2019), and a limit of 20 mg/kg dw is recommended in Germany (BfR, 2007). According to the Commission Recommendation (EU) 2018/464 (European Commission, 2018, p. 78), no maximum levels are established for arsenic, cadmium or lead, except for food supplements (European Commission, 2008, p. 173). For mercury, a limit of 0.01 mg/kg is established for food and 0.1 mg/kg (as sold) for food supplements. The European Commission does not specify if the limit for mercury in seaweed sold as food is expressed as mg/kg dry matter or wet weight,

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[*] Corresponding author.
E-mail address: marthe.blikra@nofima.no (M.J. Blikra).

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which is problematic for seaweeds since the water content is high, commonly ranging between 63 and 92% (Mæhre, Malde, Eilertsen, & Elvevoll, 2014). In the following discussion, it is assumed that the content is expressed as mg/kg wet weight. It has been shown by for instance Stevant et al. (2018) and Blikra, Wang, et al. (2021), that for *S. latissima*, the iodine content is the limiting factor for consumption, whereas the heavy metal and arsenic content pose a lower risk, taking into account the tolerable intake values for PTEs and a portion size of 1–5 g dry weight.

Fresh seaweeds require processing for biomass stabilization, since seaweed deteriorate quickly after harvesting. Dried seaweeds have advantages such as lower storage volume and providing rapid reconstitution during processing. Drying may slightly reduce the iodine content of sugar kelp (3–17%; Stévant et al., 2018). However, further reductions are usually required to obtain iodine contents below the proposed limits (BfR, 2007; CEVA, 2019). Preprocessing prior to drying can aid the reduction of PTEs.

Most available research on reduction of PTEs from brown algae, including kelp, is focused on traditional technologies such as blanching and boiling (Blikra et al., 2022; Blikra, Wang, et al., 2021). These methods reduced the iodine content in sugar kelp by 38–94%. The variation in the iodine reduction can be explained by factors such as operational settings (duration of treatment, water temperature, water-to-seaweed ratio; Blikra, Wang, et al., 2021; Bruhn et al., 2019; Luning & Mortensen, 2015; Nielsen et al., 2020), and may also be influenced by other factors, such as initial concentration of iodine post-harvest, and whether the kelp was previously frozen. However, blanching and boiling are energy demanding processes, and more sustainable technology for iodine reduction with lower associated costs are therefore interesting for the industry.

A promising candidate in this regard is pulsed electric field (PEF) processing, which works by electroporation of the cell wall and cause leakage of intracellular liquid and dissolved substances from foods during processing. Subsequent drying requires less energy and time (Toepfl, Heinz, & Knorr, 2006), which has been documented for the green marine macroalgae *Ulva* sp. (Prabhu et al., 2020). Pulsed electric field technology has also been applied for extraction of proteins (Polikovsky et al., 2016), starch and ash from *Ulva* sp. (Prabhu, Levkov, Livney, Israel, & Golberg, 2019). Robin et al. (2018) found that PEF processing could enhance the ash extraction from *Ulva* sp. by $> 100\%$. The latter authors also found a reduction in the content of cadmium (25%; non-significant), but they did not analyze the content of iodine. To the best of our knowledge, application of PEF for reducing the iodine content in algae has not been previously documented. Furthermore, application of PEF for processing of brown macroalgae has previously not been reported. Since the kelps store most of the iodine as water soluble iodide (Blikra et al., 2022), leakage of water from the food matrix could have the added benefit of reducing the iodine content, and potentially the content of other PTEs.

Furthermore, PEF technology is well suited for high-capacity continuous processing of biomaterials. Currently, production lines with capacities in the range between 3 and 70 t/h are commercially available (e.g., for potatoes). However, investment costs in the range of 300–600 k€ (for the previously mentioned lines) must be considered. Hence more detailed studies are required to strengthen the basis for the economic aspects.

Freezing and thawing prior to drying has been used commercially for stabilization of seaweed biomass while waiting for drying capacity. The resulting thawing loss is colored and of the same magnitude as the thawed biomass (Blikra, Altintzoglou, et al., 2021; Sund, 2020). The thawing loss contains significant amounts of several PTEs, including iodine, arsenic, and cadmium (Sund, 2020). It is therefore of interest to investigate whether the content of PTEs in kelp is reduced by freezing and thawing. Else, use of freezing/thawing as a pre-processing step is an economic liability (price per kg raw material) without adding benefits in terms of food safety apart from stabilization.

In this work, it was investigated whether pretreatments with PEF and freezing/thawing can reduce the content of PTEs in sugar kelp. The results can aid the industry in selecting technology for further improvements to processing lines. Furthermore, the results may contribute to making processed seaweeds more sustainable, cheaper, and safer in terms of reducing the content of PTEs.

2. Materials and methods

2.1. Raw material

About 15 *Saccharina latissima* sporophytes were collected from Kraknes, near Tromsø in Northern Norway. Sporelings were produced as described by Wang, Blikra, Evensen, Skipnes, and James (2022) and deployed at 2–6 m depth. The sporophytes were harvested in June 2021, packed in polystyrene boxes with absorbent, wet tissue paper and ice in plastic bags to keep them cold without causing freeze-damage. When the samples arrived in our laboratory for analysis the following day, they were still crisp, cold, and dry. No sorting was conducted prior to experiments, but the stipe and holdfast was removed, to simulate industrial practice.

The kelp was weighed into randomized batches of 500.5 ± 0.3 g. All batches contained a mixture of large and smaller blades, and minor biofouling. The batches were placed in clean, food-grade plastic bags and folded along the edge to avoid drying prior to further handling (within 8 h). Three batches were used per pre-treatment (=3 biological parallels).

2.2. Pre-treatments

2.2.1. Pulsed electric field

The PEF treatments were conducted using a PEF Pilot Dual (Elea GmbH, Quakenbrück, DE), equipped with a 10 L batch treatment chamber (electrode distance 24 cm). Tap water (5 L, 20 °C) was added to each batch. The water was changed between each parallel. For the treatments, the following conditions were applied: electrode voltage of 24 kV; frequency 30 Hz; and pulse width of 6 us. Two settings were applied for pulse count, one low (200) and one high (800), resulting in a measured energy supplied to kelp and water of 2.7 ± 0.3 and 14.4 ± 1.0 kJ, respectively. After the treatments, each batch was placed in a sieve and allowed to drip for 5 min, followed by weighing, placing in a clean, food-grade plastic bag and storage at 0–2 °C until further handling.

2.2.2. Soaking

Since PEF treatment involved a water phase, the samples which were not treated with PEF, i.e., positive control samples and frozen/thawed samples, were soaked prior to further handling. The same conditions as for PEF were applied, namely soaking in 5 L water (20 °C) for 1 min, followed by placement in a sieve for 5 min and packaging in plastic bags.

2.2.3. Slow freezing and thawing

Three samples (approx. 2 kg) were packaged in sous-vide bags with residual air in the bags to provide resistance to rapid freezing. They were placed in a −20 °C freezer and kept there for two months prior to thawing. The thawing was performed in a 0–2 °C chilling room for three days. Since residual ice crystals were still present in the thawing liquid in the bags, the samples were additionally thawed for 1 day at 4 °C. The samples were placed in a sieve for 5 min prior to drying to allow separation of the thawing loss from the remaining mass.

2.2.4. Drying treatments

Following pre-treatments, the samples from each batch were placed on top of baking paper on a large, perforated steel shelf. The samples were subsequently dried in a Bastramat C1500 drying/smoking cabinet equipped with a MC700 Microprocessor at 25 °C and low humidity until constant weight (4 days).

2.2.5. Dry matter

Dry matter content was determined in wet, untreated samples and samples from each pre-treatment after dehydration. For wet, untreated samples, 5.6 ± 0.5 g was weighted into pre-weighted aluminum cups. For dry samples, 2.0 ± 0.4 g was used. The samples were dried for 18–20 h at 100 °C. Two analytical replicates were taken from each parallel (=6 replicates per treatment).

2.2.6. Iodine

Elemental iodine analysis was performed by Mikroanalytisches Labor Kolbe, Oberhausen, Germany, as described by Blikra, Wang, et al. (2021). Briefly, ground seaweed samples were crushed using an IKA MF10 mill and taken through a 0.5 mm sieve. The digestion was performed in a special combustion unit from A1-Envirosciences (AQF-2100) with a manual sampler, at 1100 °C, and burned in an argon/oxygen stream. The resulting gases were measured on a Metrohm Model 883 Plus ion chromatograph. The lower limit of detection was 1 ppm. Two analytical replicates were taken from each parallel (=6 replicates per treatment).

2.2.7. Arsenic, cadmium, lead, and mercury

Analysis of the remaining PTEs was performed by ALS Scandinavia AB Luleå Aurorum 10, Sweden, as described in depth by Blikra, Wang, et al. (2021). Briefly, the samples were dissolved in nitric acid/hydrogen peroxide with traces of hydrofluoric acid in a microwave oven following B-PF51HF-MW or B-PF51-MW. The analysis was performed using ICP-SFMS/ICP-AES. One analytical replicate was taken from each parallel (=3 replicates per treatment).

2.2.8. Energy requirements

For PEF treatments, the energy required to process the kelp and water was determined by the PEF Pilot Dual equipment. Waste heat removal from the PEF equipment and water bath was not included in this estimation.

The energy input (Q) required to process 500 g of kelp by blanching (45 °C) and boiling (95–99 °C) was estimated using Equation (1). Since the iodine reduction requires that the water be exchanged between each batch during processing, the input energy was calculated as the energy needed for heating the system (water and seaweed) from initial to final temperatures. The respective masses of sugar kelp and water are given by m_s and m_w, while $C_{p.s}$ and $C_{p.w}$ denote the specific heat capacity of sugar kelp (3.2 kJ/kg*K) (Sappati, Nayak, & VanWalsum, 2019) and water (4.2 kJ/kg*K), respectively, and $T_{process}$ and T_{input} are the final (45–99 °C) and initial (10 °C) temperatures used for processing. The loss of heat to the surroundings during heating and maintaining the temperature, and the energy needed for subsequent cooling was not included in the calculation.

$$Q_{input}\left[\frac{kJ}{kg}\right] = \frac{\left(\left(m_s \bullet c_{p,s}\right) + \left(m_w \bullet c_{p,w}\right)\right) \bullet \left(T_{process} - T_{input}\right)}{m_s + m_w} \qquad \text{Eq. 1}$$

2.3. Statistical analysis

Analysis of variance (ANOVA) was performed to test for significant differences between sample groups, using Minitab® version 19.2020.1 and a 95% confidence interval. A Tukey post hoc test was applied when more than two sample groups were present. The results are given as average ± sample standard deviation.

3. Results and discussion

The high and unpredictable iodine content of *S. latissima* is the primary bottleneck for its widespread utilization in food (Blikra, Wang, et al., 2021). In previous studies, 38–94% reduction in iodine was achieved using boiling and blanching treatments (Blikra, Wang, et al., 2021; Bruhn et al., 2019; Luning & Mortensen, 2015; Nielsen et al., 2020). In our study, a significant reduction of around 40% was achieved using PEF treatments, compared to positive control samples (Table 1). This implies that PEF treatments (1 min) combined with soaking in room tempered tap water had a 40% increased effect on iodine reduction compared to soaking without PEF treatments. No significant difference between the two PEF treatments was observed with respect to iodine reduction.

During PEF processing, the permeability of cell membranes to ions or molecules of specific size ranges (nm-range) are often increased, depending on operational settings (Saulis, 2010). The electrochemistry of chemical species may also be affected. As summarized by Saulis (2010), previous studies using other types of tissue (not seaweeds), found that electroporation can result in pores large enough to let water and small ions out, but too small to allow permeation of e.g., mannitol.

In our study, a 40% reduction in the iodine content was achieved by the PEF processing, which implies that the applied electroporation settings improved the permeability of hydrophilic iodine species (most notably iodide) across the kelp's cell membrane. To the best of our knowledge, our manuscript provides the first time this is demonstrated in practice. There were no significant differences between the two PEF conditions applied in our setup, indicating that the number of pulses supplied above 200 had negligible effect on the net mass transfer of iodine. Optimizing and fine tuning the conditions may induce further mass transfer of iodine, but this remains to be seen in later studies. In the current study, minor differences in iodine reduction could also have been camouflaged by the inherent raw material variations.

As mentioned earlier, information on the effects of the PEF technology on the release of PTEs from brown algae is lacking in the literature. What is known, however, is that the conditions used in our study should lead to electroporation of the cells. The release of PTEs will also depend on the release of such elements from chemically bound compounds or complexes, in addition to the microchannels created by PEF. Further to this, the location of the compounds can be foreseen to play a role in the release. Both iodine and arsenic has previously been found to be mainly located in the peripheral tissue of *Laminaria digitata*, and their distribution showed a huge decreasing gradient from the outer meristoderm to the inner medulla (Verhaeghe et al., 2008). It may well be that the PEF treatment can cause effects additional to the perforation which contribute to the release of PTEs, but this remains a speculation until more targeted and detailed investigations are available.

In contrast to PEF, freezing and thawing ruptures and depolymerizes the cell walls and expands the extracellular spaces due to expansion of ice crystals. These complex mechanisms have been reviewed by e.g., Li, Zhu, and Sun (2018), who also explained how PEF may reduce freeze damage. A previous study focusing on sugar kelp, found that after freezing and thawing, the resulting thawing loss contained iodine in the same order of magnitude as in the untreated biomass (Sund, 2020). This suggests that freezing followed by thawing is an effective method of iodine reduction. It is therefore surprising that we found no significant difference between the iodine content in the remaining biomass after freezing and thawing compared to positive control samples (Table 1). However, a similar iodine content in the samples does not necessarily mean that iodine was not lost during thawing. Rather, this can be explained by an equally significant loss of other matter during thawing. In the aforementioned study (Sund, 2020), the thawing loss was found to contain ash (29% of dw), mannitol (9% of dw) and protein (3% of dw). For apples, freezing at −20 °C was found to lead to formation of ice crystals in the range of 10–30 μm (Chassagne-Berces et al., 2009). Ruptures of this size will allow an untargeted loss of compounds during thawing. In conclusion, bulk freezing and thawing can not be recommended as a processing method for reducing the iodine content of kelp.

The weight loss (after drying) associated with pre-processing using PEF was not significantly different when compared to freezing/thawing (93–94%, including loss of moisture, n = 2–3 per treatment). Along with the finding that the iodine content of kelp was not significantly changed by the freezing/thawing pre-treatment, whereas PEF reduced the iodine content significantly (40%), this could suggest that PEF can provide a

Table 1
The content of PTEs (mg/kg dry sample) and the dry matter content (g/100 g wet weight) of samples of *Saccharina latissima*.

Sample name	Data (mg/kg dry sample)				Limits (mg/kg)		
	Pos. control	PEF (1)	PEF (2)	Freeze-thawed	EU – food supplement*	EU – algae as food**	France***
Iodine[1]	4700 ± 600^{a}	2700 ± 100^{b}	2900 ± 300^{b}	4400 ± 300^{a}	none	none	2000
Arsenic[2]	71 ± 7^{a}	63 ± 9^{a}	63 ± 12^{a}	65 ± 1^{a}	none	none	iAs: 3
Cadmium[2]	2.1 ± 0.5^{a}	1.9 ± 0.1^{a}	2.1 ± 0.5^{a}	2.2 ± 0.3^{a}	3.0	none	0.5
Mercury[2]	0.029 ± 0.003^{a}	0.023 ± 0.002^{b}	0.024 ± 0.001^{b}	0.026 ± 0.004^{ab}	0.1	0.01	0.1
Lead[2]	0.9 ± 0.6^{a}	1.8 ± 1.1^{a}	5 ± 7^{a}	1.4 ± 0.7^{a}	3.0	none	5
Dry matter[1]	91.3 ± 0.2^{a}	90.2 ± 0.3^{b}	89.8 ± 0.2^{b}	90.3 ± 0.4^{b}			

The data are presented as mean ± sample standard deviation. Different lower-case letters indicate significant differences within the rows (between treatments). Three biological parallels were used.

[1] Each parallel was analyzed twice (6 replicates).

[2] Each parallel was analyzed once (3 replicates). The dry matter content in fresh, untreated kelp was 14 ± 4 g/100 g.

[*] mg/kg as sold (European Commission, 2008, p. 173).

[**] not specified if mg/kg dry weight or wet weight (European Commission, 2018, p. 78).

[***] mg/kg dry matter (CEVA, 2019).

more targeted reduction in certain compounds than freeze-thawing. Furthermore, it is likely that larger components which readily leak out during freezing/thawing (Sund, 2020), could be retained during PEF processing, although this should be confirmed in future experiments. Further knowledge should be obtained to optimize the PEF treatment for maximizing iodine output while minimizing the release of desired elements in the cells of the seaweeds.

Although a reduction in iodine content of −40% was achieved by the PEF treatments, the resulting concentration of iodine (2700–2900 mg/kg dw) was still above the limit proposed by France (2000 mg/kg dw; CEVA, 2019) and Germany (20 mg/g dw; BfR, 2007). Future work should aim to further reduce the iodine content, e.g., by optimizing the PEF treatment or combinations with other pre-processing technologies. To maintain an acceptable iodine content in foods with seaweed ingredients, and limit the associated risks, our recommended approach is to consider both the iodine content in the seaweed ingredient, and the amount of added iodine per portion size. In our case, a daily intake of 150 μg iodine, which is the recommended daily consumption for adults, can be achieved by ingesting approximately 0.05 g PEF treated, dried sugar kelp. This amount of kelp is suitable for instance used in a spice blend as a salt replacer or flavor enhancer (Blikra, Altintzoglou, et al., 2021).

Regarding the other PTEs, only mercury is regulated, and pose a potential limitation for sale of seaweed as food. In all cases, the sugar kelp used in our study contained mercury below the European limit of 0.1 mg/kg dw (calculated from 0.01 mg/kg ww, assuming 90% moisture). The PEF processing significantly reduced the mercury content by 19% compared to positive control samples, resulting in a final mercury content of 0.02 mg/kg dry sample. For the remaining PTEs, no significant reductions were achieved by the processing, although the mean content of arsenic decreased following all pretreatments by 11% on average compared to positive controls, with a greater net reduction after the low PEF treatment (−12%) and a lesser net reduction after freezing/thawing (−9%). For lead, the mean content after the high PEF treatment was much higher than the other treatments. This was caused by one high value (13 mg/kg dry sample), whereas the other values are within range of the other treatments (0.6–2.0 mg/kg dry sample). It is unlikely that this value is caused by the PEF treatments. Rather, inherent raw material variations or possibly, analytical error, could give rise to this unexpected result.

3.1. Energy requirements

To give an indication of the energy requirement associated with PEF processing compared to traditional boiling and blanching, the input energy was estimated by using experimental data found in previous studies (Table 2). Based on the calculations, PEF processing required approximately 50–100 times less energy input than conventional processing. Although blanching may reduce the iodine content approximately 2-fold compared to PEF, approximately three times more water and 50 times more energy was needed to achieve this result.

It should be noted that the energy consumption associated with drying has been estimated to be around 10 MJ (until 22% moisture; van Oirschot et al., 2017), which is 30–70 times higher than conventional pre-processing methods. Unless environmental drying (e.g., solar) is applied, the drying step is thus the processing step requiring the largest amount of energy. It is therefore also worth noting herein that PEF may yield the added benefit of reducing drying time and thus also energy consumption, and, e.g., 12% reduction in drying time was found for apples (Wiktor et al., 2013). Thus, PEF is a promising technology for iodine reduction while maintaining low industrial energy requirements, although further investigations and fine tuning is required.

4. Conclusion

In the last decade, sugar kelp has gained increasing interest as sustainable foodstuff, but the iodine content is so high that <1 g dry kelp provides the maximum recommended daily limit (Blikra, Wang, et al., 2021). Processing can reduce the iodine content, but traditional methods such as blanching or boiling are energy demanding and may not be economically viable industrially.

In this study, it was investigated whether the content of PTEs in *S. latissima* was affected by PEF processing and freezing/thawing pretreatments prior to drying. The iodine content was significantly reduced by PEF (−40%), but not by freezing and thawing. The mercury content was also reduced by PEF processing.

Table 2
Input energy required for processing of kelp and the associated iodine reduction.

Treatment	Iodine reduction (%)	Temperature (°C)	Time	Water to kelp ratio	Input energy (kJ/kg)	Reference
Blanching	92	45	2 min	33	150	1
Boiling	38	95	15 min	3.8	340	2
Boiling	85	99	15 min	10	370	3
PEF - low	42	r.t.	~10 s	10	2.7	**This study**

References: 1:Nielsen et al. (2020); 2: Bruhn et al. (2019); 3: Blikra et al. (2021).

Since the input energy to the product by PEF is <10% of traditional boiling and blanching, applying this technology can lead to greener seaweed processing than traditional processing. This can, in turn, bring the costs of processed seaweed down, which is a major benefit for market introduction. Future method improvement should investigate the potential for discriminating loss of valuable elements by optimizing cell membrane electroporation. Furthermore, a holistic perspective on the reduction of PTEs coupled with analysis of food quality, energy and water consumption is warranted.

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CRediT authorship contribution statement

Marthe Jordbrekk Blikra: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Dagbjørn Skipnes:** Conceptualization, Formal analysis, Investigation, Writing – review & editing, Supervision, Funding acquisition. **Torstein Skåra:** Conceptualization, Methodology, Validation, Investigation, Writing – review & editing, Supervision, Project administration.

Data availability

Data will be made available on request.

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