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## Antioxidant composition and activity of seaweed

### *Saccharina latissima*: a seasonal perspective

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Safety concerns regarding reported toxicity of artificial antioxidants lead the search for novel natural antioxidants. In this context, seaweeds have been receiving increasing attention as a promising source of antioxidants such as phenolic compounds (e.g. phenolic acids and flavonoids), carotenoids (e.g. fucoxanthin and  $\beta$ -carotene), and phycobiliproteins. Nevertheless, seaweed composition generally presents marked seasonal variations. The present study aimed at evaluating seasonal variations in the antioxidant composition and activity of sugar kelp, *Saccharina latissima*, cultivated at two different sites; in close proximity to a blue mussel and rainbow trout farm (IMTA), and at a reference/control site (REF), outside Horsens fjord, Denmark. Rope cultivated seaweed was sampled bi-monthly from three droppers ( $n=3$ ;  $\geq 10$  specimens per replicate) from May 2013 to May 2014; the biomass was freeze-dried and stored frozen until further analysis. Total phenolic compounds (TPC) were determined both spectrophotometrically (Folin-Ciocalteu) and by HPLC coupled to UV detection (255 nm). Concentration of TPC determined spectrophotometrically was higher in November and January (1,225-2,406  $\mu\text{g GAE}\cdot\text{g}^{-1}$  DW) compared to September. Noteworthy, concentration of TPC determined by HPLC was around 10 times lower compared to the previous method. Gallic acid, rutin and quercitrin were identified out of 18 polyphenol standards. Moreover, TPC concentration in November (203  $\mu\text{g}\cdot\text{g}^{-1}$  DW) was similar to that found in September and January, but significantly higher compared to May 2014. The highest concentration of flavonoids was found in September (4,558-4,830  $\mu\text{g RE}\cdot\text{g}^{-1}$  DW;  $p<0.05$ ). The pigment profile (i.e. type of pigments) did not change seasonally, whereas fucoxanthin and chlorophyll a were the most abundant. Fucoxanthin concentration was lower in September compared with all the other months (261-665  $\mu\text{g}\cdot\text{g}^{-1}$  DW;  $p<0.05$ ). Total antioxidant capacity (TAC) was significantly higher in November (3,835-4,050  $\mu\text{g GAE}\cdot\text{g}^{-1}$  DW). Regarding the antioxidant activity (DPPH), there was no significant difference in the IC<sub>50</sub> between seasons, although it was tendentially lower in September and November (0.42-0.49  $\text{mg}\cdot\text{mL}^{-1}$ ). Statistical analysis reveals a strong positive correlation between TAC and TPC, whereas flavonoids and fucoxanthin were poorly correlated to TAC. There was no significant difference for all the analyzed variables between cultivation sites. This study reveals marked seasonal variations, and high biological variability (difference among replicates), in the content of antioxidants which needs to be taken into account when considering seaweed as a source of antioxidants. Moreover, our results suggest that spectrophotometric methods may result in overestimation of TPC and that HPLC is more selective, also identifying some phenolic acids and flavonoids.