The Measurement of Antioxidant Capacity and Colour Attributes in Wild Harvest Samphire (*Tecticornia* sp.) Samples Using Mid-infrared Spectroscopy

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Abstract

Samphire (*Tecticornia* sp.) is an underutilised Australian indigenous edible halophyte and has been used as complementary vegetable, salads or salt substitute. The present study aimed to characterise as well as to differentiate wild harvested samphire samples from different sub-locations in the Kimberly Region of Western Australia using mid-infrared spectroscopy. Antioxidant capacity measured as total phenolic content (TPC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, and colour attributes were determined in the harvested samples using routine reference methods. This data was used to develop mid-infrared calibration models using partial least squares regression. The present study shows the ability of mid-infrared spectroscopy to predict TPC and DPPH radical scavenging capacity in wild harvested samphire samples.

Keywords *Tecticornia* · Wild harvest · Infrared · Antioxidant capacity · Colour · Quality

Introduction

Samphire (*Tecticornia* sp.) is an underutilised Australian indigenous edible halophyte of the subfamily Salicornioideae (family Amaranthaceae) (Moir-Barnetson 2014; Srivarathan et al. 2021). *Tecticornia*, a genus of 44 species, grows spontaneously in both arid and semi-arid land regions, and is solely distributed in Australia except for two species, namely *T. indica* (Australia, Eastern Africa and Southern Asia) and *T. australasica* (Australia, Java and New Guinea) (APC 2008; Kadereit et al. 2006; Shepherd and Wilson 2007; Bhanuvalli et al. 2018). Plants from this genus are stem-succulent halophytes dominating the salt marshes and salt lakes of inland Australia (Moir-Barnetson 2014; Srivarathan et al. 2021).

In Australia, most samphire species are popular among Indigenous communities where they have been utilised as food ingredients, animal feed and medicine for many years. However, information about the nutritional properties and dietary relevance of *Tecticornia* species is very limited despite its ecological importance and salinity tolerance (Srivarathan et al. 2021; Jansen 2004; Díaz et al. 2013). Recent reports highlight the importance of this plant as a complementary vegetable, salads or salt substitute (Srivarathan et al. 2021; Jansen 2004; Díaz et al. 2013). Regardless, the interest in halophytic species (especially samphire plants) has significantly increased in recent years due to concerns about utilising saline lands for sustainable food production (Srivarathan et al. 2021; Jansen 2004; Díaz et al. 2013). Two recent studies have investigated the nutritional composition (including anti-nutrients), bioactive compounds, antioxidant capacity (Srivarathan et al. 2021) and bioactivities (Bhanuvalli et al. 2018) of selected *Tecticornia* sp. samples. Different culinary

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"applications" of *Tecticornia* sp. plant parts have been reported in the literature such as pickling in vinegar or consumption as a condiment or seasoning ingredients (Jansen 2004). As for all edible plants, growing conditions, location and specific environmental factors can have significant impact on the nutritional composition of *Tecticornia* sp. and subsequently their quality and value as a food (Srivarathan et al. 2021; Jansen 2004; Díaz et al. 2013).

To screen and assess the nutritional quality of *Tecticornia* sp. grown under different environmental conditions, a rapid, validated, and reliable high-throughput assay would be ideal. In the past decades, vibrational spectroscopy techniques such as near-infrared (NIR) and mid-infrared (MIR) gained significant attention due to their promising features of being rapid, convenient, no sample preparation and non-destructive (Karoui et al. 2010). More recently, both NIR and MIR spectroscopies were evaluated to investigate Australian indigenous plants to assess the effects of sample preparation, different drying and storage methods, compositional characteristics, and quality parameters of different wild harvest samples (Sultanbawa et al. 2020; Cozzolino et al. 2021; Mulisa Bobasa et al. 2020).

The aim of the present study was to characterise as well as to differentiate Australian grown samphire (*Tecticornia* sp.) samples collected from different sub-locations in the Kimberly Region of Western Australia by non-destructive MIR spectroscopy.

Materials and Methods

Plant Materials

Fresh samples of *Tecticornia* sp. were harvested from three different sub-locations in June 2020 in Kimberley, WA. These regions are different in climate (e.g. rainfall, temperature) and soil properties. *Tecticornia* spp. (n = 10) from each sub-location were randomly uprooted and stored at − 80 °C. The leaves and young twigs were freeze-dried and ground into a fine powder by using a MM 400 Retsch Mixer Mill (Retsch, Haan, Germany) and kept in airtight containers at − 80 °C until further analysis.

Determination of Antioxidant Capacity

Extraction Procedure

Briefly, 0.5 g of dried powder of *Tecticornia* sp. was vortexed with aqueous methanol (80%, v/v) containing 0.1 M HCl (Hong et al. 2020). The mixture was shaken using a reciprocating shaker (RP1812, Paton Scientific, Victor Harbor, SA, Australia) for 10 min at 200 rpm and centrifuged (Eppendorf Centrifuge 5804, Eppendorf, Hamburg, Germany) at 3900 rpm for 10 min at 4 °C. The supernatant was collected and the residue was re-extracted with the extracting solvent, followed by ultra-sonication at 4 °C, shaking and centrifugation as described above until the supernatant was colourless. The supernatants were combined and filtered through a 0.2-µm PP membrane filter prior to the determination of the total phenolic content and 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity. All extractions were performed in triplicate (Hong et al. 2020).

Total Phenolic Content

Total phenolic content (TPC) (Folin-Ciocalteu assay) was determined as described previously by Phan et al. (2019) using a micro-plate absorbance reader (Sunrise, Tecan, Maennedorf, Switzerland) at 700 nm. TPC was expressed as milligrammes of gallic acid equivalents per gramme of sample (mg GAE/g), based on an external gallic acid calibration curve (0–105 mg/L) (Phan et al. 2019).

DPPH Radical Scavenging Capacity

The 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity (DPPH) was measured as outlined by Moore and Yu (2008) with slight modifications, using a micro-plate absorbance reader (Sunrise, Tecan) at 517 nm. The radical scavenging capacity was expressed as µM Trolox equivalents (TE) per gramme of sample, based on an external Trolox calibration curve (5–35 µM) (Moore and Yu 2008).

Colour Measurement

The colour of the freeze-dried samphire samples was measured using a handheld Konica Minolta CR-400 Chroma Meter (Konica Minolta, Osaka, Japan). Different known colour parameters were measured including L*, a*, b*, chroma and hue angle. The Chroma meter was calibrated with a Minolta standard reference plate at the beginning of the analysis. Freeze-dried samphire samples were placed in a Petri dish to provide adequate thickness to be measured. All the measurements were done in duplicate.

Infrared Measurement

The MIR spectra of dried powdered samples were acquired using a Bruker Alpha spectrophotometer fitted with an attenuated total reflectance platinum diamond single reflection cell (Bruker Optics GmbH, Ettlingen, Germany). The MIR spectra were recorded using OPUS software version 8.5 (Bruker Optics GmbH). Measurements were recorded in the spectral region from 4000 to

400 cm^{-1}. Each spectrum was computed using the average of 24 interferograms at a resolution of 4 cm^{-1}. Air was used as the reference background spectra and reset every 10 samples. The attenuated total reflectance (ATR) cell was cleaned between samples with a solution of 70% v/v ethanol and water to avoid cross contamination during the analysis.

Data Analysis

The Unscrambler X software (v11, CAMO ASA, Oslo, Norway) was used for multivariate analysis. The spectra were pre-processed using the second derivative (second polynomial order and a smoothing window size of 10 points) (Savitzky and Golay 1964). The second derivative was applied as it has been reported to be effective at correcting for baseline effects and slope of a spectrum (Bureau et al. 2019; Cozzolino et al. 2019). Principal component analysis (PCA) was performed to visualise the structure of the data and identify dominant features in the spectra and variation in the samples for further investigation (Bureau et al. 2019; Cozzolino et al. 2019). Calibration models between the spectra (MIR) and reference data were developed using partial least squares regression (PLS) (Bureau et al. 2019; Cozzolino et al. 2019; Williams et al. 2017). The data set was split into calibration and validation using the Kennard-Stone algorithm (Kennard and Stone 1969). The optimal number of factors for the calibration model was selected based on the minimal value of the predicted residual sum of squares (PRESS) and the highest correlation coefficient (R^2) between actual and predicted values. The PLS models were evaluated in terms of the number of factors, standard error of cross-validation (SECV) and correlation coefficient. The residual predictive value (RPD) was used to evaluate the accuracy of the models (Bureau et al. 2019; Cozzolino et al. 2019; Naes et al. 2002; Williams et al. 2017).

Results and Discussion

Figure 1 shows the second derivative of the MIR spectra of the samphire samples collected from the three different localities. Main peaks were observed around the MIR region at 1630 cm^{-1} associated with amide groups (e.g. proteins) (Schulz and Baranska 2007; McGoverin et al. 2010; Cozzolino 2015). Around 1300 cm^{-1} and
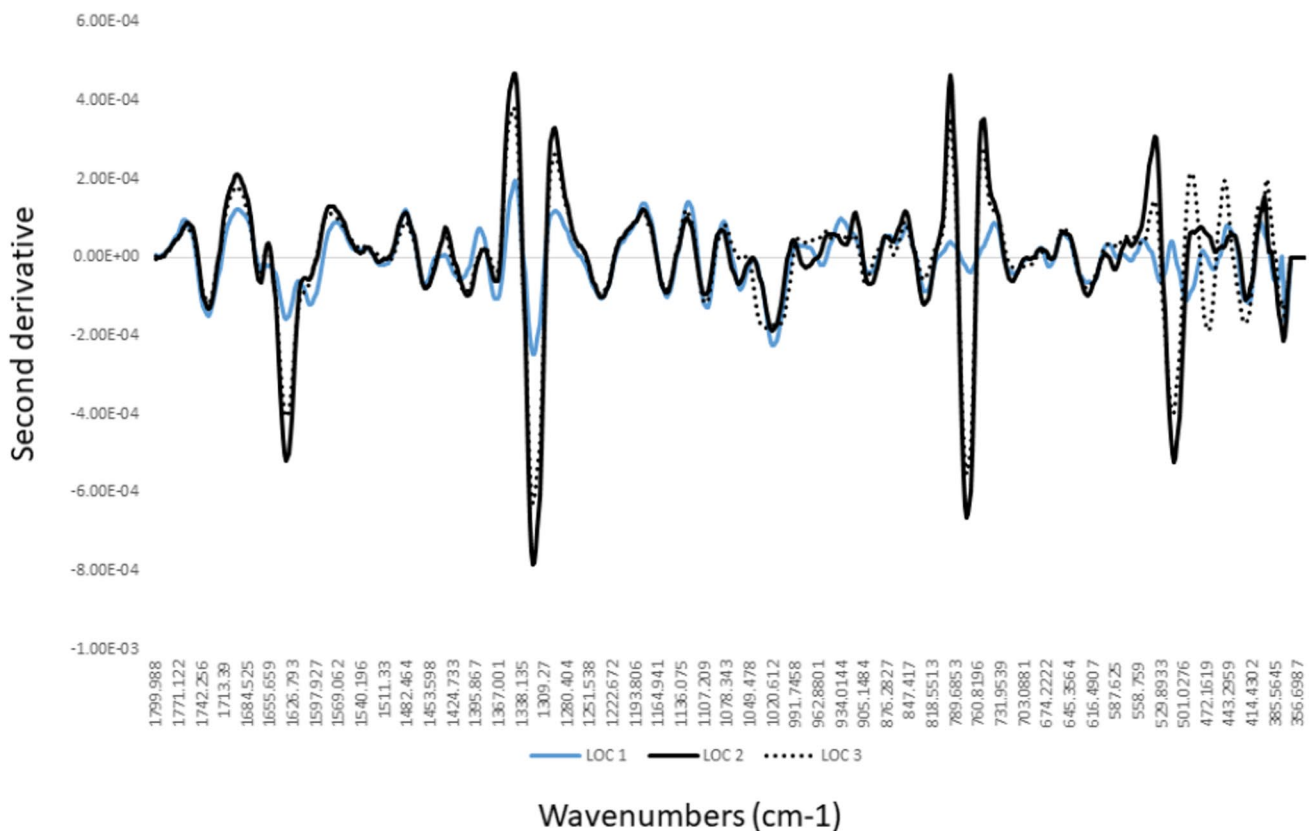

Fig. 1 Second derivative mid-infrared spectra of samphire samples sourced from three different regions

800 cm^{-1}, these frequencies were associated with O–H and C-H bends of aromatic groups and phenolics as reported by other (Schulz and Baranska 2007; McGoverin et al. 2010; Cozzolino 2015). Around 1739 cm^{-1} (-C– – O- stretching) showed the presence of ester groups (Schulz and Baranska 2007; McGoverin et al. 2010; Cozzolino 2015). Frequencies between 985 and 801 cm^{-1} are related with -C– –C-stretching of aromatic compounds (e.g. phenolic compounds) (Schulz and Baranska 2007; McGoverin et al. 2010; Cozzolino 2015). In addition, frequencies around 718, 636 and 609 cm^{-1} are associated with C–Cl stretching and might be related with the presence of excessive amounts of chloride in the sample (Lewis et al. 1994; Shipman et al. 1962).
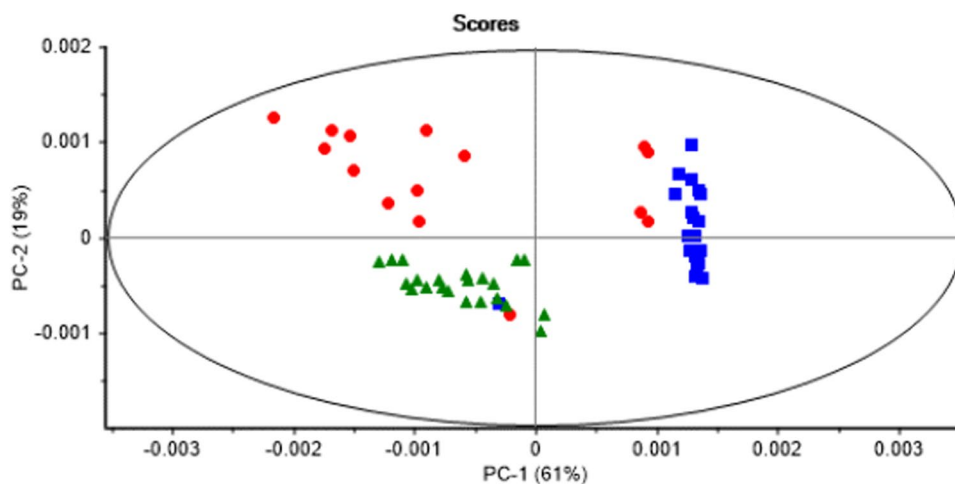
The principal component score plot of the samphire powder samples analysed is shown in Fig. 2. The first two principal components explained 81% of the variability in the data set. The samples are clustered together based on the origin (locality) along the first principal component (61% of the variability). Samples also clustered together according to locality along the PC2 (19% of the variability) as well as related with the differences between samples (sample to sample variation). The interpretation of the PCA loadings (Fig. 3) showed that wavenumbers around 1652 cm^{-1} are associated with amide groups (e.g. protein and water content), around 1377 cm^{-1} and around 820 cm^{-1} with aromatic and phenolic groups (Schulz and Baranska 2007; McGoverin et al. 2010; Cozzolino 2015). The observed variations in these frequencies contributed to explain the differences in composition among the samples due to the region.

Table 1 reports the descriptive statistics (average, range, standard deviation) for the measurement of TPC, DPPH, and colour parameters determined in the samphire powder samples used to develop the PLS calibration models. The range in composition showed a large variation in the data set attributed to the locality or origin of the plant. This variation might be attributed to the different environmental conditions (e.g. temperature and humidity) as well as the type of soils (e.g. chemical properties, pH and carbonate content) encountered in the regions where these plants grow. Overall, the range in composition was considered adequate to develop the PLS calibrations for these parameters using MIR spectroscopy. Table 2 shows the calibration and validation statistics obtained using MIR spectroscopy for the prediction of TPC, DPPH and colour parameters in samphire powder samples. The coefficient of determination (R^2) and the standard error in cross-validation (SECV) were 0.82 (SECV: 1.23), 0.75 (SECV: 6.76), 0.79 (SECV: 1.27), 0.67 (SECV: 1.1) and 0.77 (SECV: 1.52) for TPC, DPPH, L*, a* and b*, respectively. The RPD values obtained in cross-validation were equal or higher than 2, indicating that these calibrations can be used for a qualitative determination of these parameters using MIR spectroscopy (Bureau et al. 2019; Williams et al. 2017). These calibrations can deliver a semi-quantitative (low, medium or high concentration) tool for the measurement of these parameters in samphire powder samples (RPD ≥ 3) (Bureau et al. 2019; Williams et al. 2017).

The PLS loadings for the optimal calibration models developed for the measurement of the chemical parameters in samphire powder samples are shown in Fig. 4. The PLS calibrations for TPC showed the highest loadings in frequencies around 1797 cm^{-1}, 1459 cm^{-1}, 1393 cm^{-1}, 1171 cm^{-1}, 997 cm^{-1}, 870 cm^{-1} and 583 cm^{-1}. For the prediction of DPPH, the main loadings were observed around 1641 cm^{-1}, 1591 cm^{-1}, 1364 cm^{-1}, 1088 cm^{-1} and 915 cm^{-1}. The descriptions of the frequencies or MIR regions used to develop the PLS calibrations (loadings) are similar as those described in Figs. 1 and 3.

Fig. 2 Principal component score plot of the samphire powder samples analysed using mid-infrared spectroscopy and collected from three different regions. The samples are clustered together according to their locality (red: location 1; blue; location 2; green: location 3)
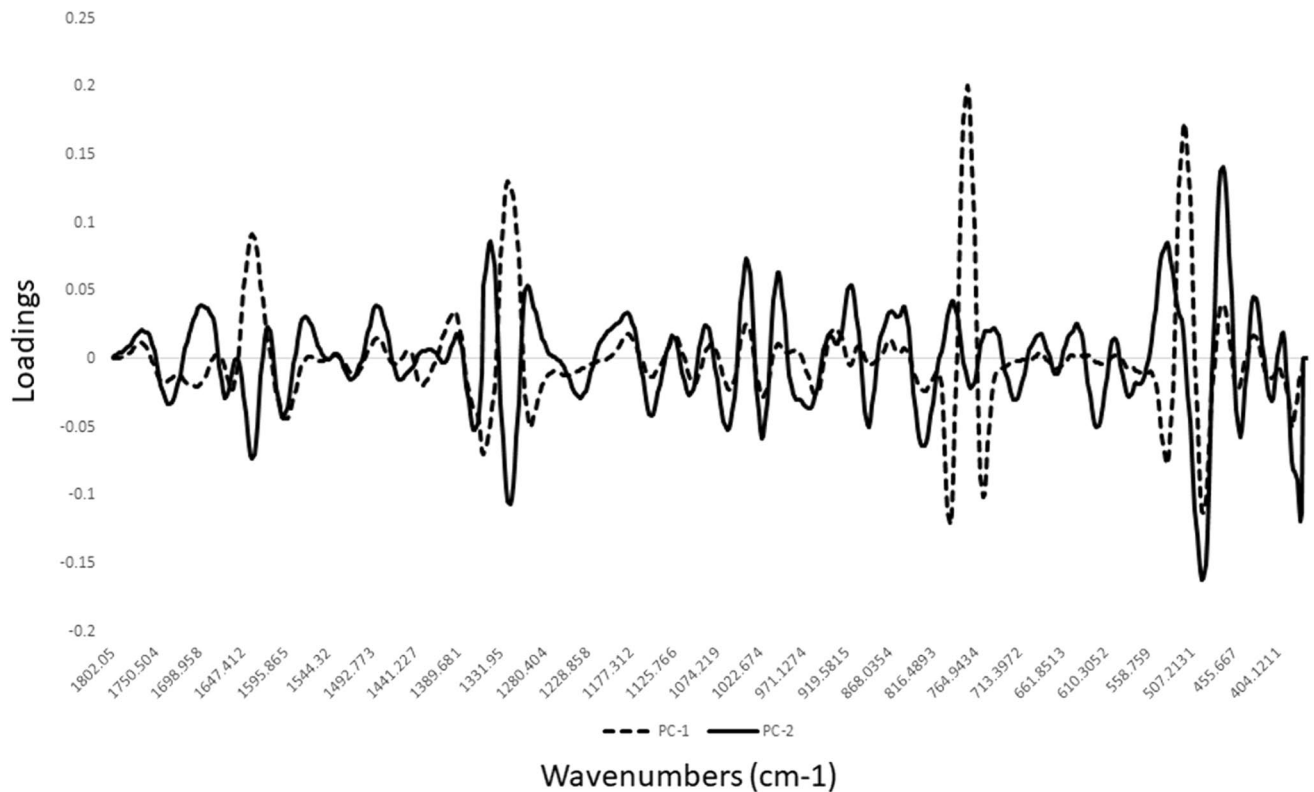
Fig. 3 Loadings derived from the principal component analysis of the samphire powder samples analysed using mid-infrared spectroscopy and collected from three different regions

Conclusion

The results of this study showed the ability of MIR spectroscopy to measure TPC and DPPH radical scavenging capacity, and colour parameters in the samphire powder samples. Although the RPD values obtained are not considered adequate to quantify the individual antioxidant compounds, they can be used as rapid screen of plants for low, medium and high antioxidant capacity. Further research is needed to optimise the prediction models for these antioxidant compounds by the addition of more samples, as well as to evaluate the effect of region/origin and harvest (years) to make the models more robust for routine applications.

Table 1 Descriptive statistics for the measurement of total phenolic content, 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity and colour attributes in Australian grown samphire samples analysed using mid-infrared reflectance spectroscopy

	L*	a*	b*	TPC (mg GAE/g DW)	DPPH (μM TE/g DW)
Mean	64.75	0.74	22.17	7.56	39.82
SD	3.01	1.8	3	3.28	16.09
Max	69.97	3.87	27.3	18.64	97.15
Min	57.88	−2.57	17.26	3.04	14.79
CV	4.6	243.2	13.5	43.4	40.4

CV, coefficient of variation (CV = SD/mean); *SD*, standard deviation; *TPC*, total phenolic content; *DPPH*, 1,1-diphenyl-2-picrylhydrazyl radical scavenging

Table 2 Cross-validation and validation statistics for the prediction of total phenolic content, 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity and colour attributes in Australian grown samphire sample analysed using mid-infrared reflectance spectroscopy

	R^2	SECV	Bias	Slope	RPD	LV
L*	0.78	1.27	0.009	0.77	2.37	7
a*	0.67	1.08	0.018	0.73	1.67	6
b*	0.77	1.52	−0.009	0.77	1.97	2
TPC	0.83	1.24	−0.0009	0.809	2.65	6
DPPH	0.75	6.76	0.11	0.84	2.38	8

LV, number of optimal latent variables used to develop the models: RPD, SD/SECV; *SECV*, standard error for cross-validation; *TPC*, total phenolic content; *DPPH*, 1,1-diphenyl-2-picrylhydrazyl radical scavenging
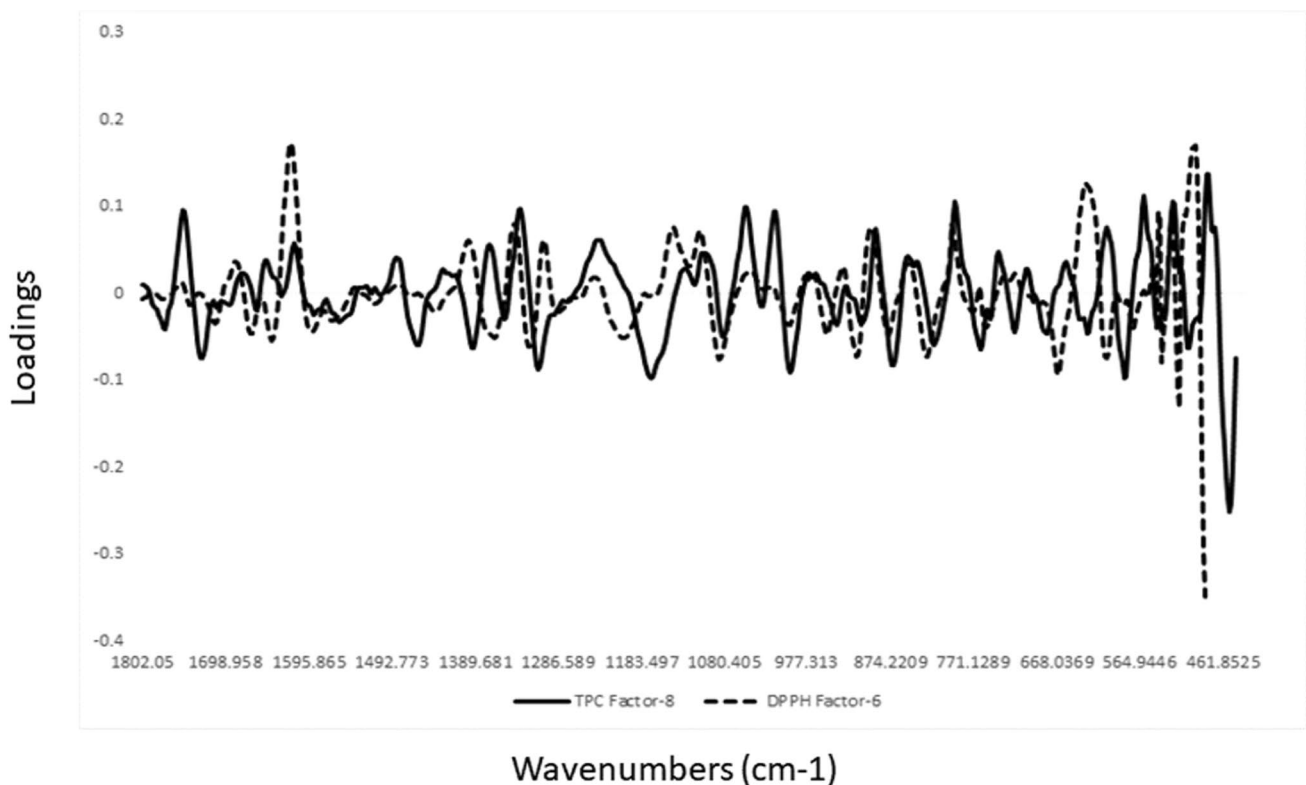
Fig. 4 Partial least squares regression loadings for the prediction of total phenolic content and 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity in the samphire powder samples analysed using mid-infrared spectroscopy and collected from three different regions

Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent not applicable.

Conflict of Interest Sukirtha Srivarathan declares that she has no conflict of interest. Anh Dao T. Phan declares that she has no conflict of interest. Olivia Wright declares that she has no conflict of interest. Yas-

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