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### Can samphire be the new salt? Understanding the potential of samphire harvested from the UK coastline

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#### ABSTRACT

Salicornia species have been explored as a substitute for salt, however the intensity of salty taste elicited remains unexplained by the sodium content alone. To investigate this, a study was conducted to determine the nutrient profile of samphire extract and relate this to its sensory quality in a nachos base. Freeze dried samphire extracts contain minerals, including Na (12–14 g/100 g), K (1–1.5 g/100 g) and Mg (0.3–0.5 g/100 g) and free amino acids such as lysine (28–41 mg/100 g), glutamic acid (20–31 mg/100 g), aspartic acid (20–56 mg/100 g) and arginine (54–109 mg/100 g), which are known to influence salty taste. The sensory panel found that 2.5 % addition of samphire extract produced a significantly saltier taste than the control product (0.7 % NaCl) at an equivalent sodium level. These findings suggest that the minerals and amino acids in samphire extract may collectively contribute to its salty taste, making it a viable option for reducing sodium in food products.

#### 1. Introduction

Salt reduction is considered to be one of the top challenges faced by the food industry. Despite substantial existing research to identify a suitable salt replacer, no single solutions are currently able to sufficiently reduce sodium. The substitutes often bring sensory defects in the final product, such as noticeable loss of salty taste, and (or) increased bitter or metallic taste (Sinopoli & Lawless, 2012). Sodium chloride plays a key role in food processing, and reduction of salt will affect the textural, microbiological, and sensorial attributes of the product which is often unacceptable to consumers. With the high demand for naturally sourced healthy products, previous research has explored a plant-based salt as alternatives to replace sodium chloride (Lee, 2011; Pires-Cabral et al., 2021). This plant is commonly known as Samphire (Salicornia sp.) in the UK, belonging to the halophyte species which has a good potential of being a sustainably sourced, clean label salt alternative.

'Halophytes' were discovered in the 1790s but gained first scientific recognition in the late 1800s. By the early twentieth century halophytes were linked to their ability to survive in saline conditions (Flowers et al., 1986). Since then, halophytes have gained limelight, but are still

underexplored. About 2500 halophyte species are known in the world and most of them can be potential candidates for use as edible plants, oilseeds, medicine, fuel, or fodder (Ksouri et al., 2012; Singh et al., 2014). These plants can withstand harsh climatic conditions ranging from the wet marine marshes to dry salt deserts. Therefore, such plants were known as 'famine foods' by the rural communities, since the vast majority of the edible crops are salt sensitive and cannot grow under saline conditions (Flowers et al., 1986). Currently, only about 11 % (i.e., 1.6 billion hectares) of global land mass is being used for crop production including both arable land and permanent crop land (FAO, 2020). Due to high stress tolerance of halophytic species, strategic exploiting of such species for food production could make good use of the land mass which is currently not used for agricultural purposes due to its high salinity. This approach will contribute to improving food security.

Halophytes belong to the group of angiosperms, plants from numerous family and genera. The genus 'Salicornia' from the Amaranthaceae family (formerly known Chenopodiaceae family) has gained significant popularity in the scientific community both in the food and the pharmaceutical sector (Flowers et al., 1986; Singh et al., 2014). Salicornia is widely known as 'samphire', 'glasswort' or 'sea asparagus',

Abbreviations: AOAC, Association of Official Agricultural Chemists; Ca, Calcium; Cu, Copper; dw/dwb, dry weight/ dry weight basis; Fe, Iron; KCl, Potassium Chloride; LC-MS, Liquid chromatography-mass spectrometry; Mg, Magnesium; Mn, Manganese; Na, Sodium; Ni, Nickel; NaCl, Sodium Chloride; Se, Selenium; UK, United Kingdom; w/w, weight by weight; Zn, Zinc.

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however the local produce from some regions is identified with different names. There are several species identified within the *Salicornia* genus, but majorly only three are recognized as nutritionally important by the food industry, namely 'Salicornia pusilla', 'S. europaea' and 'S. procumbens' (Stace, 2010). Other nutritionally important members of the *Amaranthaceae* family including spinach, quinoa, and beet (Srivarathan et al., 2021), have stimulated the scientific community to further explore other genus within the *Amaranthaceae* family, particularly *Salicornia* (referred to samphire in this paper).

Samphire broadly consists of protein, fat, sugars (simple and complex) and minerals in the leaves, root, and stem (Min et al., 2002). These basic compounds make the plant a favourable candidate for human consumption. In addition, several other nutritionally important components including dietary fibres, vitamins (majorly vitamin C and carotenoids), flavonoids, and sulfonium compounds (Loconsole et al., 2019; Min et al., 2002) are also present. The plant is naturally a rich source of essential minerals and amino acids. For example, iron, sodium, magnesium, manganese, zinc, potassium, calcium, iodine etc. are some of the commonly detected minerals and trace elements, however the mineral content varies with the Salicornia species and the growing parameters, such as the type of soil and water (Srivarathan et al., 2021). Also, the mineral content varies across different parts of the plant, for example, sodium might be slightly higher in roots than stem and leaves, whereas calcium could be higher in leaves than roots. Amino acids, including glutamic acid, leucine, glycine, lysine, and aspartic acid, are also abundant in this plant (Min et al., 2002).

Samphire has been used as one of the key ingredients in traditional fermented products (e.g., vinegar, Korean wine and nuruk) as its composition (minerals and other bioactive compounds such as phenolic compounds) has been shown to have a positive impact on the growth of beneficial microorganisms during fermentation and in the formation of the taste, flavour and subsequently the quality of the products (Seo et al., 2010). In meats, including sausage (Kim, Hwang, Song, Kim, Ham, et al., 2014) and pork loin ham (Tae-Jun et al., 2020), samphire has shown to partially substitute salt and achieve comparable salty taste alongside improved textural properties in hardness, gumminess, and chewiness. Consumers rated the samphire substituted ham with higher tenderness and juiciness compared to the control (Tae-Jun et al., 2020). The mineral and dietary fibre content of samphire may be responsible for the textural quality changes of these meat products by increasing water retention, cooking yield, emulsion stability and protein solubility; however, the extent of salty taste resulting from samphire has not yet been fully explained.

In general, minerals, including sodium and other cations, are well recognized for their contribution to salty taste (McCaughey, 2019). Amino acids such as lysine, arginine, and histidine (in combination or individually) can also impart or enhance salty taste and have recently been used alongside sodium and / or potassium chloride to achieve comparable saltiness in sodium reduced food products (Zhang et al., 2022). This may be due to taste-taste interactions between the amino acids and mineral ions; however, this needs further exploration. Some amino acids have also been reported as flavour enhancers or having ability to mask bitterness (caused, for example, by KCl and peptides) in food (da Silva et al., 2020). The role of glutamic acid in umami taste perception is well known, however, there is limited understanding in the taste, particularly salty taste perceived from other amino acids such as lysine (Delompre et al., 2019).

Considering the amino acid and mineral profile of samphire, it is unclear which compounds lead to the saltiness perception of samphire. We hypothesise that the salty taste of samphire could be attributed to the minerals or amino acids present in samphire or could be a synergistic effect of the two. Therefore, the aim of this study was to determine the nutrient composition of samphire extracts from three different regions in the UK and evaluate their impact on the sensory profile when used in a food product. Understanding of the saltiness related components in stress-tolerant plants such as samphire would benefit both the food

industry and agriculture sector.

#### 2. Materials and methods

#### 2.1. Sample collection and processing

Fresh samples of samphire (Salicornia europaea) were purchased from Totally Wild Foods, Stoke-on-Trent, UK. The samples were freshly harvested from various coastal locations (5.6 kg each) (Fig. S1) within the UK such as Cornwall (to represent Southwest England), Norfolk (to represent East of England), East Lothian (to represent Scotland) (Fig. S2) at the start of the harvest season (mid-July). All the samples were soaked in deionised water to get rid of surface dust, then thoroughly washed with deionised water and dried with paper towel. Samples from each location were prepared in three replicates. Clean samphire stalks were placed on an aluminium tray and put in the blast freezer (Blast Chiller 2004 FCC, Foster, Norfolk, UK) for minimum 30 min and then stored at -18 °C for overnight. The samples were then placed in the freeze dryer (Gamma 2-16, Christ, Osterode, Germany) for 4 days. The freeze-dried samphire was removed and grinded finely using a spice grinder (CK686, Caterlite, Bristol, UK) and the fine powder was stored in airtight containers (at -18 °C) until further analysis.

#### 2.2. Total yield

The total yield was calculated to have an understanding on the amount of freeze dried samphire that can be obtained from fresh samphire with the formula:

Total yield (%) = 
$$\frac{weight \ of \ freezedried \ samphire}{weight \ of \ fresh \ samphire} \times 100$$

#### 2.3. Colour measurement

The colour measurement of freeze-dried samphire was carried out using a colorimeter (CR-400, Konica Minolta, Osaka, Japan) with granular materials attachment (CR-A50) on the CIELAB colour scale, with coordinates L\* (lightness), a\* (redness), b\* (yellowness). L\* measures the luminosity, positive a\* indicates redness, negative a\* indicates greenness, whereas positive b\* presents yellowness and negative b\* presents blueness. Before the readings were taken, the colorimeter was calibrated with the standard white plate. The readings for L\*, a\* and b\* were obtained from the average of three readings for each sample.

#### 2.4. Proximate composition

#### 2.4.1. Moisture and ash analysis

Moisture analysis was conducted according to the AOAC 934.01 oven drying method. Silica crucibles were dried in the oven at 100 °C for 1 h, then they were transferred into a desiccator and left to cool for at least 30 min. The weight of the empty crucible was recorded and about 2 - 3 g of sample was weighed (n = 3) into it. The crucibles containing samples were placed in the oven to dry overnight at 100 °C until constant weight was achieved. The samples were cooled in a desiccator for at least 30 min and the weight was recorded. Ash content was analysed according to the AOAC method 923.03, the dried samples (with the crucibles) were placed in a muffle furnace to ash overnight at 450 °C. The samples (n = 3) were cooled in a desiccator for 1 h and the weight was recorded to calculate the ash content in samphire sample.

#### 2.4.2. Crude fat and crude protein analysis

Crude fat was analysed using 0.5 g of samphire powder (n = 3) by the Soxhlet method using petroleum ether (boiling point 40–60 °C) (laboratory reagent grade, Fisher Scientific) as a solvent (Nielsen, 2017). The crude fat % was calculated using the formula below:

Crude fat % = 
$$\frac{\text{weight of flask after extraction-weight of empty flask}}{\text{weight of sample}} \times 100$$

Crude protein was analysed with 0.5 g of samphire powder (n = 2) using Kjeldahl Method and nitrogen conversion factor of 6.17 (Nielsen, 2017) was used to express the protein content as g/100 g samphire powder. The nitrogen g/100 g sample was calculated using the formula below:

grams N/100g sample = 
$$\frac{\text{Titration volume x 0.1 x 14.01 x 100}}{1000 \text{ x Weight of sample in grams}}$$

Where, titration volume is (Final reading – Initial reading) of the titre in millilitres (cm<sup>3</sup>) of sulphuric acid in the burette which is divided by 1000 to give litres.

0.1 is the normality of the sulphuric acid titrant; 14.01 is the molar mass of nitrogen.

#### 3. Mineral and trace element analysis

The ash (from section 2.4.1) was dissolved in 0.1 M nitric acid (HNO<sub>3</sub>) and prepared to determine the minerals using Atomic Absorption spectrophotometer (novAA 350, Analytik Jena GmbH, Jena, Germany) according to AOAC method 999.11 for calcium at wavelength 422 nm (and 240 nm), magnesium 285 nm, zinc 214 nm, iron at 248 nm, manganese 279 nm and flame photometer (PFP7, Jenway, VWR, Leicestershire, UK) for sodium at 589 nm and potassium at 766 nm.

#### 3.1. Free amino acid analysis

The freeze-dried samphire extract (100 mg) was put in a 15 ml falcon tube and extracted with water in three stages using 5 ml of a  $7.5\,\mu M$  norvaline aqueous solution during the first extraction and 5 ml of distilled water in each of the two subsequent extractions. At each stage, the tube was shaken (MultiReax shaker, Heidolph, Manchester, UK) for 20 min (at setting 8) followed by sonicating in water bath (Branson 2800, Sci-Quip, Shropshire, UK) at 60 °C for 20 min. The tubes were then centrifuged at 6000 rpm for 5 min. The supernatants were combined and 1 ml of the extracted were mixed with 1 ml of acetonitrile (final norvaline concentration 1.25 µM). The 2 ml of the combined mixture was then filtered through 0.2 µm PTFE filter (Fischer Scientific, Loughborough, UK) into an autosampler LC-MS vial. Quantification was performed from calibration curves of 19 amino acids including AAS 18 amino acid standard and gamma amino-n-butyric acid (99 % purity; Sigma Aldrich, Dorset, UK) which were built from 0.000125 to 0.125 μM/L in a mixture of acetonitrile and water at ratio of 9:1 (v:v) with 1.25 µM nor-valine as an internal standard. The amino acid analysis was conducted according to the instrument settings described by Kocadağlı et al. (2021). An Agilent 1200 high-performance liquid chromatography (HPLC) system coupled to a 6410 triple quadrupole mass spectrometer was used with electrospray ion source (ESI) in positive mode. Two eluents were employed: mobile phase A (5 mM ammonium formate with 0.5 % formic acid in water), and mobile phase B [5 mM ammonium formate with 0.5 % formic acid in acetonitrile: water (9:1, v: v)]. The flow rate was 1 ml/min, the total run time was 13 min and the injection volume 5  $\mu$ L. The ESI source was set at; gas temperature 330 °C, gas flow 13 L/min, nebuliser pressure 40 psi, capillary 4000 V. Chromatographic separation was carried out at 20 °C, on a Synchronis HILIC column [150  $\times$  4.6 mm i.d., 3  $\mu m]$  with a Synchronis HILIC precolumn (10  $\times$  4.6 mm i.d.,  $3 \mu m$ , ThermoFisher Scientific, Waltham, MA, USA). Mobile phase A was increased from 10 % to 40 % for 8 min and then decreased to 10 % in 1 min and kept at 10 % for 4 min. Amino acids were identified by multiple reaction monitoring (MRM) using the ion transitions and parameters previously reported (Kocadağlı, et al., 2013).

#### 3.2. Sensory analysis

#### 3.2.1. Preparation of the food product

Freeze dried samples from East Lothian were used to prepare nachos

as per the formulations (w/w) described in Table 1. Nachos were prepared by mixing ingredients (i.e., masa flour, water, salt/ samphire extract) and kneading into dough. The dough was flattened using a rolling pin and cut into triangle shapes of 5 cm x 5 cm x 7 cm and with thickness  $2.5\pm0.5$  mm each. Green food colouring (Sainsbury's, UK) was added to the control nachos to match the greenish tinge in samphire added nachos in order to reduce the appearance bias during the sensory analysis. The nachos were baked in the oven at  $180\,^{\circ}\text{C}$  for 5 min followed by deep frying in sunflower oil at  $180\,^{\circ}\text{C}$  for 1 min and the residual surface oil on the surface of cooked nachos was removed by a paper towel. The nachos were cooled and stored in heat sealed aluminium bags till the sensory analysis.

#### 3.2.2. Descriptive profiling

The nachos were tested with a trained sensory panel (n = 14; including 2 male 12 female, age from 35 to 65 years old) based at the sensory science centre (Department of Food and Nutrition Sciences, University of Reading) and they had provided consent through their employment to taste food and use their data. All the panellists were healthy and had no known taste or olfactory defects/disorders. The nachos were evaluated on unstructured line scale for aroma, texture/ mouthfeel, taste & flavour, and aftereffects. Before collecting the data, all the panellists went through a four-day vocabulary training where they were asked to generate as many sensory terms as they could to describe the attributes (in terms of aroma, texture, taste, and aftereffects) of the nacho samples. They were provided with references for each sensory term they came up with to form a consensus vocabulary (for full details see supplementary Table S3). The four days of training was followed by two separate days of scoring on an unstructured line scale in individual sensory booths under red light at room temperature. Samples were coded and presented in a balanced order and the intensity of each attribute were rated on the scale with the anchors mutually agreed with the panellists. Between the samples, panellists were instructed to cleanse their palate with a slice of apple and filtered lukewarm water. The rating of the attributes on the scale was captured using the sensory software Compusense® (cloud version, Guelph, Ontario).

#### 3.3. Statistical analysis

Instrumental data were analysed by one-way ANOVA in SPSS statistical package (version 27, IBM). The sensory analysis data were analysed by two-way ANOVA where the panellists were treated as a random effect and samples as a fixed effect, with both treatments tested against the assessor by sample interaction, using SenPaq (QI statistics, Kent, UK). Multiple pairwise comparisons were analysed using Tukey's HSD at a significance level of 0.05.

#### 4. Results

#### 4.1. Total yield

There was no difference in total yield for samphire extracts from East Lothian (9.39 %) and Norfolk (8.41 %) (p > 0.05). However, the

Nachos formulation (w/w).

Ingredients	0.7 % NaCl (g/100 g)	1 % NaCl (g/100 g)	2.5 % SE (g/ 100 g)	3.4 % SE (g/ 100 g)
Masa Flour	50	50	50	50
Water	49	49	47	47
Salt (NaCl)	0.7	1	0	0
(Sodium level)	(2.79)	(3.51)	(2.79)	(3.51)
Samphire extract	0	0	2.5	3.4
Green food colouring (ml)	0.26	0.56	0	0

SE = samphire extract.

samphire from Cornwall had a significantly lower yield (7.20 %) than samphire from the other two locations (as shown in Table 2) (p < 0.05). This was in line with the difference in total solids content between fresh samples, where the Cornwall samphire has a lower solids content (92.6 %) than the East Lothian (96.6 %) and Norfolk (96.3 %) samples (p < 0.05).

#### 4.2. Colour

Colour is an important visual parameter and considering the potential application of samphire in food industry, the colour of samphire powder was evaluated using CIELAB colour space. Table 2 summarises the colour measurements of the freeze-dried samphire powders. The lightness (luminosity, L\*) of the Cornwall sample was significantly lower than that of the other two samples, (p < 0.05), indicating that sample from Cornwall has a darker colour than samples from Norfolk and East Lothian. The extract from Cornwall was also significantly less green (less negative a\* value) than the other two samples, and significantly less yellow (lower b\* value) than the Norfolk extract (p < 0.05).

#### 4.3. Proximate composition

The proximate compositions, including moisture, crude protein, fat, and ash contents of freeze-dried samphire samples, are shown in Table 2. The moisture contents of the dried extracts varied (from 3.4 % to 8.0 % w/w, Table 2), the values were in line with moisture content reported from previous *Salicornia* extracts from Portugal [5.2 % w/w, Lima et al. (2022)]. The overall ash content was significantly higher for Norfolk samples than samples from East Lothian and Cornwall (p < 0.05) (from 40.3 % to 49 %, Table 2).

The crude fat content in freeze dried samphire ranged from 1.93 % to 2.80 % (Table 2) with Cornwall samples significantly higher than that of East Lothian samples and Norfolk samples (p < 0.05). The crude protein content of the Norfolk sample was significantly lower than the extracts from East Lothian and Cornwall (p > 0.05), ranging from 11.5 % to 14.1 % (Table 2).

#### 4.4. Minerals and trace elements

The minerals found in the samphire extracts from three different

 Table 2

 Proximate composition, total yield, and colour of samphire extracts.

	Cornwall	East Lothian	Norfolk	Significance of Sample Region (p- value)
Yield (%)	$\begin{array}{l} \textbf{7.20} \pm \\ \textbf{0.71}^{\text{b}} \end{array}$	$\begin{array}{l} 9.39 \pm \\ 0.20^a \end{array}$	$\begin{array}{l} 8.41 \; \pm \\ 0.29^{a} \end{array}$	0.003
Color	52.2 $\pm$	58.2 $\pm$	57.3 $\pm$	0.008
L*	$1.68^{\rm b}$	$0.37^{a}$	$2.19^{a}$	0.002
a*	$-4.44~\pm$	$-7.75~\pm$	$-8.03 \pm$	0.004
b*	$1.16^{b}$	$0.52^{a}$	$0.26^{a}$	
	$18.3~\pm$	19.7 $\pm$	21.4 $\pm$	
	$1.02^{\rm b}$	$0.38^{ab}$	$0.37^{a}$	
Moisture (%)	7.97 $\pm$	3.44 $\pm$	3.82 $\pm$	< 0.001
	0.97 <sup>b</sup>	$0.45^{a}$	$0.67^{a}$	
Ash (%)	40.3 $\pm$	41.5 $\pm$	49.0 $\pm$	< 0.001
	$0.28^{a}$	$0.83^{a}$	$0.33^{b}$	
Crude Fat (%)	$2.80\ \pm$	$2.03~\pm$	$1.93~\pm$	0.007
	$0.28^{\rm b}$	$0.12^{a}$	$0.25^{a}$	
Crude Protein (%)	13.2 <sup>a</sup>	14.1 <sup>a</sup>	$11.5^{b}$	0.006
(n=2) [mean	(0.01)	(0.04)	(0.7)	
(range)]				

Values are means  $\pm$  SD (n = 3), except for protein where there were 2 replicates only due to equipment issues [n = 2; mean (range)]. The values with a different superscript (a-b) within a row are significantly different by Tukey's HSD test (p < 0.05); the proximate results are presented in % dry weight basis (dwb) of the sample.

regions in the UK (shown in Table 3) were sodium (Na) [11.6 to 13.8 g/100 g dry weight (dw)], potassium (K) (1.15. to 1.35 g/100 g dw), magnesium (Mg) (0.34 to 0.43 g/100 g dw). There were no significant differences observed for Na and K between the three regions (p > 0.05). The Norfolk sample had a significantly higher Mg content than the Cornwall sample (p < 0.05). The trace elements detected were zinc (Zn) (55.2 to 80.1 mg/kg dw), manganese (Mn) (194 to 399 mg/kg dw) and iron (Fe) (315 to 1168 mg/kg dw). Only Fe levels varied significantly between regions whereas the Norfolk sample was significantly higher than the other samples (p < 0.05). While Na is the key mineral contributing to salty taste, K, Mg, Fe also evokes salty taste but to a lesser extent (Grummer et al., 2012) but can additionally contribute to other tastes particularly bitter and (or) metallic taste (Delompre et al., 2019).

#### 4.5. Amino acids

Table 4 reports the 16 free amino acids identified in the freeze dried samphire extracts (no other amino acids were detected). The five free amino acids known to elicit salty taste directly or that may impact salty taste through inducing umami (savoury) taste namely, glutamic acid, aspartic acid, lysine, histidine, and arginine (da Silva et al., 2020; Delompre et al., 2019) are highlighted in bold. The Norfolk sample was lower in total free amino acid content, and in three of the salt taste relevant amino acids (glutamic acid, arginine and lysine), than the Cornwall and East Lothian extracts. However, the East Lothian extract was significantly higher in aspartic acid than the extracts from the other regions (p < 0.05), and it was also significantly higher in histidine than the extract from Norfolk (p < 0.05). Of the four amino acids known to contribute to sweet taste (alanine, glycine, proline, serine and valine) (Delompre et al., 2019), three were identified in the samphire extracts (ignoring proline which was only detected in the sample from East Lothian and at much lower levels than all other amino acids identified). These three (Ala, Gly, Val) all differed significantly between samples, with highest levels in Cornwall and lowest in Norfolk. There are 11 amino acids known to contribute to bitter taste (asparagine, tyrosine, threonine, methionine, tryptophan, phenylalanine, leucine, valine, isoleucine, histidine and arginine), only one was not detected (tryptophan) and three are known to contribute more to salty taste (Asp, His and Arg) so were discussed above. Of the remaining seven, 6 differed significantly between samples being generally lower in the Norfolk sample and / or higher in the Cornwall sample. The lower amino acid levels in the Norfolk samples aligns with the overall protein content which was lower in Norfolk samples than the other samples.

**Table 3** Average mean of minerals and trace elements in samphire extracts from the UK.

Mineral	Cornwall	East Lothian	Norfolk	Significance of Sample Region (p-value)			
Minerals	Minerals (g/100 g dw)						
Na	$12.0\pm1.5^{a}$	$11.6\pm2.3^{\text{a}}$	$13.8\pm0.4^a$	0.29			
K	$1.35~\pm$	$1.37~\pm$	$1.15~\pm$	0.48			
	$0.23^{a}$	$0.29^{a}$	$0.06^{a}$				
Mg	0.34 $\pm$	$0.36~\pm$	0.43 $\pm$	0.03			
	0.04 <sup>a</sup>	$0.03^{ab}$	$0.01^{\rm b}$				
Trace ele	Trace elements (mg/kg dw)						
Fe	479.8 $\pm$	315.5 $\pm$	1168.8 $\pm$	0.001			
	11.6 <sup>a</sup>	146.7 <sup>a</sup>	149.4 <sup>b</sup>				
Mn	182.9 $\pm$	194.5 $\pm$	399.1 $\pm$	0.17			
	56.5 <sup>a</sup>	155.7 <sup>a</sup>	172.0 <sup>a</sup>				
Zn	80.1 $\pm$	60.8 $\pm$	55.3 $\pm$	0.91			
	93.0 <sup>a</sup>	70.8 <sup>a</sup>	57.9 <sup>a</sup>				

Values are means  $\pm$  SD (n = 3), unless stated otherwise. The values with a different superscript (a-b) within a row are significantly different by Tukey's HSD test (p < 0.05).

**Table 4**Free amino acids in freeze dried samphire from different locations in the UK.

Amino acids	Cornwall (mg/100 g)	East Lothian (mg/100 g)	Norfolk (mg/100 g)	Significance of Sample Region (p-value)
Alanine (Ala)	$12.7 \pm 2.38^{a}$	$5.57 \pm 0.75^{b}$	$4.02 \pm 0.81^{b}$	0.001
Arginine (Arg)	101 ± 10.7 <sup>a</sup>	108 ± 6.83 <sup>a</sup>	54.7 ± 3.17 <sup>b</sup>	< 0.001
Aspartic Acid (Asp)	20.5 ± 4.76 <sup>a</sup>	56.7 ± 7.82 <sup>b</sup>	25.2 ± 1.44 <sup>a</sup>	< 0.001
GABA	$29.4 \pm 3.96$	$38.7 \pm \\7.40$	$\begin{array}{c} 41.0 \pm \\ 9.01 \end{array}$	0.19
Glutamic acid (Glu)	30.7 ± 3.49 <sup>a</sup>	$27.2 \pm 0.75^{a}$	19.9 ± 2.70 <sup>b</sup>	0.006
Glycine (Gly)	$5.99 \pm \\1.02^a$	$\begin{array}{l} \textbf{3.47} \pm \\ \textbf{0.08}^{b} \end{array}$	$\begin{array}{c} 0.92 \pm \\ 0.15^c \end{array}$	< 0.001
Histidine (His)	48.7 ± 12.1 <sup>ab</sup>	$60.6 \pm 4.07^{a}$	$32.7 \pm 1.41^{b}$	0.011
Isoleucine (Ile)	$38.6 \pm 3.31^{a}$	$\begin{array}{l} 33.8 \pm \\ 2.41^a \end{array}$	$\begin{matrix}26.8\pm\\1.27^{\rm b}\end{matrix}$	0.003
Leucine (Leu)	$32.4 \pm 3.87^{a}$	$\begin{array}{c} 24.2 \pm \\ 2.08^{b} \end{array}$	$14.2 \pm \\1.55^{\rm c}$	0.001
Lysine (Lys)	$38.8 \pm 3.26^{a}$	41.4 ± 1.37 <sup>a</sup>	28.0 ± 2.55 <sup>b</sup>	0.001
Methionine (Met)	$16.7 \pm 3.67^{a}$	$\begin{array}{l} \textbf{6.15} \pm \\ \textbf{0.75}^{\text{b}} \end{array}$	$6.31 \pm \\1.56^{\mathrm{b}}$	0.002
Phenylalanine (Phe)	$29.0 \pm 5.12^{a}$	$28.6 \pm 1.60^{a}$	$18.6 \pm \\1.57^{\mathrm{b}}$	0.012
Proline (Pro)	0	$\begin{array}{c} \textbf{0.05} \pm \\ \textbf{0.04} \end{array}$	0	0.085
Serine (Ser)	$21.1 \pm 2.47^{a}$	$15.1 \pm 1.62^{\rm b}$	14.0 ± 0.81 <sup>b</sup>	0.006
Threonine (Thr)	$10.2 \pm 1.81^{a}$	$7.57 \pm 0.83^{a}$	$\begin{array}{c} 4.12 \pm \\ 0.57^{\mathrm{b}} \end{array}$	0.002
Tyrosine (Tyr)	$30.1 \pm 5.93^{a}$	$26.6 \pm 2.00^{a}$	$24.0\ \pm$ $1.10^a$	0.207
Valine (Val)	95.4 ± 10.4 <sup>a</sup>	$79.0 \pm 4.14^{a}$	$59.1 \pm 2.39^{ m b}$	0.002
Total	$561.2 \pm 78.2$	$562.7 \pm 44.5$	$373.5 \pm 32.0$	

N.B. GABA, gamna amino-n-butyric acid. Values are means  $\pm$  SD (n = 3). The values with a different superscript (a-c) within a row are significantly different by Tukey's HSD test (p < 0.05). Rows in bold font indicate amino acids known to impact salty taste or umami (savoury) taste.

#### 4.6. Descriptive profiling

Sensory profiling data of four nachos samples, two of which contained samphire extract from the East Lothian region, resulted in 32 consensus attributes. 11 of these attributes varied between samples (for full details see supplementary table S4). Mean results for taste and flavour attributes that differed significantly between samples are summarised in Fig. 1. The difference in salt level between the two control nachos (0.7 % versus 1.0 % w/w NaCl) did not lead to a significant difference in salty taste (Fig. 1), however the 1 % salt control had a significantly higher salty aftertaste than 0.7 % salt control samples (Fig. 2). The nachos samples containing samphire extract at two different levels (2.5 % and 3.4 %), which were equivalent in sodium content to the two controls (0.7 and 1.0 % w/w NaCl respectively), did not differ from each other in salty taste nor salty aftertaste, and both were significantly higher in salty taste than the 0.7 % salt control, and higher in salty aftertaste than both control nachos samples (0.7 % and 1.0 % w/w NaCl). Therefore, these results indicate that other components within samphire extract besides sodium must have contributed to the saltiness perception. Another potential benefit of the samphire extract was that the umami taste was significantly higher than in the control samples. However, there were other differences that might not be beneficial in all products; green vegetable flavour and aftertaste were substantially and significantly higher in the samphire containing nachos compared to both control nachos, and there was a small but significant



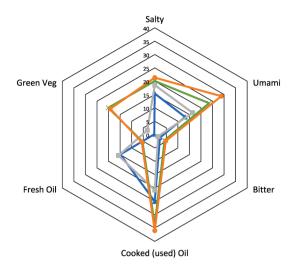


Fig. 1. Sensory profiling data of nachos for taste and flavour.



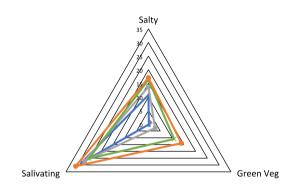


Fig. 2. Sensory profiling data of nachos for aftereffects.

difference in bitter taste compared to the higher salt control (p < 0.05). A significantly lower fresh oil note, and significantly higher cooked oil note (aroma and flavour) were perceived in the nachos containing the higher samphire level compared to the lower salt control. There were no differences in sweetness or peppery taste between the NaCl and samphire nachos (p > 0.05). Other taste/flavours described by the panellists such as sweet, cooked/ fresh oil, masa flour, peppery (black pepper) are attributed to the masa flour and the sunflower oil used for frying. However, this does not account for the significant differences in fresh and cooked oil aroma and flavour between the higher samphire nacho and the low salt control. It cannot be proved within this experiment whether this results from batch-to-batch frying differences, or whether it is a real effect of using higher levels of samphire, and hence warrants further investigation.

In terms of mouthfeel, there were no significant differences in any of the 10 attributes between any samples (Table S4, supplementary material). In addition to the aftereffects mentioned above, the only other aftereffect that varied significantly between samples was salivating (p = 0.012) (Fig. 2), which was significantly higher in samphire extract nachos (3.4 % SE i.e., 3.51 g sodium per 100 g) than the other samples. Other aftereffects that did not differ between samples were greasy, tooth

packing, residue in the mouth, sweet aftertaste, drying, throat catching, peppery and masa flour aftertaste; implying that such attributes were attributed to the masa flour and not impacted by the addition of salt or samphire.

#### 5. Discussion

The need for sodium reduction cannot be emphasized enough, and several salt substitutes have been explored, out of which *Salicornia species* (samphire) is gaining recognition and can be considered as a potential substitute to reduce sodium content of foods. As samphire grows on marsh lands, this study intended to understand the impact of location on the nutrient profile of samphire to best utilize this plant species. Further, this work also lays foundation for future work by providing evidence on the potential contribution of various nutrients, other than sodium, on the salty taste of samphire in a food product.

Halophytes such as Salicornia species, are known to be rich in minerals as they grow in high alkaline environments and are known to absorb and concentrate nutrients, predominantly minerals, from the soil and water they are grown in (Díaz et al., 2013). Srivarathan et al. (2021) analysed the nutrient profile of samphire species native to Australia (i.e., Tecticornia sp.) from different locations and highlighted the presence of minerals and trace elements, including sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), nickel (Ni) and selenium (Se). The minerals levels from our study highlight that the mineral levels from samphire grown in the UK (Table 3) are comparable to the Australian samphire species i.e., Na (8.8 to 16.7 g/100 g dw), K (0.3 to 1.7 g/100 g dw) and Mg (0.6 to 1.2 g/100g dw) (Srivarathan et al., 2021). Whereas freeze dried samphire from local Korean markets was reported to have lower Na levels (i.e., 8.9 to 9.5 g/100 g) compared to the UK and Australian samples (Kim, Hwang, Song, Kim, Ham, et al., 2014). Further, soilless cultivation of samphire (RiaFresh®, Algarve, Portugal) presents lower mineral composition Na (3.91 g/100 g); K (0.33 g/100 g); Mg (0.094 g/100 g); Ca (0.15 g/100 g) (Lima et al., 2022), which strongly suggests that the soil and irrigation practice plays a key role in the mineral composition of samphire. Our current study found higher trace elements (Zn, Fe, Mn) levels (Table 3) compared to the Australian samphire i.e., Zn (4.2 to 16.9 mg/kg dw), Fe (101 to 414 mg/kg dw) and Mn (4.9 to 44.1 mg/kg dw) and soilless cultivated samphire (RiaFresh®, Algarve, Portugal) i.e., Fe (30 mg/kg); Zn (19.8 mg/kg); Mn (24.4 mg/kg) (Lima et al., 2022). Further, the levels of calcium (Ca) and copper (Cu) were below the detectable limits in our study, however these were common minerals found in samphire species from other locations over the world. For example, freeze dried samphire from Australia had calcium at 0.35-0.51 g/100 g dw (Srivarathan et al., 2021), and the Portugal species had 0.031 g/100 g calcium (Oliveira-Alves et al., 2021). This could be due to lower levels in the growing environment in the UK, as well as variations in the species. However, since they are below the detectable limits in our samples, we would assume that the contribution of Ca and Cu to taste perception would be negligible.

Even though Matinzadeh et al. (2019)'s study suggests that halophytes from similar evolutionary genes have comparable composition irrespective of the difference in the growing environments, our results highlight otherwise. Furthermore, different halophytic species have specific osmoregulation mechanisms to deal with the salinity stress, which could impact the amount of nutrients (e.g., minerals, amino acid, etc.) the plant can uptake (Maimaiti et al., 2016), in addition to growing conditions like climate, soil, and water (Duarte et al., 2022). Therefore, it would be worth investigating the salinity of the soil and water from where the samples are harvested to gain better understanding of the effects of growing environment.

Besides the individual mineral content, the overall ash content was significantly higher for Norfolk samples than samples from East Lothian and Cornwall. It is interesting to note that the ash content (and the mineral content) of samples from the South of England (i.e., Cornwall)

and Scotland (East Lothian) were very similar even though they are on a different coastline, whereas Norfolk and East Lothian are on the same coastline, so we might expect a more similar mineral content. However, the climate of the two regions (Norfolk and East Lothian) are very different due to their latitudes, suggesting differences in alkalinity in the soil and water. Furthermore, the ash content of *Salicornia* grown in soilless conditions was 51.8 % (Lima et al., 2022) which is close to the ash content of samphire harvest from different regions in the UK (as mentioned above). Thus, this could suggest that samphire harvested from the marshlands or cultivated in a soilless system have the similar ash content, yet the concentration of individual minerals might vary as halophytes might absorb different minerals from the soil around the marshlands.

Soil salinity can influence the protein content of vegetables; however, halophytes manage to maintain their protein content (Martins-Noguerol et al., 2021) at similar levels to other plant protein sources such as quinoa (~13 %) and amaranth (~15 %) (Sá et al., 2020). The crude protein content of samphire in our study ranged from 11.53 g/ 100 g to 14.08 g/100 g dwb, which is similar to the protein content of Salicornia species produced in the soilless system (RiaFresh®, Algarve, Portugal), i.e. 17 g/100 g dw (Lima et al., 2022) as well as different species (Tecticornia sp.) from Australia (i.e., 7.6 to 12.6 g/100 g dw) (Srivarathan et al., 2021). However, there were significant differences observed between the species (Tecticornia sp.) grown across different sub-locations within Australia (Srivarathan et al., 2021), which could be due to difference in the growing environment within Australia. Variations between the UK and Australian species could be attributed to osmoregulatory mechanism of different species, and their growing environment. While Lombardi et al. (2022) did not find any significant correlation between the protein content of Salicornia (23-27 g/100 g dw) and soil salinity varying between 0 and 300 mM NaCl, in controlled conditions. While Martins-Noguerol et al. (2021) observed a decrease in the protein content from 45 % at 0 mM NaCl to 20 % at an increased salinity of 150 mM NaCl, however there were no significant differences between 150 mM and 500 mM NaCl salinity where the protein content ranged between 20 and 22 g/100 g dw. Overall, salinity may have an impact on the protein content of halophytes, but the protein content of samphire in the UK is in line with other halophytic species and plant protein sources like quinoa and amaranth.

Amino acid concentrations can also be affected by salinity (Martins-Noguerol et al., 2021), which was observed in our study as many of the amino acid concentrations varied across the three locations in the UK. Many of these amino acids are known to contribute to one of the five basic tastes; as discussed in section 3.5, and while saltiness is our focus, we cannot rule out the influence of the amino acids on sweet and bitter taste. The composition of free amino acids reported in this study included amino acids known to be relevant to salty taste (arginine, lysine, glutamic acid, aspartic acid, and histidine) which could evidence the contribution of amino acids to the saltiness perception, and this combination of amino acids may partially explain the salty taste of samphire. However only free amino acids were measured in this study, whereas amino acids bound to proteins or peptides should not be ruled out for the contribution to saltiness (Jünger et al., 2022). In future studies it would be interesting to explore the interaction between amino acids and minerals on taste perception as it would help further elucidate the specific contribution of minerals and amino acids to the salty taste of samphire. Further amino acid analysis on Salicornia species from different regions should be conducted to provide better understanding on the impact of environment on amino acid content.

The crude fat content in samphire from our study ranged from 1.93 % to 2.8 % which is similar to that reported in the Australian samphire species (1 % to 1.8 %) (Srivarathan et al., 2021). Another similar species from Portugal, Sarcocornia perennis (powdered), had 2.3 % crude fat (Clavel-Coibrié et al., 2021) and freeze dried samphire purchased from Korean market had 2.5 % fat (Kim, Hwang, Song, Kim, Ham, et al., 2014). Other halophytes like iceplant, sea fennel and seaside arrow grass

also have fat contents between 2 and 3 % (Sánchez-Faure et al., 2020). These results suggest that the fat content of samphire and other halophytes are not greatly affected by location and species, which is confirmed by Martins-Noguerol et al. (2021). Overall, samphire can be considered as a low-fat ingredient with fat levels ranging from 1 to 3 %.

Clavel-Coibrié et al. (2021) highlighted the scope of using Sarcocornia perennis, a similar species to samphire (Salicornia europaea), as a potential salt substitute in savoury snacks (crackers) and suggested the incorporation of similar species to salty products to understand the impact on nutritional and physical properties. Therefore, considering the rich mineral and amino acid profile of samphire from our results, samphire seems to be a good salt substitute as it has much lower sodium (~13 g sodium per 100 g) than table salt (38.5 g sodium per 100 g) (Food Safety Authority of Ireland, 2009), and much higher mineral content than common vegetables like spinach (e.g. 0.09 g sodium; 0.23 g potassium per 100 g dw) (Waseem et al., 2021). In terms of taste and flavour, samphire nachos (2.5 % SE at 2.79 g sodium per 100 g) had a significantly higher salty taste than 0.7 % control nachos (2.79 g sodium per 100 g) at the same sodium level in addition to a comparable salty taste to the 1 % salt control (3.51 g sodium per 100 g). Hence, we hypothesised that there are other minerals (besides sodium) and amino acids contributing to the salty taste of samphire. Higher sodium (ions) release in saliva could be associated with increased salivary flow (Neyraud et al., 2003), which could explain significantly higher salivating after effect (Fig. 2) in the higher samphire extract nachos (3.4 % SE i.e., 3.51 g sodium per 100 g) than in both the nachos containing the lower samphire level and the lower salt level.

Further experiments in our laboratory (data not published) have indicated that saltiness intensity is perceived at approximately half the level in solid food matrix compared to in aqueous solution. Therefore, in order to evaluate the contribution to saltiness perception, the mineral and amino composition of the samphire extract added in nachos were compared to literature threshold values (in water), we here assume that salty taste of samphire extract used at 2.5 % in nachos would be equivalent to 1.25 % (w/v) in water. Further, factoring in  $\sim$  50 % cooking loss during baking and frying of the nacho samples in the calculation, the levels of minerals and amino acids in nachos containing 2.5 % samphire extract were estimated in supplementary tables S5 and S6 and compared with taste thresholds levels of minerals and amino acids reported in the literature. From table S5 (supplementary material), it can be estimated that Na and Fe in nachos would be higher than the taste threshold concentration reported in the literature. This may suggest that Na and Fe would contribute to the salty taste of samphire nachos, as minerals have an additive contribution to salty taste perception (Grummer et al., 2012). Additionally, comparing the concentration of all free amino acids with literature taste thresholds (table S6, supplementary material), the concentration of individual free amino acids in samphire containing nachos would be below taste detection threshold levels. However, it is also important to consider that amino acids may have a synergistic effect (Rocha et al., 2020). Different amino acid combinations, such as arginine plus histidine (da Silva et al., 2020) and lysine plus arginine (Vidal et al., 2020), have been used as flavour enhancers and are shown to have synergistic effects with reduced sodium (or added potassium chloride), leading to improved sensorial attributes (including maintaining the salty taste) both in meat products and aqueous solutions. Recently Wang et al. (2022) also highlighted that NaCl plus glutamate was able to maintain salty taste in aqueous solutions. Therefore, although each individual amino acid was below its taste threshold level in the samphire containing nachos, we cannot rule out the possible contribution to salty taste perception through amino acid interactions or amino acid-mineral interactions. Besides the contribution of free amino acids, there may be the possibility that peptides, such as propyl and arginyl peptides, if present in samphire could contribute to salty taste, as recently demonstrated in soy sauce (Jünger et al., 2022).

In summary, samphire has a broad mineral and amino acid profile

and the results from our study strongly suggest that when incorporated in a food product like nachos, the samphire extract enhances both salty and umami taste compared to nachos containing salt only. Therefore, further investigations need to explore the contribution of different amino acids and minerals, to elucidate their additive and synergistic effects to overall saltiness perception. This will not only provide better understanding on the broader application of samphire as a salt substitute, but it will also provide a strategy for using particular combinations of minerals and amino acids as salt substitutes in food product applications. Additionally the nutrient profile of samphire such as protein, dietary fibre and bioactive compounds can serve as a functional ingredient, providing additional health benefits. It will also be interesting to explore the overall acceptance and intent to purchase samphire incorporated food products versus regular products with table salt. Overall samphire, a sustainably sourced, clean label salt substitute can transform food products by providing a healthy and diverse nutrition profile. Its incorporation in various food products can contribute to the development of more sustainable and nutritionally rich products in the food industry.

#### 6. Conclusions

The aim of this study was to analyse the nutrient profile of samphire from three different regions of the UK and evaluate the taste profile in a food product in order to understand the contribution of minerals and amino acids to salty taste of samphire. The results indicate that samphire contains approximately 1-3 % fat, 12-16 % protein, several minerals (such as K, Mg, Zn, Fe and Na) and amino acids (including salty taste relevant; lysine, glutamic acid, aspartic acid, histidine and arginine). When incorporated into a food product (nachos), the salty taste intensity of nachos containing 2.5 % samphire extract (2.79 g sodium per 100 g) was equivalent in salty taste to 1 % NaCl (3.51 g sodium per 100 g), indicating that the use of samphire could enable approximately 20 % salt reduction in solid food products. Sensory results indicate samphire can contribute to salty taste and we infer that the minerals and amino acids present in samphire may be responsible for this salty taste perception, either individually or in combination, which needs further investigation. These results can not only lead to reducing the sodium levels in food products with improved sensory properties but can also help to reduce the number of ingredients added to the product for a clean labelled product. Additionally, since plants from the Salicornia species naturally grow around marshy land and has the potential to grow on any infertile land, they may offer a solution to utilize land not used for other forms of agriculture. These underutilized plants may prove to be an asset to the food and agricultural industry.

Units

°C- degree Celsius. g- grams. kg- kilograms. mg- milligram. ml- millilitre.

Formulae

Total Yield.

$$Yield (\%) = \frac{weight \ of \ freezedried \ samphire}{weight \ of \ fresh \ samphire} \times 100$$

Crude Fat.

Crude fat 
$$\% = \frac{weight \ of \ extract - weight \ of \ empty \ flask}{weight \ of \ sample} \times 100$$

Crude Protein.

Nitrogen (g/100 g) for calculating crude protein.

grams N/100g sample = 
$$\frac{Titration\ volume\ \times\ 0.1\ \times\ 14.01\ \times\ 100}{1000\ \times\ Weight\ of\ sample\ in\ grams}$$

Where, Titration volume is (final reading – initial reading) of the titre in millilitres (cm<sup>3</sup>) which is divided by 1000 to give litres. 0.1 is the normality of the sulphuric acid titrant. 14.01 is the molar mass of nitrogen.

#### CRediT authorship contribution statement

**Saumya Sood:** Investigation, Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Lisa Methven:** Supervision, Conceptualization, Validation, Writing – review & editing. **Dimitris P. Balagiannis:** Methodology, Validation, Writing – review & editing. **Qiaofen Cheng:** Supervision, Conceptualization, Validation, Writing – review & editing.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

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#### Ethical statement for sensory panel

Ethical review and approval were not necessary for this study as the study involved tasting standard commercial practices by a trained sensory panel (n = 14; including 2 male 12 female, age from 35 to 65 years old) that are employees and have consented to taste and rate food as part of their job. Ethics approval and separate consent are only required from the trained panel where they are tasting non-standard, non-commercial or novel food ingredients. The trained panel work within the ethical and professional practices set out by the IFST: https://www.ifst.org/membership/networksand-communities/special interest-groups/sensory-science-group/ifst-guidelines.

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#### Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.138065.

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