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Levels of taurine, hypotaurine and homotaurine, and amino acids profiles in selected commercial seaweeds, microalgae, and algae-enriched food products

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Abstract

Amino acids and sulfonic acid derivatives (Taurine-Tau; Hypotaurine-HypTau; Homotaurine-HTau) of 26 different species of commercial macroalgae, microalgae and 10 algae-enriched food products from the market were quantified in a single chromatographic run. Tau and analogues were predominantly distributed in red species followed by green and brown species. *Palmaria palmata*, *Gracilaria longissima* and *Porphyra* sp. were the species with the highest content of Tau and total sulfonic acid derivatives (TAD). Notwithstanding, relatively high concentrations of HTau were found in green algae *Ulva lactuca* and *G. vermicullophyla* as well as in the brown algae *Undaria pinnatifida*. HTau and HypTau were found at lower concentrations than Tau in all species, except in *Ulva lactuca*. The samples with the highest protein content were the green species *Chlorella vulgaris*, *Nannochloropsis*, and *Afanizomenon-flos aquae*, followed by the red algae *Gracilaria longissima* and *Gracilaria vermicullophyla*.

Samples of pasta formulated with algae ingredients contained the highest levels of sulfonic acid derivatives, evidencing that these products can provide levels of TAD comparable to those found in foods of animal origin.

This study provides, for the first time, quantitative information regarding the distribution of sulfonic acid derivatives and total amino acids in multiple algae species as well as the nutritional impact of the inclusion of algae ingredients in commercial food matrices.

Highlights

-Taurine, Hypotaurine, Homotaurine were quantified in 26 algae species and 20 food samples

-Red algae species showed the highest concentrations of Tau and total sulfonic acid derivatives

-Pasta formulated with algae showed the highest levels of sulfonic acid derivatives

Keywords

Taurine, Homotaurine, Hypotaurine, algae, protein, total amino acids.

1. **Introduction**

Nowadays alternative protein sources are constantly explored to secure the future food and protein demand. This is due to the estimation that the world needs to close a 70 percent "food gap" between the crop calories available in 2006 and the expected calorie demand in 2050 (Godfray et al., 2010). Seaweeds and microalgae are gaining more and more attention since they contain high levels of protein (up to 70 % in several microalgal species) and interesting amino acid profiles. The levels of essential amino acids (EAA) in seaweeds are comparable to those defined by the FAO/WHO for dietary proteins (Černá, 2011). In microalgae, the levels of amino acids such as isoleucine, valine, lysine, tryptophan, methionine, threonine and histidine are similar or greater than those found in protein-rich sources such as eggs and soybean (Koyande et al., 2019). Seaweeds or macroalgae are rich in the amino acids responsible for the umami flavour, *i.e.*, glutamic acid, aspartic acid, glycine and alanine. However, when compared with other protein-rich food sources, seaweeds are limited by their low levels of lysine, threonine, tryptophan, cysteine, and methionine. The amino acid score and the essential amino acid index, which describe the nutritional properties of a protein, are higher for red seaweed than those for brown and green seaweeds. On the other hand, brown seaweeds have been reported to contain higher levels of acidic amino acids as compared to red and green seaweeds (Tiwari & Troy, 2015).

Considering the high protein content and the health benefits associated with algal bioactive compounds, the incorporation of algal or algae extracts into food products has been receiving a keen interest in recent years. For instance, Prabhasankar et al., (2009) developed a pasta product containing 10 % of wakame (*Undaria pinnatifida*) as an ingredient and found an increased level of total protein and amino acids threonine, isoleucine, lysine and methionine when compared with pasta without algae supplementation. The green microalgae *Nannochloropsis oculata* was used as a functional ingredient in cookies and pasta to increase the content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in these products. Other studies (Babuskin, S., Krishnan, K. R., Babu, P. A. S., Sivarajan, M., & Sukumar, M., 2014; El-Baz, F. K., Abdo, S. M., & Hussein, A. M. S., 2017; Fradique et al., 2020) formulated pasta products with *Chlorella vulgaris, Spirulina platensis* and/or *Dunaliella sp.* powder to improve their protein, fat and ash content. Batista et al., (2019) formulated wheat crackers products with different microalgae species such *Spirulina platensis*, *Chlorella vulgaris*, *Tetraselmis sp.* and *Phaeodactylon* sp. and observed that, by adding a 6 % of Spirulina and Chlorella extracts, the protein content was significantly higher than in control crackers.

In addition to the high protein and essential amino acid content, some algae species may contain uncommon bioactive molecules, as the sulfonic acid derivatives taurine (Tau), its precursor hypotaurine (HypTau) and the homologue homotaurine (HTau), which may contribute to modulate several diseases and can provide protection against free radicals and heavy metals (Colovic, M. B., Vasic, V. M., Djuric, D. M., & Krstic, D. Z., 2018).

Tau (2-aminoethanosulfonic acid), a conditionally essential amino acid for humans and essential for animals, is gaining attention for its several implications in human diseases. For instance, Wójcik, O. P., Koenig, K. L., Zeleniuch-Jacquotte, A., Costa, M., & Chen, Y. (2010) identified Tau as a preventive factor for coronary heart disease. Tau has recently been pointed out as a promising new therapeutic agent in the treatment of diseases affecting the muscles, the central nervous and the cardiovascular systems as well as against cancer and other metabolic disorders such diabetes type 2 (Gossai, D., & Lau-Cam, C. A., 2009; Schaffer, S., & Kim, H. W., 2018). Hatab et al., (2019) found that Tau showed potent antitumor activities to control hepatocellular carcinoma. Tau was also demonstrated to play a protective function in different neurodegenerative models for Parkinson's, Alzheimer's and Huntington's diseases (Jakaria et al., 2019).

HTau, also known as tramiprosate, is a low-molecular weight sulfonic compound capable of binding to the β-peptides Lys16, Lys28, and Asp23 of Aβ42 (the pathological element typical of Alzheimer's and of brain aging) in its soluble form. Tramiprosate has been shown safe and effective in several neurocognitive disorders, particularly alzheimer's disease and Mild cognitive impairment, where the results are consistent with a disease-modifying effect (Manzano, S., Agüera, L., Aguilar, M., & Olazarán, J., 2020). HTau also has a direct effect on neuronal activity; Owing to its affinity for GABA A receptors (GABAA-R), it modulates cortical inhibitory activity by reducing the response of neurons to excitatory stimuli to glutamate. According to Tian, J., Dang, H., Wallner, M., Olsen, R., & Kaufman, D. L. (2018), HTau is a safe blood-brain barrier permeable GABAA-R-specific agonist that ameliorates disease status in mouse models of multiple sclerosis. Gossai, D., & Lau-Cam, C. A. (2009) compared Tau, HTau and HypTau for their ability to modify several indices of oxidative stress and membrane damage associated with type 2 diabetes and found these 3 molecules equiprotective against diabetes-induced alterations in enzymatic and non-enzymatic indices of oxidative stress and against membrane susceptibility to oxidative damage (Gossai, D., & Lau-Cam, C. A., 2009).

HypTau is the direct precursor of Tau which may regulate nociceptive transmission in acute, inflammatory, and neuropathic pain in rat models by activating glycinergic neurons in the spinal cord, and it may be a promising candidate for treating various pain states (Hara et al., 2012). HypTau and Tau have anti-inflammatory and antioxidant functions and are important stimulants of immune cell metabolism (Ali et al., 2019). Association of Tau and HypTau with adaptive immune cells and platelets suggests the importance of these amino acids in triggering immunity in the setting of Hepatitis C virus (HCV) infection. Recently, Wan et al., (2020) found that HypTau may regulates a variety of metabolic pathways that lead to the upregulation of some age-related genes to extend lifespan.

In the context of the quantification of these sulfonic acid derivatives available in the literature, Kawasaki et al. (2017) reported the content of free amino acids and Tau in 29 Japanese algae species. The authors found the highest Tau contents in red algae species. Furthermore, Mehdinia, A., Rostami, S., Dadkhah, S., & Fumani, N. S. (2017) quantified HTau and Tau in 8 algae species from coastal zones from the Persian Gulf and found the greatest amounts of these molecules in 3 red species, *i.e.*, *Hypnea boergesenii*, *Gracilaria corticate*, and *Gracilaria pygmaea*. McCusker, S., Buff, P. R., Yu, Z., & Fascetti, A. J. (2014) quantified Tau and essential amino acids (EAA) in 18 algae species and found higher content of Tau in red species. Hwang, E. S., Ki, K. N., & Chung, H. Y. (2013) reported the Tau content of two Porphyra species, *P. tenera* and *P. haitanensis*, while Bogolitsyn, K. G., Kaplitsin, P. A., & Pochtovalova, A. S. (2014) studied the amino acid and Tau composition in 4 species of artic brown algae. Cao, Y., Duan, J., Guo, J., Guo, S., & Zhao, J. (2014) quantified Tau content in 24 brown algae species from China and found Tau only in five species, with levels ranging between 0.64 mg/100 g d.w. and 3.66 mg/100 g d.w.

Nevertheless, to the best of our knowledge, no study has earlier quantified the three sulfonic acid derivatives (Tau, Htau and Hyptau) simultaneously, and considering the significant health implications related to the ingestion of these molecules, there is still a lack of information about the presence of these compounds in many algae species. Furthermore, there are no data available about the content of Tau, HTau and HypTau in commercial food products containing algae ingredients.

Hence, the main scope of this work was to provide information on the levels of Tau, HypTau and HTau in different algae species as well as in algae-containing pasta and crackers available on the market, by using a validated UHPLC-DAD-MS/MS method (Terriente-Palacios et al., 2019). In addition, their amino acid profile, total protein content, Essential Amino Acid Index (EAAI), Limiting Amino Acid (LAA) and Essential to Non-Essential amino acid ratio (EAA/NEAA) were also assessed to evaluate the impact of adding algal ingredients to different food products.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (ACN) and methanol (MeOH) were HPLC gradient-grade (Merck KGaA (Darmstadt, Germany). Perchloric (60%) and hydrochloric acid (37%) were from J.T. Baker (NJ, United States). Sigma-Aldrich Chemie (Sant Quentin Fallavier, France) supplied formic acid, ammonium acetate, ammonium formate, phenyl isothiocyanate (PITC), triethylamine (TEA), Trichloroacetic acid (TCA), NaK tartrate tetrahydrate, Folin-Ciocalteu's phenol reagent, Bovine serum albumin, Na_2CO_3, NaOH and pure standards for the target 19 amino acids, Tau, HypTau and HTau. Ultrapure water was obtained with a Milli-Q system from Millipore (Bedford,MA, USA).

Single stock solutions were prepared for each of the 22 target compounds [Histidine (His), HypTau, Hydroxyproline (Hyp), Tau, HTau, Arginine (Arg),Serine (Ser), Glycine (Gly), Aspartic acid (Asp), Glutamic acid (Glu),Cysteine (Cys), Threonine (Thr), Proline (Pro), Alanine (Ala), Gamma aminobutyric acid (GABA), Lysine (Lys), Tyrosine (Tyr), Methionine(Met), Valine (Val), IsoLeucine (Ile), Leucine (Leu), and Phenylala-nine (Phe)] by dissolving the corresponding pure standards in 0.1 M HCl.

2.2. Algae and food sampling

Twenty-six different species of algae (including fresh and dried seaweeds and microalgae), ten algae-enriched food products and ten food products of the same categories not containing algae ingredients were purchased in commercial establishments in Spain, France, Japan, and Germany (Table S1, *Supplementary material*). Three different batches were acquired for each product. Dried products were homogenized with a mixer mill (Retsch GmbH & Co, KG Germany). Fresh products were freeze dried (LYOMICRON 55 – Cool vacuum Technologies, Barcelona - Spain) and homogenized as as previously described (Terriente-Palacios et al., 2019).. The powdered samples were stored at ambient temperature under dry and dark conditions and analysed within one month.

2.3. Quantification of amino acids and sulfonic acid derivatives

Algae and food samples were processed as previously described (Terriente-Palacios, C., Diaz, I., & Castellari, M., 2019). Briefly, 10 mg of seaweed sample were hydrolysed with 1 mL of 8 M perchloric acid for 24 h at 110 °C. After cooling at room temperature, the samples were filtered through 0.2 µm membrane syringe filters (GMP filter membranes, Merck KGaA, Darmstadt, Germany), and then derivatized with a methanol-water-TEA-PITC solution (7:1:1:1, v/v/v/v). The derivatized samples were re-dissolved with 24 µL of mobile phase B and 226 µL of mobile phase A, centrifuged at 11,000 × g for 5 min, and filtered through a Single Step Standard Filter Vials (Thomson Instrument Company, CA, USA).

Four µL of the sample were injected into the chromatographic system, consisting of an Acquity UPLC® equipped with a PDA detector, an electrospray (ESI) as a source of ionization operated in the positive mode, and a TQD triple quadrupole mass spectrometer (Waters, Milford, MA, USA). The system was controlled by MassLynx 4.1 software (Waters, Milford, MA, USA). Chromatographic separation was carried out on a BEH-C_{18} , 1.7 µm, 100 mm x 2.1 mm i.d. column (Waters, Milford, MA, USA). Source temperature was fixed at 135 ºC, the capillary voltage was set at 3.0 kV and the desolvation temperature was set at 350 ºC. The cone gas (nitrogen) flow rate was 350 L/h and cone voltage was set at 30 V. MS experiments were carried out in "Scan" mode to obtain *m/z* values of the molecular ions. MS/MS experiments in "Daughter Ions" mode were also performed, to obtain the fragmentation patterns of molecular ions. The collision energies varied between 10 and 20 eV. The gas used in the collision cell was argon at a flow rate of 0.1 mL/min. Peak identity was confirmed by comparing their retention times, UV spectra, MS and MS/MS spectra with the corresponding data obtained from pure standards.

Quantitation of the target compounds was done based on an external calibration curve and considering the sample dilution during the extraction and derivatization steps. Calibration curves were made for each target compound by injecting derivatized amounts of pure standards at different concentrations in the range from 0.1 mM to 10.0 mM and by plotting the signal obtained from the diode array detector at λ=254 nm versus the corresponding concentrations. Three replicates of analysis were carried out for each sample.

Even if the method was already validated in algae samples, additional validation was carried out for the new food matrices (pasta and crackers), according to harmonized guidelines for single-laboratory validation of methods of analysis (Thompson, M., Ellison, S. L. R., & Wood, R., 2002; AOAC International 2002).

The precision of the method (RSD_r%), was calculated from Eq. 1

$$RSD\% = \left(\frac{s}{X}\right) x\ 100 \qquad \text{(Eq. 1)}$$

under repeatable conditions by spiking one pasta and one cracker sample without algae supplementation with a solution containing a mix of the 22 standards of the target compounds at three concentration levels (0.1 mM, 5 mM and 10 mM); six replicates of analysis were carried out on the same day. Similarly, the intermediate precision was calculated by analysing the above spiked samples at three concentration levels along 6 days (RSD_R%).

Trueness was evaluated by analysing recovery of the 22 target compounds in a pasta and a cracker sample spiked before hydrolysis with known amounts of pure standards at three levels (0.1, 5.0 and 10.0 mM). Three spiked sample replicates were analysed for each spiking level. Linearity was assessed based on the external calibration curves by checking the working range, the coefficient of determination (R^2), the residual value of replicates, and the Lack-of-fit (LoF) test significance (Thompson, M., Ellison, S. L. R., & Wood, R., 2002). The instrument limit of detection (ILOD) and the instrument limit of quantification (ILOQ) were calculated as 3.3 σ/b and 10 σ /respectively, where "σ" is the Residual Standard Deviation of the Calibration Curve (Sx/y) and "b" is the slope of regression line from the calibration curves of each compound. The Breush-Pagan test, to establish the presence or absence of heteroscedasticity, was also applied. The method limits of detection (MLOD) and quantification (MLOQ) were estimated from ILOD and ILOQ considering the dilution factor and the mass fraction of each sample.

2.4. Total Protein.

Protein extraction was carried out by following Slocombe, S. P., Ross, M., Thomas, N., McNeill, S., & Stanley, M. S. (2013) with minor modifications. Five mg of sample were re-suspended in 200 µL 24 % (w/v) TCA and, after homogenization by agitation, were incubated for 15 minutes in a water bath at 95 ºC, and then allowed to cool at room temperature. After addition of 600 µL MilliQ water and mixing, the samples were centrifuged at 15,000 g for 20 min at 4 ºC (Microcentrifuge 5415 R, Eppendorf AG, Hamburg Germany) and their supernatants discarded. The pellets were re-suspended in 0.5 mL Lowry Reagent D and incubated 10 min at 55 ºC. Samples were then cooled at room temperature, centrifuged at 15,000 g for 20 min and the supernatant retained. For protein quantification, a stock of Lowry Reagent D was prepared daily in a 48:1:1 ratio (v/v/v) of Lowry Reagents A, B and C. A suitable volume (up to 50 µL) of the protein extract was added into a 1.5 mL microfuge tube, together with 950 µL of Lowry Reagent D followed by immediate mixing. After incubation for 10 min at room temperature, 0.1 mL of 0.2 N Folin-Ciocalteu phenol reagent was added to each tube and mixed immediately. Absorbance was read at 600 nm (1800 UV-Vis spectrophotometer, Shimadzu Co, Madison, WI) using UVProbe™ software (Shimadzu Co, Madison, WI) after 30 minutes at room temperature. Calibration curves were prepared for each assay with a bovine serum albumin (BSA) stock solution (200 mg/mL) and using a polynomial line of best fit generated in Microsoft Excel 365. Samples were prepared in triplicate and analysed.

2.5. Essential Amino Acid Index (EAAI), limiting amino acid (LAA) and ratio of Essential to Non-Essential Amino Acids (EAA/NEAA).

The Essential Amino Acid Index (EAAI) (Friedman, M., 1996), a measure of the protein nutritional quality, was calculated using Equation 2:

$$EAAI = \sqrt[n]{CS1x\ CS2\ x\ CS3\ x \ldots..CSn}$$

(Eq. 2)

where CS1, CS2, …CSn are the chemical scores of each essential amino acid, calculated according to Equation 3,

$$Chemical\ score = \frac{EAA\ in\ sample\ protein\ (mg\ g^{-1})}{EAA\ in\ standard\ protein\ (mg\ g^{-1})} \times 100$$

(Eq. 3)

where "EAA in sample protein" indicates the concentration of a given essential amino acid in the test protein and "EAA in standard protein" represents the concentration of the same essential amino acid in a reference protein, in this case egg protein (FAO/WHO, 2007). From CS, the limiting amino acid (LAA) for each algae sample was determined as the EAA in the sample protein which showed the greatest difference in concentration from the same EAA in the standard protein.

The ratio of Essential to Non-Essential Amino Acids (EAA/NEAA) was determined considering EAA as the sum of the concentrations of His, Arg, Thr, Lys, Met, Val, Ile, Leu and Phe, and NEAA as the sum of the concentrations of Hyp, Ser, Gly, Asp, Glu, Cys, Pro, Ala, GABA and Tyr.

To estimate the variations of EAA and NEAA between products enriched with algae and controls, the percentile variations of the Essential Amino Acid Index (% ΔEAAI) and of the ratio of Essential to Non-Essential Amino Acids (% ΔEAA/NEAA) were calculated using Equations 4 and 5:

$$\%\ \Delta\mathrm{EAAI}\ = \left(\frac{EAAI_P - EAAI_C}{EAAI_C}\right) \times 100$$

(Eq. 4)

where,

$EAAI_P$ is the Essential amino acid index of a given product enriched with algae,

$EAAI_C$ is the mean EAAI value of the corresponding control food category

$$\%\Delta\, EAA/NEAA = \left(\frac{(EAA/NEAA)_P - (EAA/NEAA)_C}{(EAA/NEAA)_C}\right) \times 100$$

(Eq. 5)

where $(EAA/NEAA)_P$ is the ratio of Essential to Non-Essential Amino Acids of a given product enriched with algae, $(EAA/NEAA)_C$ is the mean ratio of Essential to Non-Essential Amino Acids of the corresponding control food category.

2.6. Statistical analysis

Statistical analyses were all performed using the software Minitab® version 19.2, 2019 (Minitab Inc., State College, PA, USA). The Kolmogorov-Smirnov test was applied to verify whether the distribution of the variables was normal ($p < 0.05$). Kruskal-Wallis non-parametric test and Mann-Whitney pairwise comparisons of median values were carried out to evidence differences between groups. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. UHPLC method validation for pasta & crackers

Precision (RSD_r%), ranged from 1.0 % to 4.4 % in pasta and between 1.0% to 4.3 % in crackers. Intermediate precision (RSD_R%) ranged from 1.0 % to 4.7 % in pasta and between 1.0 % to 5.1 % in crackers (Table S2, Table S3, *Supplementary material*). Recovery values for the 22 compounds were within 85.5 % and 107.0 % in pasta and within 85.5 % and 106.0 % in crackers (Table S4, *Supplementary material)*. These results fulfil the acceptable values of precision and recovery recommended by the IUPAC Technical report (Thompson, M., Ellison, S. L. R., & Wood, R., 2002). Calibration curves showed R^2 value always higher than 0.997 for all the compounds and satisfied the homoscedasticity criterion, and the residual standard deviation approach could be applied (Terriente-Palacios, C., Diaz, I., & Castellari, M., 2019). The linear range was initially tested between 0.1 mM–2.5 mM. Preliminary analysis of samples indicated the need to extent the upper limit of the calibration curve to 10 mM for several amino acids. So, the working range was confirmed for all the amino acids and was finally established between 0.1 mM–10.0 mM.

The MLOD and MLOQ were from 0.01 mg/kg d.w. to 0.11 mg/kg d.w. and from 0.04 mg/kg d.w. to 0.31 mg/kg d.w. respectively, for all the target compounds. Glycine had the lowest detection and quantification limits while HypTau the highest ones (Table S5, *Supplementary material*).

3.2. Quantification of sulfonic acid derivatives

Concentrations of HypTau, Tau and HTau are listed in **Table 1**, while **Figure 1** shows the sum of the three sulfonic acid derivatives (TAD) in the 26 algae species**.** The highest concentrations of Tau, HTau and TAD were found in red algae, with TAD values ranging between 2.52 mg/100 g d.w. and 868 mg/100 g d.w., followed by green and brown algae with TAD concentrations up to 339 mg/100 g d.w. and 252 mg/100 g d.w., respectively. Among all the species, *Gracilaria longissima*, *Palmaria palmata* and *Porphyra sp* were those with the highest contents of TAD. No significant differences were found among the three groups for the HypTau content.

Tau was the predominant sulfonic acid derivative in all the algal species, except for the green algae *Ulva lactuca*, which showed similar concentrations of HTau and Tau. As it was pointed out in other studies (Tevatia et al., 2015; Kawasaki et al., 2017), the high content of Tau in red macroalgae and some green microalgae species may be related with their growing environment and the hypothesis that this compound is involved in osmoregulation. Our findings agree (Table S9 *Supplementary material*) with the levels of Tau found by Vieira et al., (2018) in almost all the species, except for the samples of *Palmaria palmata* and *Porphyra sp* which showed lower contents of this compound.

In the case of *Porphyra sp*, results presented here are in the middle of the range reported by other authors (Dawczynski, C., Schubert, R., & Jahreis, G., 2007; McCusker, S., Buff, P. R., Yu, Z., & Fascetti, A. J., 2014) and are lower than those found by Hwang et al., (2013). Results for the species *Gracilaria vermiculophylla* (“Ogonori”) are in good agreement with the data reported in two previous studies (Mehdinia, A., Rostami, S., Dadkhah, S., & Fumani, N. S., 2017; Kawasaki et al., 2017), while in the case of *Eisenia arborea, Laminaria spp*., and *Ulva spp*., McCusker, S., Buff, P. R., Yu, Z., & Fascetti, A. J. (2014) reported lower concentrations of Tau, ranging between 1 mg/100 g d.w. and 7 mg/100 g d.w.

It should be underlined that relatively high levels of Tau have been found in products of animal origin, such as mussels and clams, with concentrations of 655 mg/100 g d.w. and 240 mg/100 g d.w. respectively, while beef and lamb meat contain lower amounts around 40 mg/100 g d.w. (Lourenço R. & Camilo M. E., 2002). Considering the results obtained in this study, some species of algae, such as *Gracilaria longissima* (842 mg/100 g d.w.), *Palmaria palmata* (555 mg/100 g d.w.) and *Porphyra sp.* (527 mg/100 g d.w.), can be considered among the best alimentary sources of Tau.

Regarding the concentration of HTau, red algae species showed the highest median concentration; *Porphyra sp.*, *Palmaria palmata* and *Gracilaria vermicullophyla*, had the highest concentrations in agreement with the scarce data available in literature about this compound (Mehdinia, A., Rostami, S., Dadkhah, S., & Fumani, N. S., 2017; Kawasaki et al., 2017), while brown algae samples contained the lowest amounts among the species included in this study (Table 1).

Notwithstanding, relatively high concentrations of HTau were found in green algae *Ulva lactuca* (159 mg/100 g d.w.) and *G. vermicullophyla* (60.00 mg/100 g d.w.) as well as in the brown algae *Undaria pinnatifida* (56,7 mg/100 g d.w.). We already reported (Terriente-Palacios, C., Diaz, I., & Castellari, M., 2019) high levels of this compound in a distinct sample of *Ulva lactuca*. In the case of *G. vermicullophyla* and *U. pinnatifida*, this is the first time that HTau is quantified in these species. Even though more data need to be gathered, our findings seem to indicate that relatively high levels of HTau can be found not only in red algae species but also in green and brown species.

HypTau was present in almost all the products analyzed but in lower quantities than those found for the other two sulfonic acid derivatives, with concentrations ranging from 0.03 mg/100 g d.w. (*Tetraselmis sp.*) to 37.9 mg/100 g d.w. (*Chlorella vulgaris*). Previously HypTau has been detected, but not quantified, only in *Ulva lactuca* (Gupta, V., & Kushwaha, H. R., 2017), while Tevatia et al., (2015) found 0.02 mg/100 g d.w. of HypTau in *Tetraselmis sp*., which is consistent with our result for this specie. So, quantitative information about the content of HypTau is provided for the first time for most of the seaweed and microalgae species included in our study.

The content of HypTau, Tau and HTau in pasta and cracker products including some algae and Controls is presented in **Table 2**. None of the three sulfonic acid derivatives were found at detectable levels in the Control products. On the contrary, in all pasta and cracker products containing algae, at least one of the three sulfonic acid derivatives was found at detectable levels.

For algae fortified pasta products, the TAD content ranged between 0.19 mg/100 g d.w and 23.1 mg/100 g d.w., while in algae fortified cracker samples TAD ranged between 0.75 mg/100 g d.w. and 159 mg/100 g d.w. It should be underlined that “Crackers with Porphyra” contained the highest content of Tau, which is in line with the high content of this compound observed in the raw algae material (**Table 1**).

As expected, lower amounts of TAD were found in products formulated with low percentages of algae inclusion or with algae species relatively poor in sulfonic acid derivatives, as in the case of “Noodles with wakame” (2.1 % *Undaria pinnatifida*) or “Crackers with Wakame” (1.5 % *Undaria pinnatifida*).

3.3. Total protein and amino acids indexes

Table 1 shows the total protein content and amino acid indexes in the 26 algae samples (see also Table S6 *Supplementary material***)**. Our results for the total protein content agreed with the values provided on the product label for 15 over 26 samples; in 4 samples the protein content was not specified on the label and in 7 samples (*Chondrus crispus*, *Himanthalia elongata*, *Hizikia fusiforme*, *Laminaria japonica*, *Undaria pinnatifida*, *Ascophyllum nodosum* and *Spirulina platensis*) the differences were > 10 %.

Red and green algae showed the highest total protein contents; the green microalgae *Chlorella vulgaris, Nannochloropsis sp*. and *Afanizomenon flos-aquae* exhibited the higher total protein content (52.2 g/100 g d.w., 40.0 g/100 g dw. and 36.4 g/100 d.w. respectively), followed by the red algae *Gracilaria vermiculophylla* and Gracilaria *longissima*. These protein contents are in line with the results of other authors (Vieira et al., 2018) and with studies indicating that the protein content in brown algae is generally lower than in green and red species (Olsson, J., Toth, G. B., & Albers, E., 2020).

The EAA/NEAA ratio was in the range of 0.41 and 1.20 in both red and brown algae, and between 0.47 and 0.96 in green algae species (Table 1). The highest EAA/NEAA ratio (1.20) was found in red the seaweed *Gracilaria longissima* and in the brown microalgae *Odontella aurita*, indicating that these two algae species can be considered a good source of essential amino acids (Friedman, M. 1996). In the case of *U. pinnatifida* (0.96) and *Porphyra sp*. (0.64) samples the results are in good agreement with the estimation of Mišurcová et al., (2014).

Other authors reported EAA/NEAA ratios from 1.06 to 2.17 in commercial algae products, *i.e. Porphyra sp*. and *Ulva spp*., respectively (Vieira et al., 2018) and from 0.5 to 0.8 in products from the species *Porphyra sp*., *Laminaria sp*. and *Undaria pinnatifida* (Dawczynski, C., Schubert, R., & Jahreis, G., 2007; Mišurcová et al., 2014). In this case the differences could be partially associated to the amino acids included in the calculation.

The essential amino acid index (EAAI) was in the range of 84.8–185 in red algae samples, 79.9-169 in brown algae and 36.8–137 for green algae; the highest EAAIs were found in the samples of the red seaweeds *Gigartina pistillata*, and *Gracilaria longissima*. These values are comparable with those estimated by Vieira et al., (2018) and Mišurcová et al., (2014). Methionine was the limiting amino acid (LAA) in 12 samples of 26, followed by lysine and leucine, in agreement with data available in the literature (Dawczynski, C., Schubert, R., & Jahreis, G., 2007; Mišurcová et al., 2014; Vieira et al., 2018). Since EAAI is closely related with the biological quality of the protein, these data confirm that algae, and more specifically certain red algae species, could be a source of high-quality protein (Friedman, M. 1996).

Total protein content and amino acid indexes in pasta and crackers products including some algae and Controls are listed in **Table 2** (see also Table S7 and S8, *Supplementary material*).

In general, our results for the total protein content were in good agreement with was indicated on the label of pasta and cracker products, except for the products “Noodles with Nori” (19 % less than declared on the label) and “Crackers with Spirulina” (44 % less than declared on the label).

In general, and as pointed out by other authors (Prabhasankar et al., 2009; Fradique et al., 2010; Rodríguez De Marco, E., Steffolani, M. E., Martínez, M., & León, A. E., 2018), the higher the percentage of seaweed incorporated, the higher the protein content in the final product (*e.g.*, “Fusilli with Spirulina” with 4% of Spirulina, and “Crackers with Porphyra with 62 % of *Porphyra sp.*). It is noteworthy that when compared to the corresponding “Control” category, the inclusion of algae ingredients increased significantly the total protein content only in crackers but did not significantly ($p<0.05$) modify neither the EEA/NEAA ratio nor the EAAI in both pasta and cracker samples. Likewise, the use of algae ingredients did not modify limiting amino acid (LAA), which was always Methionine in pasta and Lysine in crackers.

Percentual variations in EAAI and EAA/NEAA ratios in algae containing specific products vs. the corresponding “Control” food categories are represented **in Figure 2 and Figure 3**. Two commercial pasta samples including algae in their formulation, *i.e.*, “Noodles with Porphyra” (NN) and “Fusilli with Spirulina” (FS) exhibited an improvement of their EAAI and EAA/NEAA ratio close to or higher than 5 % compared to the corresponding control products (**Figure 2**). In one product (SS) the variations were lower than 1 %, in one product (NU) the EAAI increased but the EAA/NEAA ratio was unchanged and in one product (NW) the EAAI increased but the EAA/NEAA ratio decreased if compared to the corresponding “Control” category. In the case of the commercial crackers, only one sample (CN, “Seaveg crispies” with 62 % *Porphyra sp.*) showed an improvement of both EAAI and EAA/NEAA parameters when compared to the corresponding control products (**Figure 3**). The other samples had limited variations in these parameters (lower than 1 %).

So, it seems that products with the highest percentages of algae addition provided not only a significant improvement of their total protein content compared with the products without algae addition, but also increased the nutritional quality of the protein fraction.

4. Conclusion

An UHPLC-DAD-MS/MS analytical method to quantify the concentrations of taurine, hypotaurine, homotaurine and main amino acids in algae was further validated in two food matrices, pasta and crackers.

The distribution of the target compounds was assessed in 26 samples of macro- and microalgae available on the market, as well as in 20 samples of commercial pasta and cracker products including or not algae as an ingredient. All the algae species included in this study contained at least one of the three target sulfonic acid derivatives. Red algae species stand out for their high Taurine and Homotaurine content, where the species belonging to the genus *Gracilaria* presented the highest concentration. Hypotaurine was also found in almost all species, but at lower concentrations than the other two compounds.

It is important to highlight that, to the best of our knowledge, we report for the first time the concentration of Tau in 9 algae species, *i.e. Gracilaria longissima*, *Mastocarpus stellatus*, *Gigartina pistillata*, *Eisenia bicyclis*, *Odontella aurita*, *Enteromospha intestinalis*, *Codium sp*, *Dunaliella salina* and *Aphanizomenon flos-aquae*. HTau and HypTau were herein quantified for the first time ever in almost all the species, except in *Gracilaria* sp, *Porphyra* sp, *Laminaria japónica*, *Ulva Lactuca*, *Chlorella vulgaris* and *Tetraselmis chuii* (Tevatia et al., 2015; Terriente-Palacios et al., 2019).

Commercial pasta and crackers formulated with different species of algae contained at least one of the three target sulfonic acid derivatives at detectable levels. In some cases, *i.e*., “Seaveg crispies” or “Noodles with Nori” the total sulfonic acid derivatives concentration reached levels comparable to those found in foods of animal origin. Inclusion of seaweeds and microalgae also contributed to significantly increasing the total protein content in crackers compared to commercial products without algae ingredients, and increased other nutritional properties of the protein fraction (EEA/NEAA, EAA) in products with the higher percentage of algae addition, *e.g.*, “Fusilli with Spirulina” (4%), “Noodles with Nori” (4.4%) and “Seaveg crispies” (62 %). Nevertheless, these effects may differ according to the algae species and the amount used in the formulation.

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Table 1. Concentrations of taurine. homotaurine and hypotaurine (mg/100 g d.w.), Sum of sulfonic acid derivatives (TAD, mg/ 100 g d.w), Protein content (g/100 g d.w.), Essential to Non – Essential amino acid ratio (EAA/NEAA), Essential amino acid Index (EAAI) and Limiting amino acid (LAA) in the 26 algae species. (Each value is the mean of *n*=3 independent determinations ± standard deviation)

nd: not detected, lower than the limit of detection

Small letters on the same column indicate differences among median at p<0.05

*Algae specie as indicated on the label of the commercial product

Algae species*	Taurine	Homotaurine	Hypotaurine	TAD	Protein	EEA/NEAA	EAAI	LAA
	mg / 100 g d.w.				g / 100 d.w.			
Red algae								
Condrus crispus	84.5 ± 0.31	3.45 ± 0.05	nd	87.9	12.8 ± 0.03	0.77 ± 0.01	123 ± 0.01	Met
Gracilaria longissima	842 ± 0.12	12.2 ± 0.11	14.6 ± 0.10	868	34.6 ± 0.07	1.20 ± 0.02	172 ± 0.02	Met
Mastocarpus stellatus	0.58 ± 0.01	1.69 ± 0.13	0.25 ± 0.05	2.52	11.0 ± 0.01	0.51 ± 0.02	106 ± 0.04	Lys
Gracilaria vermicullophyla	227 ± 0.94	50.1 ± 0.81	1.85 ± 0.07	279	35.1 ± 0.06	0.41 ± 0.02	84.8 ± 0.02	Leu
Palmaria palmata	555 ± 0.10	67.2 ± 0.11	7.56 ± 0.02	630	17.8 ± 0.02	0.56 ± 0.02	112 ± 0.02	Met
Porphyra sp.	527 ± 0.95	70.2 ± 0.53	5.81 ± 0.08	603	31.0 ± 0.07	0.64 ± 0.01	134 ± 0.03	Phe
Gigartina pistillata	185 ± 0.17	14.4 ± 0.21	nd	199	28.1 ± 0.01	1.14 ± 0.01	185 ± 0.01	Met
Median	**227 a**	**14.4 a**	**1.85**	**279 a**	**28.1 a**	**0.64**	**129**	
Brown algae								
Eisenia byciclis	0.88 ± 0.07	0.57 ± 0.01	0.62 ± 0.05	2.07	10.4 ± 0.01	0.63 ± 0.01	104 ± 0.03	Met
Himanthalia elongata	0.28 ± 0.05	0.28 ± 0.05	0.20 ± 0.09	0.76	9.20 ± 0.06	0.56 ± 0.01	95.9 ± 0.02	Leu
Hizikia fusiforme	57.0 ± 0.01	0.38 ± 0.05	0.33 ± 0.04	57.7	22.5 ± 0.05	0.49 ± 0.02	99.6 ± 0.02	Leu
Laminaria japonica	65.0 ± 0.02	2.05 ± 0.04	1.60 ± 0.11	68.6	22.9 ± 0.02	0.41 ± 0.04	81.4 ± 0.04	Phe
Laminaria ochroleuca	57.0 ± 0.06	3.50 ± 0.06	2.19 ± 0.01	62.4	6.90 ± 0.01	0.50 ± 0.01	36.8 ± 0.01	Lys
Undaria pinnatifida	195 ± 0.09	56.7 ± 0.09	0.67 ± 0.01	252	13.5 ± 0.03	0.98 ± 0.03	137 ± 0.01	Leu
Fucus vesiculosus	152 ± 0.08	0.96 ± 0.08	0.21 ± 0.05	153	10.7 ± 0.02	0.55 ± 0.01	85.3 ± 0.01	Leu
Ascophyllum nodosum	135 ± 0.01	0.61 ± 0.01	nd	136	8.80 ± 0.03	0.96 ± 0.01	134 ± 0.01	Met
Odontella aurita	1.36 ± 0.01	0.36 ± 0.01	0.30 ± 0.01	2.02	15.1 ± 0.01	1.20 ± 0.01	121 ± 0.04	Met
Median	**62.0 ab**	**0.61 b**	**0.33**	**62.7 b**	**11.0 b**	**0.55**	**134**	
Green algae								
Enteromorpha intestinalis	30.8 ± 0.02	60.0 ± 0.05	nd	90.8	9.28 ± 0.05	0.83 ± 0.01	169 ± 0.05	Met
Caulerpa lentillifera	nd	0.60 ± 0.05	0.14 ± 0.02	0.74	13.2 ± 0.04	0.51 ± 0.02	99.2 ± 0.03	Lys
Codium sp.	12.1 ± 0.05	nd	nd	12.1	11.4 ± 0.05	0.96 ± 0.01	143 ± 0.03	Val
Ulva lactuca	158 ± 0.14	159 ± 0.05	22.1 ± 0.02	339	24.1 ± 0.02	0.85 ± 0.02	168 ± 0.02	Met
Nannochloropsis	101 ± 0.51	2.05 ± 0.07	0.16 ± 0.01	103	40.0 ± 0.02	0.64 ± 0.04	79.9 ± 0.02	Met
Chlorella vulgaris	138 ± 0.12	10.5 ± 0.01	37.9 ± 0.01	186	52.2 ± 0.03	0.67 ± 0.02	152 ± 0.02	Ile
Dunaliella salina	26.2 ± 0.02	0.96 ± 0.08	0.20 ± 0.03	27.3	17.0 ± 0.05	0.94 ± 0.01	100 ± 0.02	Met
Tetraselmis chuii	132 ± 0.12	1.82 ± 0.02	0.03 ± 0.04	134	13.4 ± 0.03	0.79 ± 0.01	122 ± 0.01	Phe
Afanizomenon flos-aquae	10.1 ± 0.2	nd	nd	10.1	36.4 ± 0.04	0.76 ± 0.01	139 ± 0.04	Val
Spirulina platensis	4.12 ± 0.03	121 ± 0.02	0.12 ± 0.01	125	31.7 ± 0.08	0.47 ± 0.02	105 ± 0.04	Met
Median	**28.5 bc**	**1.94 ab**	**0.14**	**97.0 ab**	**20.6 a**	**0.78**	**141**	

Table 2. **Concentration of sulfonic acid derivatives (mg/100 g d.w.),** Sum of sulfonic acid derivatives (TAD, mg/ 100 g d.w), **Protein content (g/100 g d.w.), Essential to Non – Essential amino acid ratio (EAA/NEAA), Essential amino acid Index (EAAI) and Limiting amino acid (LAA) in algae-enriched food products and controls.** (Each value is the mean of n=3 independent determinations ± standard deviation). nd: not detected, lower than the limit of detection.

Small letters in the same column indicate significant differences between the median within the same food category

*Product description and algae species as indicated in the label of the commercial product

Product description*	**Taurine**	**Homotaurine**	**Hypotaurine**	**TAD**	Protein	EEA/NEAA	EAAI	LAA
	mg / 100 g d.w.				g / 100 d.w.			
Pasta + Algae								
(NN) Noodles with Nori	20.8 ± 0.01	2.15 ± 0.03	0.14 ± 0.01	23.1	12.9 ± 0.01	0.50 ± 0.03	97.5 ± 0.01	Met
(NU) Noodles with Ulva	13.9 ± 0.02	2.94 ± 0.02	0.10 ± 0.01	16.9	11.8 ± 0.05	0.49 ± 0.02	95.9 ± 0.02	Met
(NW) Noodles with Wakame	2.82 ± 0.03	1.08 ± 0.02	0.06 ± 0.00	3.96	11.4 ± 0.04	0.49 ± 0.04	97.1 ± 0.02	Met
(FS) Fusilli with Spirulina	3.45 ± 0.04	11.9 ± 0.03	0.19 ± 0.03	0.19	26.4 ± 0.03	0.47 ± 0.01	98.6 ± 0.01	Met
(SS) Spaghetti with seaweeds	17.7 ± 0.03	1.21 ± 0.01	0.09 ± 0.02	3.47	10.2 ± 0.05	0.50 ± 0.03	94.0 ± 0.01	Met
Median	**6.82 a**	**2.15 a**	**0.10 a**	**11.6 a**	**10.9**	**0.49**	**97.1**	
Pasta Control								
(P1) Spaghetti	nd	nd	nd	nd	10.1 ± 0.07	0.48 ± 0.01	97.7 ± 0.01	Met
(P2) Noodles	nd	nd	nd	nd	10.4 ± 0.06	0.47 ± 0.03	94.9 ± 0.01	Met
(P3) Pasta spirals	nd	nd	nd	nd	10.0 ± 0.05	0.50 ± 0.03	90.6 ± 0.02	Met
(P4) Noodles	nd	nd	nd	nd	10.9 ± 0.02	0.52 ± 0.02	90.3 ± 0.01	Met
(P5) Noodles	nd	nd	nd	nd	10.2 ± 0.01	0.50 ± 0.01	97.2 ± 0.01	Met
Median	**<0.003 b**	**<0.006 b**	**<0.01 b**	**<0.01 b**	**10.2**	**0.50**	**94.9**	
Crackers + Algae								
(CH) Crackers with Himanthalia	2.58 ± 0.02	2.07 ± 0.00	0.06 ± 0.00	4.71	10.4 ± 0.01	0.51 ± 0.01	97.9 ± 0.03	Lys
(CW) Crackers with Wakame	2.20 ± 0.01	0.75 ± 0.00	nd	0.75	12.3 ± 0.05	0.50 ± 0.01	97.1 ± 0.02	Lys
(CU) Crackers with Ulva	4.90 ± 0.02	nd	nd	4.90	13.2 ± 0.04	0.50 ± 0.02	97.2 ± 0.02	Lys
(CS) Crackers with Spirulina	2.21 ± 0.03	0.70 ± 0.00	nd	2.91	12.9 ± 0.03	0.50 ± 0.01	97.5 ± 0.01	Lys
(CP) Crackers with Porphyra	159 ± 2.18	0.01 ± 0.00	0.09 ± 0.00	159	24.4 ± 0.05	0.52 ± 0.02	101.3 ± 0.01	Lys
Median	**3.74 a**	**0.70 a**	**<0.02 a**	**4.71 a**	**12.9 a**	**0.50**	**97.5**	
Crackers + Control								
(C1) Brown rice crackers	nd	nd	nd	nd	7.81 ± 0.07	0.49 ± 0.02	97.1 ± 0.01	Lys
(C2) Brown rice crackers	nd	nd	nd	nd	7.67 ± 0.06	0.49 ± 0.01	97.1 ± 0.01	Lys
(C3) Salad crackers	nd	nd	nd	nd	8.01 ± 0.05	0.51 ± 0.03	96.7 ± 0.01	Lys
(C4) Crackers with olive oil	nd	nd	nd	nd	10.9 ± 0.02	0.51 ± 0.01	97.4 ± 0.01	Lys
(C5) Flaxseed. Sesame crackers	nd	nd	nd	nd	12.8 ± 0.01	0.51 ± 0.03	97.9 ± 0.01	Lys
Median	**<0.003 b**	**<0.006 b**	**<0.01 b**	**<0.01 b**	**8.00 b**	**0.51**	**97.1**	

Figure 1. Sum of Taurine, Hypotaurine and Homotaurine, (∑TAD, g / 100 g d.w). in the 26 algae species

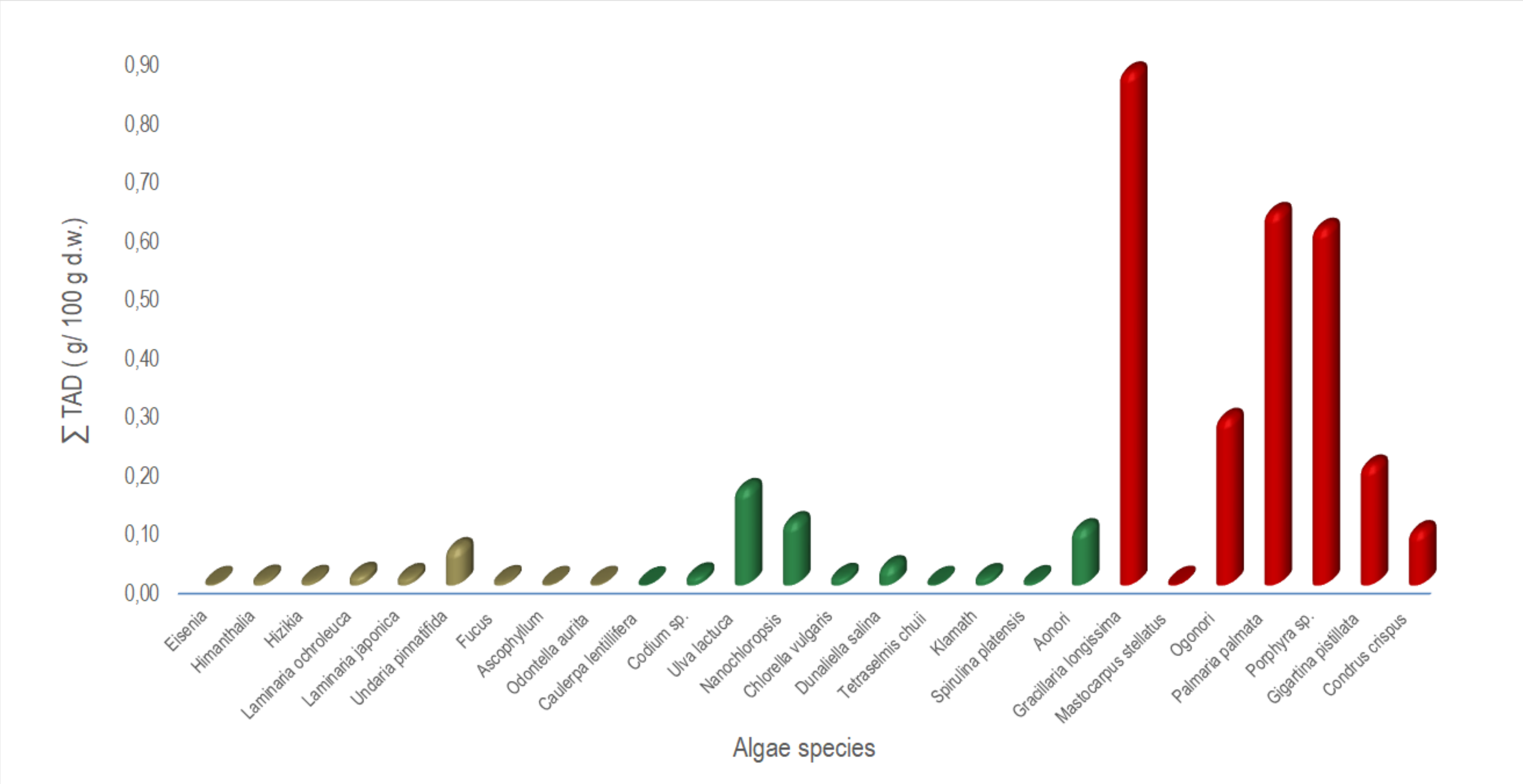

Figure 2. Percentual variations of the Essential Amino Acid Index (% Δ EAAI) and of the Essential to Non-Essential Amino Acid ratio (% Δ EAA/NEAA) for pasta products enriched with different algae species in comparison to the control samples

SS (Spaghetti with 3% seaweeds), FS (Fusilli with 4% spirulina), NW (Noodles with 2.1% wakame), NU (Noodles with 2.4% ulva), NN (Noodles with 4.4% nori).

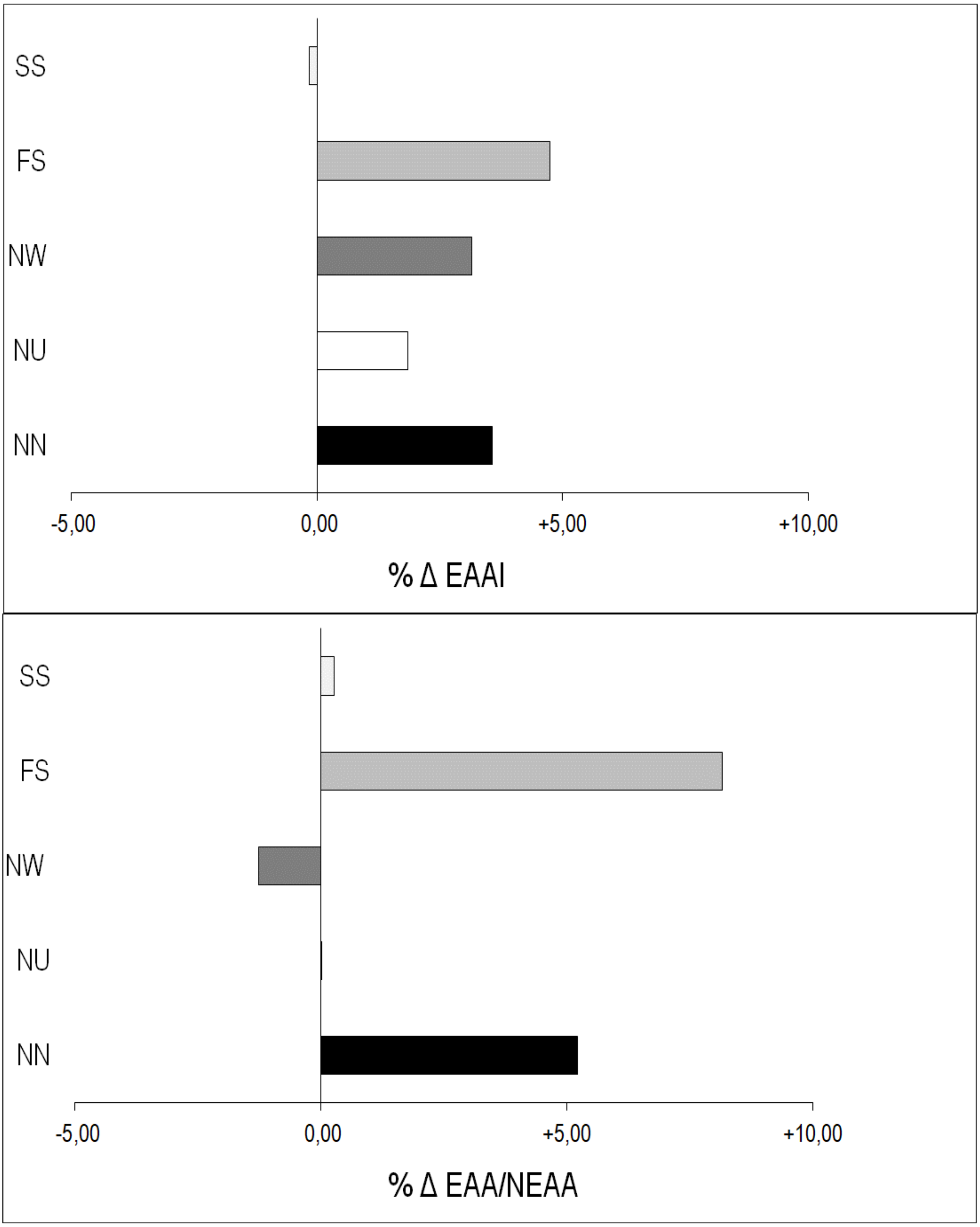

Figure 3. Percentual variations of the Essential Amino Acid Index (% Δ EAAI) and of the Essential to Non-Essential Amino Acid ratio (% Δ EAA/NEAA) for crackers products enriched with different algae species in comparison to the control samples. CN (Crackers with 62 % Nori), CS (Crackers with 2.6 % Spirulina), CU (Crackers with 1.5 % *Ulva spp*), CW (Crackers with 1.5 % Wakame), CH (Crackers with 5 % *Himanthalia elongata*).

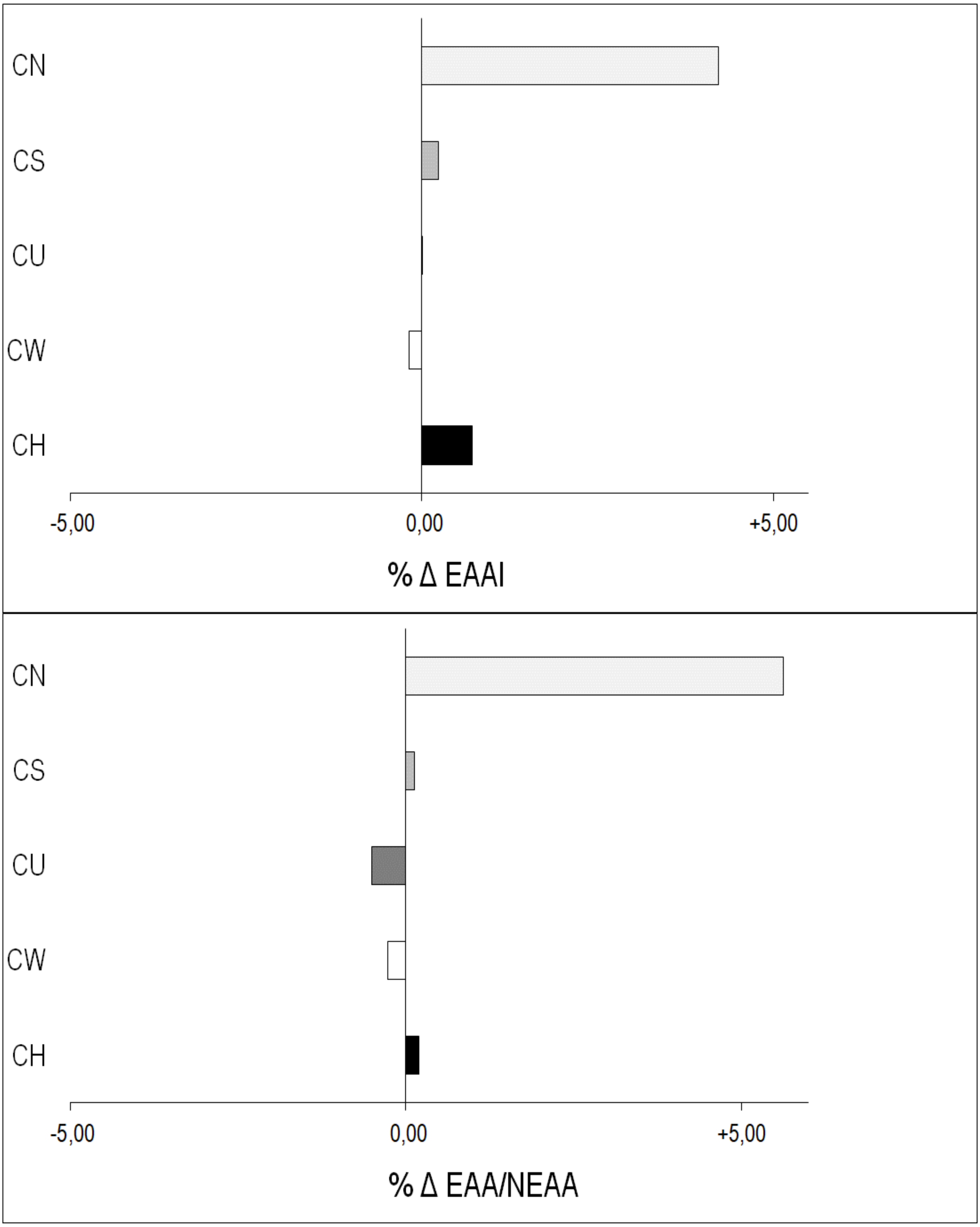

Table S1. Product information recovered from the label.

Algae sp./Composition	Sample	Product description	Form	Country	Protein Content (g/100 g d.w.)
		Algae			
Condrus crispus	Chc	Dried Irish Moss	Dried	Spain	10
Gracillaria longissima	Gl	Gracillaria	Fresh	Spain	34.2
Mastocarpus stellatus	Ms	Organic Irish Moss	Dried	Spain	10.8
Gracillaria sp.	Gsp	Red Ogonori	Fresh	Spain	not specified
Palmaria palmata	Pp	Dulse	Dried	Spain	16.9
Porphyra sp.	Po	Nori	Dried	Spain	30.5
Gigartina pistillata	Gp	Fresh Gigartina	Fresh	Spain	not specified
Eisenia byciclis	Eb	Arame	Dried	Japan	10.1
Himanthalia elongata	He	Sea Spaguetti bio	Dried	Spain	9.3
Hizikia fusiformis	Hf	Iziki seaweed	Dried	Spain	26.2
Laminaria japonica	Lj	Kombu	Dried	Spain	19.6
Laminaria ochroleuca	Lo	Kombu bio	Dried	Spain	7.1
Undaria pinnatifida	Up	Wakame	Dried	Spain	11.9
Fucus vesiculosus	Fv	Fucus	Capsules	Spain	10.4
Ascophyllum nodosum	An	Kelp	Dried	Spain	5.7
Odontella aurita	Oa	Odontella	Capsules	France	14.4
Enteromorpha intestinalis	Ei	Green Aonori	Fresh	Japan	not specified
Caulerpa lentillifera	Cl	Green Caviar	Dried	Germany	12.6
Codium sp.	Csp	Barnacle seaweed	Dried	Spain	10.9
Ulva lactuca	Ul	Sea Lettuce	Dried	Germany	26.8
Nanochloropsis	Nsp	Nanochloropsis	Dried	Spain	46.4
Chlorella vulgaris	Cv	Chlorella	Dried	Spain	54.7
Dunaliella salina	Ds	Dunaliella	Dried	Spain	18.5
Tetraselmis chuii	Tch	Holofit TetraSod	Capsules	Spain	not specified
Afanizomenon flos-aquae	Afa	Klamath eco	Dried	Spain	34.8
Spirulina platensis	Sp	Spìrulina	Dried	Spain	37.9
		Pasta			
Nori seaweed (Porphyra spp., 4.44%)	NN	Noodles with Nori	Dried	Spain	16
Sea lettuce seaweed (Ulva spp., 2.4%)	NU	Noodles with Ulva	Dried	Spain	12
Wakame seaweed (Undaria pinnatifida, 2.1%)	NW	Noodles with Wakame	Dried	Spain	12
Spirulina (4%)	FS	Fusilli with Spirulina	Dried	France	8
Spirulina (1.5%), Fucus (1.5%)	SS	Spaghetti with seaweeds	Dried	Spain	14
without algae	P1	Spaghetti	Dried	Spain	12
without algae	P2	Noodles	Dried	Spain	12
without algae	P3	Pasta spirals	Dried	Spain	12
without algae	P4	Noodles	Dried	Spain	12
without algae	P5	Noodles	Dried	Spain	12
		Crackers			
Sea spaguetti seaweed (Himanthalia elongata, 5%)	CH	Rice crackers with organic seaweed.	Dried	Spain	8.2
Oatmeal, whole wheat flour, sesame, Atlantic Wakame seaweed (Undaria pinnatifida, 1.5%)	CW	Crackers with seaweed, oatmeal and sesame	Dried	Spain	11.5
Sea lettuce seaweed (Ulva spp, 1.5%)	CU	Crackers with seaweed, tomatoe and chia seeds	Dried	Spain	14.3
Italian spirulina (2.6%)	CS	Wholemeal crackers Spirulina eco	Dried	Spain	6.5
Roasted nori seaweed (62%)	CN	Seaveg crispies	Dried	United Kingdom	30
without algae	C1	Brown rice crackers	Dried	Spain	8
without algae	C2	Brown rice crackers	Dried	Spain	7
without algae	C3	Salad crackers	Dried	Belgium	7.8
without algae	C4	Crackers with olive oil	Dried	Spain	11
without algae	C5	Flaxseed, Sesame and Quinoa crackers	Dried	Spain	13.2

Table S2. Results for precision (RSD_r%) in pasta and cracker matrices at three different spiking concentrations (0.1 mM, 5 mM and 10 mM).

	RSD$_r$ (%) n=6							
	Pasta				Crackers			
Amino acid	0.1 mM	5 mM	10 mM	Mean	0.1 mM	5 mM	10 mM	Mean
Hyptau	1.30	2.01	1.88	1.73	3.71	2.36	3.27	3.11
Tau	2.40	1.93	2.03	2.12	3.91	1.57	2.52	2.67
Htau	3.42	2.08	4.45	3.32	1.15	2.19	2.28	1.87
His	3.01	2.90	4.48	3.46	2.27	2.54	2.75	2.52
Arg	3.10	1.90	3.01	2.67	3.45	1.05	2.46	2.32
Thr	3.80	3.07	1.83	2.90	3.88	1.03	2.24	2.38
Lys	2.70	2.56	3.09	2.78	1.47	2.50	1.35	1.77
Met	2.01	2.62	3.51	2.71	1.89	3.29	3.96	3.05
Val	2.84	3.18	1.23	2.42	1.76	2.81	1.45	2.01
Ileu	2.94	3.58	4.29	3.60	3.21	2.84	2.46	2.84
Leu	2.57	2.72	3.98	3.09	4.23	3.56	1.61	3.13
Phe	2.22	2.27	3.09	2.53	3.90	2.22	1.12	2.41
Hyp	4.02	2.85	2.32	3.06	4.31	4.02	4.03	4.12
Ser	3.51	1.15	3.54	2.73	3.74	2.15	2.23	2.71
Gly	1.19	1.22	1.29	1.23	2.86	2.87	4.46	3.40
Asp	3.65	3.04	1.05	2.58	1.54	2.34	3.44	2.44
Glu	4.12	2.32	1.64	2.69	2,00	3.38	2.22	2.53
Cys	3.22	1.58	2.23	2.34	1.45	3.12	1.19	1.92
Pro	3.34	4.34	3.62	3.77	1.35	4.32	1.92	2.53
Ala	1.33	2.44	1.44	1.74	3.62	2.95	2.93	3.17
GABA	3.96	2.36	1.05	2.46	2.59	3.50	2.97	3.02
Tyr	2.96	4.04	2.95	3.32	1.37	3.01	2.84	2.41

Table S3. Results for intermediate precision (RSD_R%) in pasta and cracker matrices at three different spiking concentrations (0.1 mM, 5 mM and 10 mM)

	RSD_R (%) n=6							
	Pasta				Crackers			
Amino acid	0.1 mM	5 mM	10 mM	Mean	0.1 mM	5 mM	10 mM	Mean
Hyptau	1.58	4.21	1.53	2.44	3.45	1.91	4.46	3.27
Tau	4.29	3.51	2.41	3.40	3.94	3.22	3.52	3.56
Htau	1.95	4.15	2.18	2.76	2.60	1.67	3.78	2.68
His	3.21	1.73	3.82	2.92	2.01	4.12	2.71	2.95
Arg	4.02	4.44	4.21	4.22	3.70	2.32	1.01	2.34
Thr	2.19	3.85	3.52	3.19	1.17	3.22	1.31	1.90
Lys	4.05	2.74	2.59	3.13	2.28	1.40	1.55	1.74
Met	1.84	1.27	3.99	2.37	4.36	3.77	4.01	4.05
Val	3.99	2.70	4.25	3.65	4.37	1.22	4.20	3.26
Ileu	3.08	4.23	4.01	3.77	2.36	2.43	1.35	2.05
Leu	3.65	3.87	3.87	3.80	2.44	2.32	2.08	2.28
Phe	2.69	2.38	2.44	2.50	3.97	1.66	3.42	3.02
Hyp	4.41	3.56	3.99	3.99	3.40	3.34	3.33	3.36
Ser	2.06	2.55	2.98	2.53	3.35	3.29	2.35	3,00
Gly	3.86	1.19	3.84	2.96	3.32	3.77	3.89	3.66
Asp	3.76	1.02	3.61	2.80	2.13	3.72	1.21	2.35
Glu	3.74	2.75	3,00	3.16	4.23	3.10	1.71	3.01
Cys	1.86	2.03	2.94	2.28	3.94	4.40	2.79	3.71
Pro	3.92	2.08	2.54	2.85	3.09	2.48	4.21	3.26
Ala	2.77	1.73	2.84	2.45	2.44	1.45	1.58	1.82
GABA	4.19	1.47	1.67	2.44	1.12	1.83	2.55	1.83
Tyr	3.18	4.47	1.34	3,00	3.79	1.31	1.35	2.15

Table S4. **Recovery** values in pasta and cracker matrices (mean ± standard deviation, n=3) at 3 different spiking concentrations (0.05 mM, 0.5 mM and 2.5 mM).

	Recovery (%) n=6							
	Pasta				Crackers			
Amino acid	0,05 mM	0,5 mM	2,5 mM	Mean	0,05 mM	0,5 mM	2,5 mM	Mean
Hyptau	92.4 ± 0.81	105 ± 1.70	99.9 ± 0.42	102	95.2 ± 1.43	91.2 ± 0.55	97.5 ± 1.45	94.6
Tau	93.2 ± 0.52	103 ± 2.45	91.4 ± 0.99	95.9	106 ± 1.04	97.1 ± 1.28	92.8 ± 0.73	98.6
Htau	90.1 ± 2.25	95.9 ± 1.52	91.2 ± 0.90	92.4	93.5 ± 0.82	88.2 ± 0.77	101 ± 1.26	94.2
His	92.1 ± 0.91	93.0 ± 2.41	95.1 ± 2.41	93.4	96.0 ± 1.61	97.6 ± 0.23	94.7 ± 0.12	96.1
Arg	90.1 ± 1.01	91.4 ± 1.38	94.2 ± 0.21	91.9	102 ± 1.11	93.0 ± 2.16	101 ± 1.94	98.7
Thr	96.4 ± 1.65	94.5 ± 0.19	93.9 ± 0.75	94.9	103 ± 1.92	99.5 ± 0.15	102 ± 2.05	102
Lys	85.5 ± 0.16	98.2 ± 1.28	100 ± 2.58	94.6	97.3 ± 0.68	104 ± 1.34	98.5 ± 2.32	99.9
Met	93.9 ± 0.92	92.5 ± 1.47	93.8 ± 2.56	93.4	87.9 ± 2.13	104 ± 2.32	91.8 ± 2.23	94.6
Val	95.8 ± 0.88	101 ± 1.95	100 ± 0.64	98.9	88.0 ± 2.58	96.2 ± 0.39	94.5 ± 2.35	92.9
Ileu	90.8 ± 1.22	95.1 ± 2.54	92.3 ± 1.22	92.7	105 ± 0.92	102 ± 2.15	102 ± 0.68	103
Leu	90.8 ± 2.01	104 ± 0.25	96.4 ± 1.44	97.1	85.5 ± 1.83	89.9 ± 2.42	102 ± 1.84	92.5
Phe	94.0 ± 1.05	100 ± 0.22	99.7 ± 0.14	97.9	93.4 ± 1.54	100 ± 0.11	94.4 ± 1.35	95.9
Hyp	93.1 ± 0.62	93.4 ± 1.27	95.0 ± 1.57	93.8	104 ± 1.02	87.1 ± 0.12	92.6 ± 0.83	94.6
Ser	107 ± 1.95	102 ± 0.57	101 ± 2.42	103	88.2 ± 1.75	100 ± 0.90	101 ± 2.02	96.4
Gly	102 ± 1.23	100 ± 0.43	101 ± 2.42	101	90.9 ± 2.43	100 ± 0.82	103 ± 1.94	98.0
Asp	88.7 ± 0.22	92.0 ± 1.59	99.4 ± 1.82	93.4	86.1 ± 2.08	103 ± 0.81	96.2 ± 0.25	95.1
Glu	91.5 ± 1.11	95.9 ± 0.52	93.2 ± 0.97	93.5	98.8 ± 0.11	96.1 ± 0.82	98.5 ± 0.82	97.8
Cys	86.9 ± 1.20	101 ± 2.00	100 ± 1.83	96.0	102 ± 0.19	94.8 ± 1.90	101 ± 0.52	99.3
Pro	102 ± 0.52	87.1 ± 1.01	97.3 ± 0.62	95.9	97.2 ± 0.10	91.5 ± 1.63	99.0 ± 0.94	95.9
Ala	102 ± 0.85	90.1 ± 0.62	94.0 ± 2.54	95.4	103 ± 1.32	102 ± 2.16	92.3 ± 1.35	99.1
GABA	95.3 ± 1.52	101 ± 1.82	91.9 ± 0.82	96.1	97.9 ± 0.55	90.7 ± 1.25	96.7 ± 0.52	95.1
Tyr	85.5 ± 0.98	93.8 ± 1.83	97.1 ± 1.59	92.1	98.9 ± 0.53	93.3 ± 2.12	97.9 ± 1.01	96.7

Table S5. Linearity, explained by regression equation and correlation coefficient (R^2), limit of detection (LOD) and limit of quantification (LOD) expressed as mg/Kg d.w. in pasta and cracker matrices.

*Terriente et al. 2019

Amino acids	Regression equation*	R^2*	Pasta / Crackers MLOD	Pasta / Crackers MLOQ
			mg / kg d.w.	
Hyptau	$y = 7.2 * 10^2 x - 10.6$	0.999	0.11	0.29
Tau	$y = 2.3 * 10^2 x - 27.0$	0.999	0.03	0.12
Htau	$y = 4.8 * 10^2 x - 74.6$	0.999	0.06	0.13
His	$y = 2.7 * 10^2 x - 40.3$	0.999	0.04	0.14
Arg	$y = 6.1 * 10^2 x - 21.0$	0.999	0.04	0.13
Thr	$y = 7.2 * 10^2 x - 11.6$	0.999	0.03	0.11
Lys	$y = 4.3 * 10^2 x - 11.4$	0.999	0.04	0.15
Met	$y = 8.2 * 10^2 x - 12.3$	0.998	0.02	0.08
Val	$y = 9.9 * 10^2 x - 53.4$	0.999	0.02	0.07
Ileu	$y = 1.3 * 10^2 x - 20.3$	0.998	0.09	0.30
Leu	$y = 1.1 * 10^2 x - 10.4$	0.999	0.07	0.23
Phe	$y = 8.7 * 10^2 x - 58.4$	0.999	0.09	0.28
Hyp	$y = 4.1 * 10^2 x - 22.2$	0.998	0.04	0.11
Ser	$y = 1.3 * 10^2 x - 75.6$	0.999	0.02	0.08
Gly	$y = 2.2 * 10^2 x - 32.4$	0.999	0.01	0.04
Asp	$y = 8.7 * 10^2 x - 14.5$	0.999	0.03	0.11
Glu	$y = 7.0 * 10^2 x - 26.4$	0.999	0.02	0.08
Cys	$y = 4.7 * 10^2 x - 10.1$	0.998	0.06	0.21
Pro	$y = 10.4 * 10^2 x - 32.3$	0.999	0.02	0.08
Ala	$y = 6.6 * 10^2 x - 55.4$	0.998	0.03	0.12
GABA	$y = 4.0 * 10^2 x - 42.7$	0.999	0.04	0.13
Tyr	$y = 1.4 * 10^2 x - 41.4$	0.999	0.04	0.09

Table S6. Protein content (g/100 g d.w.) and Essential (EAA) and non-essential (NEEA) amino acids (g/100 g protein) in the 26 algae species. Chc (*Chondrus crispus*); Gl (*Gracilaria longissima*); Ms (*Mastocarpus stellatus*); Gsp (*Gracilaria sp*); Eb (*Eisenia biciclys*); He (*Himanthalia elongate*); Hf (*Hizikia fusiformis*); Lj (*Laminaria japonica*); Ei (*Enteromorpha intestinalis*); Cl (*Caulerpa lentillifera*); Csp (*Codium sp*); Ul (*Ulvalactuca*); Nsp (*Nanochloropsis sp*).

	Red algae				**Brown algae**				**Green algae**				
	Chc	Gl	Ms	Gsp	Eb	He	Hf	Lj	Ei	Cl	Csp	Ul	Nsp
Protein	12.8 ± 0.03	34.6 ± 0.07	10.9 ± 0.01	35.1 ± 0.06	10.3 ± 0.01	11.0 ± 0.06	22.5 ± 0.05	22.8 ± 0.02	9.28 ± 0.05	13.1 ± 0.04	11.4 ± 0.05	24.1 ± 0.02	40.0 ± 0.02
His	1.98 ± 0.03	4.09 ± 0.05	2.29 ± 0.03	1.27 ± 0.05	2.35 ± 0.01	10.5 ± 0.04	1.97 ± 0.01	4.20 ± 0.04	2.76 ± 0.02	2.03 ± 0.01	3.75 ± 0.02	3.15 ± 0.03	1.02 ± 0.02
Arg	5.98 ± 0.03	9.70 ± 0.05	4.01 ± 0.03	11.0 ± 0.05	5.26 ± 0.01	1.86 ± 0.04	7.83 ± 0.01	5.09 ± 0.04	4.16 ± 0.02	3.06 ± 0.01	4.11 ± 0.02	7.03 ± 0.03	2.12 ± 0.02
Thr	3.30 ± 0.03	8.12 ± 0.02	4.48 ± 0.02	4.35 ± 0.03	5.38 ± 0.01	3.72 ± 0.03	4.99 ± 0.01	4.85 ± 0.01	3.29 ± 0.02	2.42 ± 0.01	2.92 ± 0.02	8.45 ± 0.01	3.59 ± 0.02
Lys	17.7 ± 0.03	5.15 ± 0.02	13.0 ± 0.02	2.96 ± 0.03	3.21 ± 0.02	7.97 ± 0.03	5.87 ± 0.01	4.46 ± 0.01	7.62 ± 0.02	0.74 ± 0.01	6.31 ± 0.02	8.82 ± 0.01	5.44 ± 0.02
Met	0.43 ± 0.03	1.20 ± 0.02	0.89 ± 0.02	14.7 ± 0.03	5.54 ± 0.01	0.86 ± 0.03	4.68 ± 0.01	0.89 ± 0.01	2.69 ± 0.02	1.98 ± 0.01	8.61 ± 0.02	1.70 ± 0.01	0.22 ± 0.02
Val	6.72 ± 0.03	6.68 ± 0.02	4.77 ± 0.02	3.76 ± 0.03	4.82 ± 0.01	6.72 ± 0.03	5.41 ± 0.01	2.16 ± 0.01	12.1 ± 0.02	8.94 ± 0.01	14.7 ± 0.02	6.53 ± 0.01	11.8 ± 0.02
Ileu	9.12 ± 0.03	5.53 ± 0.02	4.48 ± 0.02	11.9 ± 0.03	3.77 ± 0.01	1.65 ± 0.03	4.17 ± 0.01	4.84 ± 0.01	7.08 ± 0.02	5.21 ± 0.01	5.09 ± 0.02	5.20 ± 0.01	14.2 ± 0.02
Leu	9.67 ± 0.03	10.2 ± 0.02	6.35 ± 0.02	1.12 ± 0.03	7.89 ± 0.01	3.69 ± 0.03	12.3 ± 0.01	6.24 ± 0.01	11.3 ± 0.02	8.33 ± 0.01	8.19 ± 0.02	9.74 ± 0.01	6.23 ± 0.02
Phe	5.79 ± 0.03	8.09 ± 0.02	5.07 ± 0.02	4.18 ± 0.03	4.07 ± 0.01	1.59 ± 0.03	4.43 ± 0.01	0.27 ± 0.01	6.99 ± 0.02	5.15 ± 0.01	5.39 ± 0.02	5.43 ± 0.01	0.64 ± 0.02
Hyp	0.30 ± 0.03	1.47 ± 0.05	0.66 ± 0.03	0.18 ± 0.05	0.14 ± 0.01	1.85 ± 0.04	0.35 ± 0.01	0.51 ± 0.04	0.18 ± 0.02	0.24 ± 0.01	0.31 ± 0.02	3.28 ± 0.03	0.10 ± 0.02
Ser	11.6 ± 0.03	4.85 ± 0.02	4.89 ± 0.02	5.52 ± 0.03	4.62 ± 0.01	6.48 ± 0.03	3.68 ± 0.01	3.15 ± 0.01	4.92 ± 0.02	6.41 ± 0.01	4.04 ± 0.02	23.5 ± 0.01	12.7 ± 0.02
Gly	13.3 ± 0.03	5.80 ± 0.02	8.44 ± 0.02	24.7 ± 0.03	5.29 ± 0.01	4.00 ± 0.03	6.43 ± 0.01	12.4 ± 0.01	5.60 ± 0.02	7.37 ± 0.01	6.62 ± 0.02	5.57 ± 0.01	9.18 ± 0.02
Asp	7.59 ± 0.03	8.80 ± 0.02	10.5 ± 0.02	4.16 ± 0.03	9.78 ± 0.01	17.5 ± 0.03	15.5 ± 0.01	18.7 ± 0.01	8.65 ± 0.02	17.6 ± 0.01	5.71 ± 0.02	8.18 ± 0.01	10.5 ± 0.02
Glu	10.5 ± 0.03	9.18 ± 0.02	8.95 ± 0.02	8.83 ± 0.03	21.0 ± 0.01	10.0 ± 0.03	23.3 ± 0.01	21.5 ± 0.01	15.5 ± 0.02	17.2 ± 0.01	14.72 ± 0.02	6.51 ± 0.01	13.7 ± 0.02
Cys	9.18 ± 0.03	0.62 ± 0.02	3.50 ± 0.02	2.63 ± 0.03	6.71 ± 0.01	3.83 ± 0.03	3.52 ± 0.01	2.39 ± 0.01	8.35 ± 0.01	0.29 ± 0.01	1.07 ± 0.02	1.49 ± 0.01	1.83 ± 0.02
Pro	8.06 ± 0.03	5.01 ± 0.02	4.87 ± 0.02	6.21 ± 0.03	3.25 ± 0.01	2.18 ± 0.03	4.15 ± 0.01	10.0 ± 0.01	10.8 ± 0.02	8.22 ± 0.01	4.92 ± 0.02	4.85 ± 0.01	5.95 ± 0.02
Ala	3.49 ± 0.02	5.84 ± 0.02	8.31 ± 0.02	9.73 ± 0.03	6.25 ± 0.01	16.8 ± 0.03	9.78 ± 0.01	9.38 ± 0.01	10.0 ± 0.02	11.5 ± 0.01	5.83 ± 0.02	5.68 ± 0.01	11.6 ± 0.02
GABA	3.39 ± 0.03	1.41 ± 0.02	15.6 ± 0.02	6.01 ± 0.03	0.54 ± 0.01	3.85 ± 0.03	1.85 ± 0.01	1.12 ± 0.01	0.75 ± 0.02	1.09 ± 0.01	1.78 ± 0.02	1.38 ± 0.01	1.10 ± 0.02
Tyr	2.58 ± 0.03	6.10 ± 0.02	3.59 ± 0.02	10.8 ± 0.03	2.06 ± 0.01	2.45 ± 0.03	2.08 ± 0.01	1.70 ± 0.01	4.89 ± 0.04	4.36 ± 0.01	4.03 ± 0.02	5.17 ± 0.01	4.10 ± 0.02
Total Aas	131	108	115	134	102	108	122	114	128	112	108	122	116

Table S6 (continued). Pp (*Palmaria palmata*); Po (*Porphyra sp*); Gp (*Gigartina pistillata*); Lo (*Laminaria ochroleuca*); Up (*Undaria pinnatifida*); Fv (*Fucus vesiculosus*); An (*Ascophyllum nodosum*); Oa (*Odontella aurita*); Cv (*Chlorella vulgaris*); Ds (*Dunaliella salina*); Tch (*Tetraselmis chuii*); Afa (*Afanizonemon flos-aquae*); Sp (Spirulina pacifica).

	Red algae			**Brown algae**					**Green algae**				
	Pp	Po	Gp	Lo	Up	Fv	An	Oa	Cv	Ds	Tch	Afa	Sp
Protein	17.8 ± 0.02	31.0 ± 0.07	28.1 ± 0.01	6.90 ± 0.01	13.4 ± 0.03	10.6 ± 0.02	8.80 ± 0.03	15.1 ± 0.01	52.2 ± 0.03	17.0 ± 0.05	13.4 ± 0.03	36.4 ± 0.04	31.7 ± 0.08
His	1.62 ± 0.04	2.19 ± 0.05	1.69 ± 0.03	2.15 ± 0.01	1.85 ± 0.01	2.17 ± 0.02	4.07 ± 0.02	1.04 ± 0.02	2.58 ± 0.02	1.31 ± 0.04	5.03 ± 0.02	1.67 ± 0.01	1.54 ± 0.01
Arg	6.15 ± 0.04	7.27 ± 0.05	7.88 ± 0.03	1.66 ± 0.01	10.0 ± 0.01	3.63 ± 0.02	2.43 ± 0.02	1.21 ± 0.02	8.17 ± 0.02	0.94 ± 0.04	3.70 ± 0.02	7.87 ± 0.01	4.73 ± 0.01
Thr	5.37 ± 0.02	6.23 ± 0.02	10.4 ± 0.02	0.95 ± 0.01	12.5 ± 0.03	4.64 ± 0.04	7.48 ± 0.03	4.54 ± 0.02	4.20 ± 0.02	3.54 ± 0.04	5.18 ± 0.02	7.12 ± 0.01	1.72 ± 0.01
Lys	4.51 ± 0.02	5.68 ± 0.02	13.5 ± 0.02	1.69 ± 0.01	10.0 ± 0.03	11.8 ± 0.04	12.3 ± 0.03	17.7 ± 0.02	7.88 ± 0.02	5.79 ± 0.04	6.91 ± 0.02	8.06 ± 0.01	4.60 ± 0.01
Met	0.89 ± 0.02	1.93 ± 0.02	1.73 ± 0.02	0.12 ± 0.01	1.32 ± 0.03	0.42 ± 0.04	0.97 ± 0.03	0.37 ± 0.02	3.53 ± 0.02	0.51 ± 0.04	1.80 ± 0.02	1.33 ± 0.01	1.52 ± 0.01
Val	5.42 ± 0.02	6.53 ± 0.02	14.7 ± 0.02	3.04 ± 0.01	3.94 ± 0.03	4.79 ± 0.04	7.73 ± 0.03	4.00 ± 0.02	5.38 ± 0.02	3.97 ± 0.04	10.3 ± 0.02	2.07 ± 0.01	4.56 ± 0.01
Ileu	3.51 ± 0.02	4.38 ± 0.02	5.24 ± 0.02	1.81 ± 0.01	7.52 ± 0.03	2.26 ± 0.04	5.13 ± 0.03	21.9 ± 0.02	3.52 ± 0.02	18.0 ± 0.04	5.37 ± 0.02	6.95 ± 0.01	3.80 ± 0.01
Leu	6.17 ± 0.02	6.90 ± 0.02	7.89 ± 0.02	3.26 ± 0.01	1.88 ± 0.03	3.88 ± 0.04	6.39 ± 0.03	15.0 ± 0.02	8.56 ± 0.02	14.4 ± 0.04	9.73 ± 0.02	8.96 ± 0.01	8.55 ± 0.01
Phe	5.18 ± 0.02	3.12 ± 0.02	5.90 ± 0.02	0.46 ± 0.01	4.57 ± 0.03	1.48 ± 0.04	2.85 ± 0.03	5.13 ± 0.02	5.08 ± 0.02	3.67 ± 0.04	0.43 ± 0.02	6.88 ± 0.01	5.55 ± 0.01
Hyp	0.31 ± 0.04	0.05 ± 0.03	1.85 ± 0.01	0.34 ± 0.05	0.19 ± 0.01	0.64 ± 0.02	0.15 ± 0.02	0.36 ± 0.02	0.58 ± 0.02	0.16 ± 0.04	1.35 ± 0.02	0.07 ± 0.01	1.54 ± 0.01
Ser	12.7 ± 0.02	17.6 ± 0.01	5.03 ± 0.04	6.76 ± 0.02	14.5 ± 0.03	5.14 ± 0.04	4.83 ± 0.03	2.80 ± 0.02	21.4 ± 0.02	5.73 ± 0.04	6.01 ± 0.02	18.8 ± 0.01	22.5 ± 0.01
Gly	11.8 ± 0.02	10.6 ± 0.01	4.96 ± 0.04	5.70 ± 0.02	7.57 ± 0.03	10.2 ± 0.04	4.28 ± 0.03	17.7 ± 0.02	7.18 ± 0.02	7.65 ± 0.04	6.58 ± 0.02	6.60 ± 0.01	6.29 ± 0.01
Asp	12.1 ± 0.02	6.40 ± 0.01	7.14 ± 0.04	2.60 ± 0.02	9.90 ± 0.03	13.1 ± 0.04	9.64 ± 0.03	3.22 ± 0.02	9.53 ± 0.02	9.83 ± 0.04	10.8 ± 0.02	9.87 ± 0.01	11.0 ± 0.01
Glu	10.2 ± 0.02	14.3 ± 0.01	11.4 ± 0.04	2.14 ± 0.02	11.2 ± 0.03	12.4 ± 0.04	15.4 ± 0.03	9.05 ± 0.02	11.1 ± 0.02	12.3 ± 0.04	12.1 ± 0.02	14.6 ± 0.01	16.9 ± 0.01
Cys	3.72 ± 0.02	2.40 ± 0.01	2.40 ± 0.04	1.98 ± 0.02	1.36 ± 0.03	1.13 ± 0.04	1.02 ± 0.03	0.12 ± 0.02	1.64 ± 0.02	3.09 ± 0.04	1.06 ± 0.02	0.42 ± 0.01	1.70 ± 0.01
Pro	8.56 ± 0.02	4.72 ± 0.01	12.0 ± 0.04	4.31 ± 0.02	3.06 ± 0.03	7.61 ± 0.04	5.47 ± 0.03	11.3 ± 0.02	5.81 ± 0.02	7.38 ± 0.04	5.78 ± 0.02	6.49 ± 0.01	5.06 ± 0.01
Ala	7.53 ± 0.02	4.87 ± 0.01	15.5 ± 0.04	5.19 ± 0.02	3.97 ± 0.03	10.4 ± 0.04	5.61 ± 0.03	1.38 ± 0.02	8.18 ± 0.02	6.88 ± 0.04	10.7 ± 0.02	8.76 ± 0.01	8.64 ± 0.01
GABA	0.87 ± 0.02	2.54 ± 0.01	2.37 ± 0.04	0.76 ± 0.02	1.83 ± 0.03	0.74 ± 0.04	3.39 ± 0.03	10.4 ± 0.02	3.60 ± 0.02	1.19 ± 0.04	2.59 ± 0.02	0.51 ± 0.01	0.98 ± 0.01
Tyr	1.58 ± 0.02	5.28 ± 0.01	0.45 ± 0.04	0.42 ± 0.02	0.93 ± 0.03	2.18 ± 0.04	1.71 ± 0.03	2.92 ± 0.02	3.76 ± 0.02	1.31 ± 0.04	4.38 ± 0.02	0.71 ± 0.01	3.27 ± 0.01
Total Aas	108	113	130	45	108	99	101	131	122	108	110	118	115

Table S7. **Essential amino acids in pasta and crackers samples with and without algae addition.**

Product	His	Arg	Thr	Lys	Met	Val	Ileu	Leu	Phe
mg / 100 g					**Pasta**				
Noodles with Porphyra (NN)	0.16 ± 0.01	0.53 ± 0.01	0.31 ± 0.02	0.35 ± 0.02	0.05 ± 0.01	0.48 ± 0.01	0.38 ± 0.01	0.82 ± 0.01	0.54 ± 0.02
Noodles with Ulva (NU)	0.15 ± 0.02	0.52 ± 0.02	0.31 ± 0.01	0.33 ± 0.01	0.05 ± 0.01	0.47 ± 0.02	0.38 ± 0.01	0.83 ± 0.02	0.54 ± 0.02
Noodles with Wakame (NW)	0.14 ± 0.01	0.53 ± 0.01	0.32 ± 0.01	0.34 ± 0.01	0.06 ± 0.01	0.48 ± 0.01	0.38 ± 0.01	0.82 ± 0.01	0.54 ± 0.01
Fusilli with Spirulina (FS)	0.11 ± 0.02	0.53 ± 0.03	0.32 ± 0.03	0.32 ± 0.03	0.06 ± 0.03	0.48 ± 0.03	0.38 ± 0.03	0.95 ± 0.03	0.64 ± 0.01
Spaghetti with seaweeds (SS)	0.14 ± 0.02	0.52 ± 0.02	0.31 ± 0.02	0.33 ± 0.02	0.05 ± 0.02	0.47 ± 0.02	0.38 ± 0.02	0.82 ± 0.02	0.54 ± 0.02
Spaguetti (P1)	0.14 ± 0.05	0.53 ± 0.05	0.31 ± 0.02	0.34 ± 0.02	0.06 ± 0.02	0.48 ± 0.02	0.39 ± 0.02	0.81 ± 0.02	0.53 ± 0.01
Noodles (P2)	0.13 ± 0.04	0.55 ± 0.04	0.31 ± 0.04	0.34 ± 0.03	0.05 ± 0.03	0.50 ± 0.03	0.38 ± 0.01	0.81 ± 0.03	0.54 ± 0.03
Pasta Spirals (P3)	0.12 ± 0.01	0.51 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.04 ± 0.03	0.45 ± 0.01	0.35 ± 0.01	0.82 ± 0.01	0.55 ± 0.01
Noodles (P4)	0.15 ± 0.04	0.51 ± 0.04	0.30 ± 0.01	0.33 ± 0.01	0.03 ± 0.01	0.47 ± 0.01	0.37 ± 0.01	0.84 ± 0.01	0.55 ± 0.01
Noodles (P5)	0.14 ± 0.05	0.52 ± 0.05	0.31 ± 0.02	0.32 ± 0.02	0.07 ± 0.02	0.45 ± 0.02	0.40 ± 0.02	0.83 ± 0.02	0.53 ± 0.01
mg / 100 g					**Crakers**				
Crackers Himanthalia (CH)	0.25 ± 0.01	0.38 ± 0.01	0.29 ± 0.01	0.22 ± 0.01	0.16 ± 0.01	0.44 ± 0.01	0.33 ± 0.01	0.66 ± 0.01	0.48 ± 0.01
Crackers Wakame (CW)	0.24 ± 0.01	0.38 ± 0.02	0.29 ± 0.02	0.19 ± 0.02	0.16 ± 0.02	0.44 ± 0.01	0.34 ± 0.02	0.66 ± 0.02	0.48 ± 0.03
Crackers Ulva (CU)	0.24 ± 0.01	0.38 ± 0.01	0.28 ± 0.02	0.21 ± 0.01	0.16 ± 0.01	0.44 ± 0.01	0.33 ± 0.01	0.66 ± 0.01	0.47 ± 0.01
Crackers Spirulina (CS)	0.24 ± 0.03	0.38 ± 0.03	0.28 ± 0.03	0.21 ± 0.03	0.16 ± 0.03	0.44 ± 0.03	0.34 ± 0.03	0.66 ± 0.03	0.48 ± 0.03
Crackers Nori (CN)	0.74 ± 0.02	0.79 ± 0.02	0.33 ± 0.02	0.52 ± 0.02	0.36 ± 0.02	1.76 ± 0.02	0.74 ± 0.02	1.16 ± 0.02	1.88 ± 0.02
Brown rice crackers (C1)	0.24 ± 0.05	0.38 ± 0.04	0.29 ± 0.02	0.19 ± 0.02	0.16 ± 0.02	0.44 ± 0.02	0.34 ± 0.02	0.66 ± 0.02	0.48 ± 0.02
Brown rice crackers (C2)	0.24 ± 0.04	0.39 ± 0.01	0.28 ± 0.03	0.19 ± 0.03	0.15 ± 0.03	0.44 ± 0.03	0.34 ± 0.02	0.65 ± 0.03	0.48 ± 0.02
Salad crackers (C3)	0.24 ± 0.01	0.38 ± 0.02	0.28 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.44 ± 0.01	0.33 ± 0.01	0.66 ± 0.01	0.48 ± 0.01
Crackers with olive oil (C4)	0.24 ± 0.04	0.38 ± 0.04	0.29 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.44 ± 0.01	0.34 ± 0.01	0.66 ± 0.01	0.48 ± 0.01
Crackers sesame and quinoa (C5)	0.24 ± 0.05	0.38 ± 0.05	0.29 ± 0.02	0.19 ± 0.02	0.16 ± 0.02	0.44 ± 0.02	0.34 ± 0.02	0.67 ± 0.02	0.49 ± 0.02

Table S8. Non-essential amino acids in pasta and crackers samples with and without algae addition.

Product	Hyp	Ser	Gly	Asp	Glu	Cys	Pro	Ala	GABA	Tyr
mg / 100 g					**Pasta**					
Noodles with Nori (NN)	0.13 ± 0.01	0.51 ± 0.01	0.41 ± 0.01	0.47 ± 0.01	3.16 ± 0.01	0.09 ± 0.01	1.24 ± 0.01	0.35 ± 0.01	0.80 ± 0.01	0.26 ± 0.01
Noodles with Ulva (NU)	0.13 ± 0.01	0.51 ± 0.02	0.42 ± 0.02	0.48 ± 0.02	3.25 ± 0.02	0.09 ± 0.01	1.23 ± 0.02	0.35 ± 0.02	0.81 ± 0.02	0.26 ± 0.02
Noodles with Wakame (NW)	0.13 ± 0.01	0.51 ± 0.02	0.41 ± 0.01	0.41 ± 0.01	3.15 ± 0.01	0.09 ± 0.00	1.23 ± 0.01	0.35 ± 0.01	0.81 ± 0.01	0.26 ± 0.01
Fusilli with Spirulina (FS)	0.13 ± 0.02	0.52 ± 0.03	0.41 ± 0.03	0.50 ± 0.03	3.28 ± 0.03	0.09 ± 0.01	1.24 ± 0.03	0.35 ± 0.03	0.81 ± 0.02	0.26 ± 0.01
Spaghetti with seaweeds (SS)	0.13 ± 0.01	0.51 ± 0.01	0.40 ± 0.02	0.47 ± 0.02	3.18 ± 0.02	0.09 ± 0.00	1.23 ± 0.02	0.35 ± 0.02	0.81 ± 0.01	0.26 ± 0.00
Spaguetti (P1)	0.12 ± 0.01	0.50 ± 0.01	0.41 ± 0.02	0.47 ± 0.02	3.33 ± 0.02	0.09 ± 0.02	1.19 ± 0.02	0.35 ± 0.02	0.88 ± 0.02	0.27 ± 0.02
Noodles (P2)	0.13 ± 0.03	0.50 ± 0.02	0.40 ± 0.03	0.46 ± 0.03	3.44 ± 0.03	0.09 ± 0.00	1.25 ± 0.03	0.35 ± 0.03	0.82 ± 0.03	0.26 ± 0.03
Pasta Spirals (P3)	0.11 ± 0.01	0.51 ± 0.01	0.41 ± 0.01	0.48 ± 0.01	2.90 ± 0.01	0.10 ± 0.01	1.25 ± 0.01	0.33 ± 0.01	0.81 ± 0.01	0.25 ± 0.01
Noodles (P4)	0.14 ± 0.04	0.51 ± 0.01	0.41 ± 0.01	0.46 ± 0.01	2.66 ± 0.01	0.10 ± 0.01	1.28 ± 0.01	0.33 ± 0.01	0.75 ± 0.01	0.29 ± 0.01
Noodles (P5)	0.14 ± 0.01	0.51 ± 0.02	0.40 ± 0.02	0.47 ± 0.02	3.15 ± 0.02	0.09 ± 0.02	1.19 ± 0.02	0.35 ± 0.02	0.72 ± 0.02	0.21 ± 0.02

mg / 100 g					**Crackers**					
Crackers Himanthalia (CH)	0.10 ± 0.01	0.56 ± 0.01	0.40 ± 0.01	0.52 ± 0.01	2.82 ± 0.01	0.20 ± 0.01	1.08 ± 0.01	0.31 ± 0.01	0.20 ± 0.01	0.30 ± 0.01
Crackers Wakame (CW)	0.10 ± 0.02	0.56 ± 0.02	0.41 ± 0.02	0.51 ± 0.02	2.72 ± 0.02	0.20 ± 0.01	1.08 ± 0.02	0.30 ± 0.02	0.21 ± 0.02	0.30 ± 0.01
Crackers Ulva (CU)	0.10 ± 0.02	0.56 ± 0.01	0.40 ± 0.01	0.52 ± 0.01	2.78± 0.01	0.20 ± 0.01	1.08 ± 0.01	0.30 ± 0.01	0.21 ± 0.02	0.29 ± 0.01
Crackers Spirulina (CS)	0.10 ± 0.01	0.57 ± 0.03	0.40 ± 0.01	0.51 ± 0.03	2.73 ± 0.03	0.20 ± 0.02	1.08 ± 0.03	0.31 ± 0.03	0.20 ± 0.03	0.29 ± 0.03
Crackers Nori (C1)	0.10 ± 0.01	0.57 ± 0.02	0.42 ± 0.02	0.54 ± 0.02	2.85 ± 0.02	0.21 ± 0.02	1.10 ± 0.02	0.31 ± 0.02	0.20 ± 0.02	0.29± 0.02
Brown rice crackers (C2)	0.09 ± 0.01	0.57 ± 0.02	0.41 ± 0.02	0.51 ± 0.02	2.71 ± 0.02	0.20 ± 0.02	1.04 ± 0.02	0.31 ± 0.02	0.40 ± 0.02	0.30 ± 0.02
Brown rice crackers (C3)	0.11 ± 0.03	0.55 ± 0.02	0.40 ± 0.03	0.50 ± 0.03	2.71 ± 0.03	0.20 ± 0.01	1.08 ± 0.03	0.30 ± 0.03	0.27 ± 0.03	0.30 ± 0.03
Salad crackers (C4)	0.09 ± 0.01	0.57 ± 0.01	0.40 ± 0.01	0.48 ± 0.01	2.69 ± 0.01	0.21 ± 0.01	1.08 ± 0.01	0.29 ± 0.01	0.13 ± 0.01	0.29 ± 0.01
Crackers with olive oil (C5)	0.10 ± 0.02	0.57 ± 0.01	0.41 ± 0.01	0.52 ± 0.01	2.75 ± 0.01	0.20 ± 0.01	1.10 ± 0.01	0.30 ± 0.01	0.09 ± 0.01	0.29 ± 0.01
Crackers sesame and quinoa (C5)	0.10 ± 0.01	0.55 ± 0.02	0.39 ± 0.02	0.52 ± 0.02	2.70 ± 0.02	0.22 ± 0.03	1.11 ± 0.02	0.31 ± 0.02	0.12 ± 0.02	0.30 ± 0.02

Table S9. Tau, HTau and HypTau content in algae samples from literature.

Concentrations expressed in mg/100 g d.w.

Algae specie	Tau	HTau	HypTau	Reference
Chondrus crispus	78.1			Vieira, E. F., et al, 2018
Gracilaria vermicullophyla	≈ 250	≈ 40		Mehdinia, A., et al, 2017
	≈ 200			Kawasaki, A., et al. 2017
Palmaria palmata	201.6			Vieira, E. F., et al, 2018
Porphyra sp	1160			Dawczynski, C., et al, 2007
	396			Vieira, E. F., et al, 2018
	122			McCusker, S., et al, 2014
	979; 646			Hwang, E. S., et al, 2013
Eisenia bicyclis	7			McCusker, S., et al, 2014
Himanthalia elongata	63.2			Vieira, E. F., et al, 2018
Hizikia fusiformis	69.6			Dawczynski, C., et al, 2007
	≈ 50			Kawasaki, A., et al. 2017
Laminaria japonica	19			Dawczynski, C., et al, 2007
	70.7			Vieira, E. F., et al, 2018
	≈2			McCusker, S., et al, 2014
Laminaria ochroleuca	≈2			McCusker, S., et al, 2014
Undaria pinnatifida	19.8			Dawczynski, C., et al, 2007
	165.1			Vieira, E. F., et al, 2018
Fucus vesiculosus	143.9			Vieira, E. F., et al, 2018
Ascophyllum nodosum	162.5			Vieira, E. F., et al, 2018
Codium sp	≈10			Kawasaki, A., et al. 2017
Ulva lactuca	171.4			Vieira, E. F., et al, 2018
	≈1			McCusker, S., et al, 2014
Tetraselmis chuii	68.7		0.02	Tevatia et al., 2015