2011

Effect Of Processing Conditions On Phytochemical Constituents Of Edible Irish Seaweed Himanthalia Elongata.

Sabrina Cox
Technological University Dublin, sabrina.cox@tudublin.ie

Nissreen Abu-Ghannam
Technological University Dublin, nissreen.abughannam@tudublin.ie

Shilpi Gupta
Technological University Dublin, shilpi.19may@gmail.com

Recommended Citation

Cox, S., Abu-Ghannam, N., Gupta, S. (2011). Effect Of Processing Conditions On Phytochemical Constituents Of Edible Irish Seaweed Himanthalia Elongata. *Journal of Food Processing and Preservation*, 36 (4), pp.348-363. doi:10.1111/j.1745-4549.2011.00563.x

Funder: ABBEST

Effect of processing conditions on phytochemical constituents of edible Irish seaweed *Himanthalia elongata*

Sabrina Cox, Nissreen Abu-Ghannam* and Shilpi Gupta

Journal of Food Processing and Preservation.

Accepted: 25/04/11

School of Food Science and Environmental Health,

Dublin Institute of Technology, Cathal Brugha St.,

Dublin 1, Ireland.

***Corresponding author: Dr. Nissreen Abu-Ghannam**

Tel: +35314027570; **Email:** nissreen.abughannam@dit.ie

Abstract

Seaweed is well recognised as an excellent source of phytochemicals. This study was a preliminary screening to investigate the effects of various food processing methods on the phytochemicals of *Himanthalia elongata*. Hydrothermal processing was carried out until an edible texture was achieved. The total phenolic content (TPC) of fresh *H. elongata* was 175.27 mg GAE/100 g fresh weight (FW) while boiling significantly reduced the TPC to 25.4 mg GAE/100 g FW ($p < 0.05$). A drying pre-treatment before boiling reduced the cooking time therefore leading to less leaching of antioxidants upon boiling. In terms of extract, drying of *H. elongata* followed by boiling had the most significant effect on the phytochemicals as TPC increased by 174%. Boiled extracts had the most effective DPPH scavenging activity (EC_{50} of 12.5 µg/ml). As a comparison, seaweed subjected to the same treatments were studied in terms of antimicrobial activity. Overall, extracts from fresh *H. elongata* achieved the highest inhibition.

Keywords: Seaweed, *Himanthalia elongata*, processing, antioxidants, antimicrobials.

1. Introduction

In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods (Kuda *et al*., 2005). Seaweeds are a known source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities (Bansemir *et al*., 2006). Compounds with antiviral, antifungal, antimicrobial and antioxidant activities have been detected in green, brown and red algae (Vairappan *et al*., 2001; Bansemir *et al*., 2006; Duan *et al*., 2006; Chandini *et al*., 2008; Cox *et al*., 2010). Antioxidant activity of marine algae may arise from pigments such as chlorophylls and carotenoids, vitamins and vitamin precursors including α-tocopherol, β-carotene, niacin, thiamine and ascorbic acid, phenolics such as polyphenolics and hydroquinones and flavonoids, phospholipids particularly phosphatidylcholine, terpenoids, peptides, and other antioxidative substances, which directly or indirectly contribute to the inhibition or suppression of oxidation processes (Shahidi, 2009). The environment in which seaweeds grow is harsh as they are exposed to a

56 combination of light and high oxygen concentrations. These factors can lead to the formation
57 of free radicals and other strong oxidizing agents but seaweeds seldom suffer from any
58 serious photodynamic damage during metabolism. This fact implies that their cells have some
59 protective antioxidative mechanisms and compounds (Matsukawa *et al.,* 1997).
60 Reactive oxygen species (ROS) such as hydroxyl, superoxide and peroxyl radicals are formed
61 in human cells by endogenous factors and result in extensive oxidative damage which can
62 lead to age related degenerative conditions, cancer and a wide range of other human diseases
63 (Reaven and Witzum, 1996; Aruoma, 1999). Phenolic compounds can act as antioxidants by
64 chelating metal ions, preventing radical formation and improving the antioxidant endogenous
65 system (Al-Azzawie and Mohamed-Saiel, 2006). These phenolic compounds are commonly
66 found in plants, including seaweeds (Duan *et al*., 2006). Polyphenols represent a diverse class
67 of compounds including flavonoids (i.e. flavones, flavonols, flavanones, flavononols,
68 chalcones and flavan-3-ols), lignins, tocopherols, tannins and phenolic acids (Shukla *et al*.,
69 1997).
70 Interest in new sources of natural antioxidants has increased in recent years in order to reduce
71 the use of synthetic forms such as Butylated Hydroxyanisole (BHA) and Butylated
72 Hydroxytoluene (BHT). Natural antioxidants from plant origin can react rapidly with these
73 free radicals and retard or alleviate the extent of oxidative deterioration (Akoh and Min,
74 1997). Furthermore, antioxidants from natural sources can also increase the shelf life of
75 foods. Phenolic phytochemicals inhibit autoxidation of unsaturated lipids, thus preventing the
76 formation of oxidized low-density lipoprotein (LDL), which is considered to induce
77 cardiovascular diseases (Amic *et al*., 2003). Therefore, the consumption of foods with high
78 levels of these phytochemicals or addition of such extracts could protect the body as well as
79 the foods against these events (Chandini *et al*., 2008).

Marine algae have been consumed in Asia since ancient times, but to a much lesser extent in the rest of the world. Many plant-based foods can be eaten raw or after cooking. Cooking can be performed in various ways but, for vegetables, most common are steaming, boiling and microwaving. These cooking processes would bring about a number of changes in physical characteristics and chemical composition of the vegetables (Zhang and Hamauzu, 2004). Reports on the effects of cooking on the antioxidant compounds in vegetables have been inconclusive. There are reports demonstrating an enhancement or no change in antioxidant activity of vegetables (Gahler *et al*., 2003; Turkman *et al*., 2005) while others have indicated a deterioration of activity after thermal treatment (Ismail *et al*., 2004; Zhang and Hamazu, 2004).

The presence and diversity of phytochemicals in vegetables are important factors for human health. The phytochemical contents in untreated vegetables have been the most studied. Since a large part of ingested vegetables are generally thermally processed prior to consumption, it is also important to investigate how the processing affects the levels of these compounds (Volden *et al*., 2009). Processing of vegetables for consumption exposes the phytochemicals present to detrimental factors that may lead to alterations in concentrations and health related quality. For example wet-thermal treatment causes denaturation of enzymes that can catalyse breakdown of nutrients and phytochemicals. On the other hand, processing by heat can result in reduction of constituents by leaching or due to thermal destruction (Rungapamestry *et al*., 2007). Turkmen *et al*. (2005) revealed that different cooking methods (boiling, steaming and microwaving) caused losses of phenolics from squash, peas and leek. However, under similar conditions, an increase in the phenolic content of vegetables such as green beans, peppers and broccoli was reported (Turkman *et al*., 2005). Watchtel-Galor *et al*. (2008) found that steaming and microwaving led to losses in the total phenolic content of broccoli, choy-sum

104 and cabbage, although steaming had significantly less loss than microwaved samples. Volden
105 *et al*. (2009) also reported loss of phytochemicals in steamed cauliflower (19%).
106 The traditional process to preserve seaweeds is by sun drying (Lim and Murtijaya, 2007) as
107 several seaweeds are perishable in their fresh state and could deteriorate within a few days
108 after harvest. Drying is one of the most common food processing methods that can be used to
109 extend the shelf-life and to achieve the desired characteristics of a food product. Reducing the
110 water activity (a_w) of food via this process can minimize deterioration from chemical
111 reactions and microbial activity (Chiewchan *et al*., 2010). Dried seaweeds are rehydrated by
112 various methods such as boiling before consumption, therefore in the present study drying
113 was considered as a pre-treatment before cooking.
114 Food poisoning is a concern for both consumers and the food industry despite the use of
115 various preservation methods. Food processors, food safety regulators and regulatory
116 agencies are continuously concerned with the high and growing number of illness or
117 outbreaks caused by some pathogenic and spoilage microorganisms in foods. Recently,
118 consumers are demanding foods which are fresh, natural and minimally processed. Along
119 with this, consumers are also concerned about the safety of foods containing synthetic
120 preservatives. This has put pressure on the food industry and has fuelled research into the
121 discovery of alternative natural antimicrobials (Shan *et al*., 2007).
122 Being rich in phytochemicals responsible for antioxidant and antimicrobial activity, there
123 have been many studies conducted on seaweeds to quantify these compounds (Duan *et al*.,
124 2006; Chandini *et al*., 2008 and Cox *et al*., 2010), however little information is available on
125 the effect of hydrothermal treatment on these phytochemicals in seaweeds. The purpose of
126 this study was a preliminary screening to investigate the effect of different processing
127 methods on the phytochemical constituents present in an Irish edible brown seaweed,
128 *Himanthalia elongata.* The effect was studied in terms of both the extract of *H. elongata* and

as a whole food. The aim was on one hand, to study the effect of common cooking treatments on the phytochemicals of the brown seaweed. At the same time, the effect of drying as a pre-treatment on the cooking time and phytochemical content was also assessed. Moreover, the antimicrobial properties of *H. elongata* extracts against common food pathogenic and food spoilage bacteria was also investigated after varied processing treatments.

2. Methods

2.1 Chemicals

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate (Na_2CO_3), sodium benzoate, vanillin, hydrochloric acid (HCl), (+)-catecin, aluminium chloride ($AlCl_3$) and quercetin were purchased from Sigma Aldrich Chemie (Steinheim, Germany). Tryptic Soy Broth (TSB) was purchased from Sparks (Dublin, Ireland).

2.2 Seaweed material

H. elongata (Phaeophyta) was purchased from Quality Sea Veg., Co Donegal, Ireland. Samples were collected in September and November 2009, washed thoroughly with freshwater to remove epiphytes and stored at 4°C until analysis.

2.3 Preparation of samples

H. elongata was washed thoroughly with tap water, dried with absorbent paper and then cut into 3 cm long pieces before processing. The effect of processing on *H. elongata* in terms of antioxidant and antimicrobial activity was evaluated by drying, boiling, steaming, microwaving, and combinations of drying as a pre-treatment before boiling and steaming until an edible texture was achieved as described in section 2.4.

154 2.4 Determination of cooking time and texture evaluation

155 Cooking time of seaweed was selected from preliminary experiments and was determined by
156 the tactile method. To overcome the subjectivity of the tactile method, a combination of
157 tactile and instrumental textural methods were used in order to decide the cooking time of
158 seaweed. Edible texture was determined by a sensory panel consisting of 6 judges. At 5 min
159 cooking time intervals, seaweed samples were removed to undergo tactile and instrumental
160 texture analysis. Shear tests were performed using an Instron Universal Testing Machine
161 (Model 4301, Canton MA, USA) attached to Bluehill 2 version 2.14 analysis software for
162 materials testing. A Warner Bratzler cutter was used in the shear tests. An aluminium plate
163 with dimensions of 10 x 6 cm^2, thickness 1.3 cm with an opening of 3 mm in the centre was
164 supported in the Instron base. Seaweed samples (5 g) were sheared at a speed of 200
165 mm/min. The cutting implement was allowed to travel the depth of the seaweed, cutting
166 through the sample and seaweed hardness was defined as the peak of force-deformation curve
167 recorded in Newtons per mm (N/mm). Ten replications of each sample were carried out.

168

169 2.5.1 Drying pre-treatment

170 Seaweed samples were placed in 5 g lots on a drying tray in a single layer. Drying of seaweed
171 was investigated in a drier (Innova 42, Mason Technology, Ireland) at 25°C air drying
172 temperature over a period of 12 - 24 hours. Air velocity was 2.0 ± 0.1 m s^{-1} measured with
173 VWR Enviro-meter digital anemometer (VWR, Ireland).

174

175 2.5.2 Boiling

176 The seaweed samples (dried or fresh) were boiled by immersion in 2 L of distilled water kept
177 at the specified boiling temperatures (80 and 100°C) using a water bath (Lauda, Aqualine
178 AL5, Mason Technology, Ireland) until an edible texture was achieved (30 – 32 N/mm) as

described in section 2.4. After boiling, the cooked seaweeds were drained using a wire mesh strainer and placed on ice to cool before the extraction procedure.

2.5.3 Steaming

Regular steaming was performed on dried and fresh seaweeds using an atmospheric steam cooker (Kenwood, FS360, United Kingdom). The seaweed samples (5 g), were placed in the centre tray of the steam cooker, covered with the lid and steamed over 2 L of boiling water. Steaming time was selected according to preliminary experiments, in which steaming time was determined when an edible texture was achieved (30 – 32 N/mm) as described in section 2.4. After the steaming process, the cooked seaweeds were drained and placed on ice to cool before the extraction procedure.

2.5.4 Microwaving

Fresh seaweed samples (5 g) were placed in a pyrex bowl, covered with a plastic film to prevent water loss and microwaved in a domestic microwave oven (Sharp Platinum Collection, R-957, United Kingdom) at 450 and 900 watts (W) for 30 and 20 seconds (s), respectively. Cooking time was selected according to preliminary experiments, in which microwaving time was determined when an edible texture was achieved (30 – 32 N/mm) as described in section 2.4. After microwaving, the seaweeds placed on ice to cool before the extraction procedure.

2.6 Extraction of phytochemicals

Seaweed samples after respective processing (5 g original weight) were powdered in liquid nitrogen using a mortar and pestle, then extracted with 50 ml of methanol (60%) under nitrogen atmosphere for 2 hours. Liquid nitrogen was used as it can reduce the particle size of

204 a large amount of seaweed in a short period of time. The extraction was carried out at 40°C at
205 100 rpm in a shaker incubator (Innova 42, Mason Technology, Ireland) as optimised in our
206 previous work (Cox *et al*., 2010). Samples were filtered using Whatman Number 1 filter
207 papers (Sigma Aldrich Chemie, Steinheim, Germany) and centrifuged at 10,000 rpm for 15
208 min (Sigma 2K15, Mason Technology, Ireland). Resulting extracts were evaporated to
209 dryness using vacuum polyevaporator (Buchi Syncore Polyvap, Mason Technology, Ireland)
210 at 60°C. A pressure gradient program was designed for evaporation of the solvents with
211 vacuum conditions of 337 and 72 mbar for methanol and water, respectively.
212
213 2.7 Total phenolic content
214 The total phenolic content of seaweed samples (concentration 1 mg/ml of extract in water)
215 was measured using the Folin-Ciocalteau method as reported by Taga *et al*. (1984). The total
216 phenolic contents of the whole seaweeds were expressed as mg gallic acid equivalent per 100
217 gram fresh weight (mg GAE/100 g FW) and as mg GAE/g for extracts.
218
219 2.8 DPPH radical scavenging activity
220 Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH)
221 according to the method of Yen and Chen (1995) with some modifications. Briefly, a 100 µl
222 aliquot of test sample was placed in a 96-well microtitre plate and 100 µl of 0.16 mM DPPH
223 methanolic solution was added. The mixture was shaken and incubated for 30 min in
224 darkness at 25ºC. Changes in the absorbance of the samples were measured at 517 nm using a
225 microplate reader (Powerwave, Biotek, VT, USA).
226
227 The ability to scavenge the DPPH radical was calculated using the following equation given
228 by Duan *et al*. (2006):

229 Scavenging effect (%) = $\left[1-\left(\frac{A_{sample}-A_{sampleblank}}{A_{control}}\right)\right]\times 100$ Equation 1

230 Where: $A_{control}$ is the absorbance of the control (DPPH solution without sample), A_{sample} is the
231 absorbance of the test sample (DPPH solution plus test sample) and $A_{sample\ blank}$ is the
232 absorbance of the sample only (sample without any DPPH solution). DPPH results were
233 interpreted as the "efficient concentration" or EC_{50} value which is the concentration of
234 substrate that causes 50% loss of the DPPH activity.
235
236 2.9 Total flavonoid content
237 Total flavonoid contents were determined according to the method of Zhishen *et al*. (1999).
238 Quercetin was used to prepare the standard curve and results were expressed as mg quercetin
239 equivalents (QE)/100 gram fresh weight (mg QE/100 g FW) for whole seaweeds and as mg
240 QE/g for extracts.
241
242 2.10 Total condensed tannin content
243 Total condensed tannin contents were determined according to the method of Julkunen-Titto
244 (1985). (+)-Catechin was used to prepare the standard curve and results were expressed as mg
245 catechin equivalents (CE)/100 gram fresh weight (mg CE/100 g FW) for whole seaweeds and
246 as mg CE/g for extracts.
247
248 2.11 Antimicrobial activity
249 2.11.1 Microbial culture
250 Two species of common food pathogenic and two species of food spoilage bacteria selected
251 for this study were *Listeria monocytogenes* ATCC 19115, *Salmonella abony* NCTC 6017,
252 *Enterococcus faecalis* ATCC 7080 and *Pseudomonas aeruginosa* ATCC 27853, respectively

253 (Medical Supply Company, Dublin, Ireland). All cultures were maintained at -70°C in 20%
254 glycerol stocks and grown in Tryptic Soy Broth (TSB) at 37°C; apart from *P. aeruginosa*
255 which was incubated at 30°C to obtain sub-cultures. Working cultures were prepared from
256 sub-cultures and grown at optimal conditions for each bacterium for 18 hours before analysis.
257 Bacterial suspensions were then prepared in saline solution (NaCl 0.85%, BioMérieux,
258 France) equivalent to a McFarland standard of 0.5, using the Densimat photometer
259 (BioMérieux Inc, France) to obtain a concentration of 1 x 10^8 colony forming units
260 (CFU)/ml. This suspension was then diluted in TSB to obtain a working concentration of 1 x
261 10^6 CFU/ml.
262
263 2.11.2 Antimicrobial activity assay
264 The influence of varying concentrations of extract on efficacy was assessed against *L.*
265 *monocytogenes*, *S. abony*, *E. faecalis* and *P. aeruginosa* using 96-well microtitre plates
266 (Sarstedt Ltd., UK). Seaweed extracts after respective processing (5 g original seaweed
267 weight) were dissolved in 2.5 ml of TSB and 200 µl was added to the first row of each plate.
268 All other wells were filled with 100 µl of TSB and 100 µl from the first well was serial
269 diluted two-folds along each column. Finally, 100 µl of bacterial suspension containing 1 x
270 10^6 CFU/ml was added to the wells. The last column was used for bacterium and media
271 controls and sample blanks were prepared for all of the extracts. Absorbance readings were
272 then taken at 0 and 24 hours at 600 nm using a microplate spectrophotometer (Powerwave,
273 Biotek) with 20 seconds agitation before each optical density (OD) measurement. Analysis of
274 growth over time was also performed on most effective extracts. OD measurements were
275 taken every three hours for 24 hours. Sodium benzoate and sodium nitrite were used as
276 controls. Percentage inhibition was calculated as follows:

277 Bacterial inhibition (%) = $\left[\left(\frac{O-E}{O}\right)\right]\times 100$ Equation 2

278 Where: *O* is (OD of the Organism at 24 h - OD of the Organism at 0 h) and *E* is (OD of the
279 Extract at 24 h – Blank at 24 h) – (OD of the Extract at 0 h- Blank at 0 h).
280 Results were interpreted by categorising percentage inhibitions based on inhibition intensity
281 according to Dubber and Harder (2008).

282

283 2.12 Statistical analysis

284 All experiments were performed in triplicate and replicated twice. All statistical analyses
285 were carried out using STATGRAPHICS Centurion XV. Statistical differences between
286 different processing treatments were determined using ANOVA followed by Least
287 Significant Difference (LSD) testing. Differences were considered statistically significant
288 when $p < 0.05$.

289

290 **3. Results and Discussion**

291 3.1 Effect of processing on total phenolic content

292 Increased intake of vegetables is generally associated with a reduced risk of cancer and
293 cardiovascular disease (Kris-Etherton *et al*., 2002). Processing and preparation of vegetables,
294 especially thermal treatment, which are applied prior to consumption may affect the
295 phytochemicals. Heat applications such as boiling, steaming or microwaving are common
296 practices in the processing of food products in order to render them palatable and
297 microbiologically safe. Since seaweed would need to undergo some heat treatment prior to
298 usage, it was relevant to assess the effects of heat treatment on the stability of seaweed
299 antioxidant properties. In the present study, cooking of *H. elongata* was carried out by three
300 commonly used procedures and the antioxidant properties of the cooked product were

evaluated and compared with fresh. It is a well known fact that cooking time as well as cooked texture, appearance and flavour are important cooking quality characteristics (Xu and Chang, 2008). Firmness or softness is one of the most important criteria in determining the acceptability of foods. Acceptable texture parameters are outlined in the literature for various foods such as legumes (Xu and Chang, 2008) but there are no such values previously outlined for seaweed. Because sensory evaluation is based on human senses which detect myriad characteristics of material properties simultaneously, it is difficult to find a good correlation between orally perceived texture and instrumentally measured texture (Nishinari, 2004). Therefore, in the present study the cooking time of fresh seaweed boiled at 100°C was calculated using a tactile and instrumental texture measurement based on the length of time it took the seaweed to become edible. Samples were taken every 5 minutes and edibility was judged based on the hardness and chewiness of the samples until an acceptable edible texture was achieved. From these methods, it was found that softening of fresh *H. elongata* from 45 N/mm to 30 – 32 N/mm was an acceptable edible texture as can be seen in Table 1.

The total phenolic content (TPC) of processed *H. elongata* can be seen in Fig. 1 (A). In order to achieve an edible texture, it was necessary that *H. elongata* was boiled for 40 and 35 min at 80ºC and 100ºC, respectively. This resulted in almost 82.31 and 85.5% loss in TPC, respectively as compared to fresh seaweed. A reduction of TPC from 175.27 to 25.4 mg GAE/100 g FW was observed. Similar results were obtained with steaming although it was not as detrimental as boiling. The seaweed was steamed for 45 min which resulted in a loss of 32.06% TPC. The reason for such high reduction during boiling could be due to leaching of nutrients in water which was proved by a reduction in the amount of extract obtained also. Fresh *H. elongata* had a 3.14% yield of extract whereas steaming or boiling resulted in a significant ($p < 0.05$) reduction in the yield of extract to as low as 0.26% (Table 2). The reduction of vitamin levels during processing and cooking can vary largely depending on the

326 cooking method and type of food (Leskova *et al*., 2006). Studies have shown that phenolic
327 compounds are sensitive to heat, whereby boiling of vegetables for few minutes could cause a
328 significant loss of phenolic content which can leach into boiling water (Amin *et al*., 2006).
329 Xu and Chang (2008) reported a 40 – 50% loss in the TPC of legumes due to leaching of
330 phenolics in boiling water whereas Oboh (2005) found up to 200% increase in the phenolic
331 content of boiled tropical green leafy vegetables. Reduction in TPC was also found in other
332 vegetables such as broccoli, kale and spinach (Zhang and Hamauzu, 2004; Ismail *et al*.,
333 2004). These authors stated the probable reason was due to dissolution of polyphenols into
334 cooking water which could be the case with *H. elongata* as it requires long cooking times to
335 become edible. Although microwave cooking has been reported to be the most deleterious
336 with respect to the antioxidant properties of vegetables (Sultana *et al*., 2008), the results in
337 the present study were encouraging as microwaving increased the TPC by 22.49% (450 W)
338 and 36.58% (900 W), as compared to fresh.
339 In order to reduce the cooking time and eventual loss of phytochemicals, drying was used as
340 a pre-treatment. The process of drying in itself was not detrimental as a drying period of 12 h
341 and 24 h retained 80.1 and 85.96% of the original phenolic content as compared to fresh
342 seaweed. Possible losses could be attributed to stressing the plant during the drying process
343 due to loss of water through the cell walls. Moreover, drying for 12 and 24 h before boiling
344 not only reduced the cooking time but also the loss in TPC. Drying for 24 h before boiling at
345 100ºC decreased cooking time by 15 mins and reduced the loss of total phenols by 8.83%, as
346 compared to boiling at 100°C without a drying pre-treatment. Drying as a pre-treatment was
347 not effective for steaming as 44.19% reduction in TPC was seen when seaweed was dried for
348 24 h followed by steaming ($p < 0.05$). Microwaving was not carried out on dried seaweeds as
349 it was not a hydrothermal process and therefore re-hydration would not take place.

Although heat processing seriously degraded the quality of seaweed, an interesting observation was an increase in the potency of the extract (Fig. 1 (B)). When the activity of the heat processed extract was compared in terms of per gram dried extract, it was found that boiling at 80°C and 100°C resulted in an increase of 104.03% and 71% TPC per g of dried extract, respectively, as compared to fresh ($p < 0.05$). Fresh *H. elongata* contained 55.75 mg GAE/g of extract. Drying for 12 and 24 h led to a significant decrease in TPC up to 22.42% as compared to fresh ($p < 0.05$). Drying pre-treatment for 12 h followed by boiling at 80 and 100°C for 30 and 25 mins, respectively, had a 161.43 and 125.11% increase per g of extract as compared to fresh samples ($p < 0.05$). Samples pre-treated by drying for 24 h followed by boiling at 100°C for 20 min increased the TPC by 165.32% as compared to fresh. Drying of *H. elongata* for 24 h followed by boiling at 80°C for 30 mins had the most significant effect ($p < 0.05$) on the TPC of all treatments resulting in 173.99% increase in TPC. Steaming for 45 mins had a 36.62% decrease in TPC per g of extract while a drying pre-treatment before steaming for 50 mins had a 40% decrease. Microwaving at 450 W caused no significant increase in the TPC per g of extract as compared to fresh ($p < 0.05$), while microwaving at 900 W increased the TPC to 82 mg GAE/g of extract (47.08% increase). As the TPC of *H. elongata* per g of extract was increased due to processing, a lower concentration of the extract would be required to have a potential effect on preventing oxidation in food products which is a promising finding.

3.2 Effect of processing on DPPH radical scavenging activity

The results of the DPPH free radical scavenging ability of the seaweed processed under different conditions are shown in Table 2. DPPH reagent has been used extensively for investigating the free radical scavenging activities of compounds (Shon *et al.*, 2003). The results indicated that free radical scavenging ability of the processed seaweeds ranged from

375 52.51 to 100% (concentration 100 µg/ml extract) with extracts from seaweed dried for 12 h
376 followed by boiling at 100°C being most effective. Significant differences ($p < 0.05$) in
377 DPPH values were found for all processing treatments. At 100 µg/ml extract concentration,
378 drying led to slight decrease in DPPH radical scavenging activity from 75.5% to 74.69% (12
379 h) and 67.87% (24 h) while boiling led to a significant increase ($p < 0.05$). Boiling at 100ºC
380 increased DPPH scavenging by 23.89%, from 75.5 to 93.54%. Drying of *H. elongata* for 12 h
381 followed by boiling at 100ºC had the most significant increase in antioxidant activity as
382 100% inhibition of the DPPH radical was achieved with 100 µl/ml of extract. Steaming
383 significantly reduced the DPPH radical scavenging activity ($p < 0.05$) to 52.51%. Extracts
384 from *H. elongata* given drying pre-treatments followed by steaming had 53.79% (12 h) and
385 53.5% (24 h) scavenging of the DPPH radical. Seaweed microwaved at 450 W had 76.29%
386 activity against DPPH radical while microwaving at 900 W had 75.35% activity.
387 DPPH results are often interpreted as the "efficient concentration" or EC_{50} value, which is
388 defined as the concentration of substrate that causes 50% loss of the DPPH activity
389 (Molyneux, 2004). The EC_{50} values of DPPH radical scavenging activity from dried
390 methanolic extracts of seaweeds are also presented in Table 2. Processing of *H. elongata*
391 resulted in significantly different EC_{50} values ($p < 0.05$), depending upon treatment. The EC_{50}
392 levels ranged from 12.5 to 100 µg/ml of extract with all treatments in which boiling was
393 found to have the most effective EC_{50} values (12.5 µg/ml of extract). Extracts from fresh
394 seaweed had an EC_{50} of 25 µg/ml. Drying of seaweed led to a significant ($p < 0.05$) reduction
395 in the DPPH radical scavenging activity of the extract to 50 µg/ml (12 and 24 h). Steaming
396 had the most detrimental effect on the DPPH radical scavenging activity of the extract as
397 100µg/ml was required to reduce the DPPH radical by 50% and activity at 100 µg/ml
398 concentration was almost half that of the most effective processed seaweed (53.5 and 100%,

399 respectively). There was no significant difference between microwaved seaweed extracts
400 compared to fresh ($p > 0.05$) as all had an EC_{50} value of 25 µg/ml.
401 3.3 Effect of processing on total flavonoid content
402 The bioavailability of phytochemicals is influenced by the matrix and microstructure of the
403 food they occur in, the storage conditions (light, oxygen, and temperature regime) and
404 thermal processing they are subjected. As a consequence, knowledge of the content and
405 stability of phytochemicals in foods after processing is essential to evaluate the nutritional
406 value of foods rich in these phytochemicals, like seaweed to (Parada and Aguilera, 2007).
407 The total flavonoid content (TFC) of processed whole *H. elongata* is presented in Fig. 2 (A).
408 The TFC of fresh *H. elongata* was 53.18 mg QE/100 g FW. Drying for 12 and 24 h had no
409 significant effect on the TFC as there was only a slight increase of 0.72 and 0.25%,
410 respectively ($p > 0.05$). All treatments which included boiling significantly reduced the TFC,
411 within a range of 88.86 to 90.18%. This highest reduction was seen in fresh seaweed boiled at
412 100ºC which led to a 90.18% reduction in TFC ($p < 0.05$). Flavonoids commonly accumulate
413 in epidermal cells of plant organs, being found as glycosides and in non-glycosidic forms
414 (aglycones) (Sakihama *et al*., 2002). Release of flavonoids and increased chemical extraction
415 of these compounds could be induced by the effect of boiling (Olivera *et al*., 2008). This
416 release of flavonoids coupled with contact and leaching into water could have resulted in high
417 reduction in TFC for boiled samples. The results of the present study are similar to Olivera *et*
418 *al*. (2008) who found that boiling decreased TFC in brussels sprouts. Steaming also led to a
419 significant reduction in TFC compared to fresh but this was significantly less as compared to
420 boiled seaweeds ($p < 0.05$). Steaming retained 17.4% more TFC than boiled samples as
421 compared to fresh samples. This could be due to the fact that steamed seaweeds were not in
422 direct contact with water which resulted in considerably less leaching of flavonoids.
423 Microwaving at 450 W had a 12.69% increase in TFC while microwaving at 900 W raised

424 the TFC by 10.65% in whole *H. elongata* ($p < 0.05$). These results are in line with Francisco
425 *et al*. (2010) and Rodrigues *et al*. (2009) who also reported significant losses of flavonoids up
426 to 67% in cooked conventional vegetables.
427 Total flavonoid content of processed *H. elongata* extracts are presented in Fig. 2 (B). Extracts
428 from fresh *H. elongata* contained 42.29 mg QE/g of extract. All treatments significantly
429 changed the TFC content as compared to fresh ($p < 0.05$). Simple drying for 12 and 24 h
430 significantly reduced ($p < 0.05$) the TFC in the range of 2.45% to 9.35% whereas boiling at
431 80°C and 100°C resulted in an increase up to 15.76%. However, a combination of drying pre-
432 treatment followed by boiling had the most significant effect on the TFC of *H. elongata*.
433 Drying for 12 h followed by boiling at 80°C and 100°C resulted in 18.72 and 21.67%
434 increase in TFC, respectively ($p < 0.05$). Drying for 24 h followed by boiling at 100°C for 20
435 mins had a 26.6% increase in TFC. The most significant increase of 32.02% was seen in
436 samples dried for 24 h followed by boiling at 80°C for 30 mins. Steaming alone and in
437 combination with 12 and 24 h drying pre-treatments resulted in 14, 11.43 and 11.98%
438 increase in TFC, respectively. The increase in the case of microwaved samples ranged from
439 8.72% to 14.29%.
440
441 *3.4 Effect of processing on total condensed tannin content*
442 Phlorotannins are a group of phenolic compounds which are restricted to polymers of
443 phloroglucinol and have been identified from several brown algae. Many studies have shown
444 that phlorotannins are the only phenolic group detected in brown algae (Jormalainen and
445 Honkanen, 2004; Koivikko *et al*., 2007). Total condensed tannin content (TTC) of processed
446 *H. elongata* can be seen in Fig. 3 (A). Condensed tannins of the studied seaweeds ranged
447 from 70.61 to 5.5 mg QE/100 g FW. Fresh *H. elongata* contained 70.05 mg CE/100 g FW
448 while drying for 12 and 24 h reduced the TTC by 2.92 and 4.73%, respectively.

449 Similarly to total flavonoid contents, TTC was significantly reduced upon boiling ($p < 0.05$).
450 Boiling at 80°C for 40 mins significantly reduced the TTC from 70.05 to 6.22 mg CE/100 g
451 FW (91.11% reduction). The most significant reduction of 92.13% in TTC was seen in *H.*
452 *elongata* boiled at 100°C for 35 mins ($p < 0.05$). Similar to TFC, steaming had a lower
453 reduction of TTC as compared to boiling. Steaming retained 40.5% more TTC than boiled
454 samples as compared to fresh ($p < 0.05$). Microwaving at 450 and 900 W had a 20.27 and
455 22.54% reduction in TTC, respectively ($p < 0.05$). The basis of the significant decrease in
456 cooked seaweeds could also be attributed to the possible break-down of tannins present in the
457 seaweed to simple phenol (Akindahunsi and Oboh, 1999). Khandelwal *et al*. (2010) and
458 Somsub *et al*. (2008) also found decreases in tannin levels of cooked legumes and vegetables.
459 In contrast to TPC and TFC, a significant reduction in the total condensed tannins of
460 processed *H. elongata* extracts was observed as compared to fresh (Fig. 3 (B)). Extracts from
461 fresh *H. elongata* contained 55.7 mg CE/g, while drying for 12 and 24 h had 7.73 and 8.65%
462 reduction in TTC, respectively. Boiling at 80°C for 40 mins led to a 58.97% reduction in
463 TTC while 62.89% reduction was seen in *H. elongata* boiled at 100°C for 35 mins. Drying
464 pre-treatment followed by boiling also had significant losses of TTC but less than that of
465 boiled seaweed. Drying for 12 h before boiling at 80 and 100°C had 53.52 and 53.78%
466 reduction respectively. Drying for 24 h followed by boiling at 80°C for 30 min caused a loss
467 of TTC by 55.71% while drying for 24 h in combination with boiling at 100°C for 20 mins
468 had a 55.91% reduction from 55.7 to 24.55 mg CE/g of extract. Steaming resulted in 18.91%
469 reduction while in combination with a drying pre-treatment there was a 28% reduction as
470 compared to extracts from fresh *H. elongata*. Microwaving at 450 and 900 W for 30 and 20 s
471 had a 19.14 and 16.88% reduction in TTC, respectively ($p < 0.05$).
472
473 3.5 Antimicrobial activity of processed *H. elongata* extracts against *L. monocytogenes*

474 Consumers are concerned about the safety of foods containing synthetic preservatives. This
475 has put pressure on the food industry and has fuelled research into the discovery of
476 alternative natural antimicrobials (Shan *et al*., 2007). Antimicrobial activity of processed
477 seaweed extracts was studied in order to analyse the effect of food processing on their
478 activity. The entire yield of extract from 5 g original weight of each of the processed
479 seaweeds were dissolved in 2.5 ml TSB and utilized in the assay. Therefore, different
480 concentrations were achieved at each dilution level as can be seen in Table 3. In the present
481 study antimicrobial activity was tested against common food spoilage (*E. faecalis* and *P.*
482 *aeruginosa*) and food pathogenic (*L. monocytogenes* and *S. abony*) bacteria. These organisms
483 were studied after discussions with the Food Safety Authority of Ireland because they have
484 been identified as being problematic in the Irish food industry. The entire spectrum of
485 inhibitory effects is reported as outlined by Dubber and Harder (2008).
486 *L. monocytogenes* is a Gram-positive pathogenic bacterium commonly isolated from foods in
487 many countries including Ireland (Chitlapilly-Dass *et al*., 2010). The percentage inhibition of
488 the processed seaweed extracts against *L. monocytogenes* are presented in Table 4 and the
489 concentrations of extract for each dilution of processed *H. elongata* are outlined in Table 3.
490 At highest extract concentrations, extract from fresh seaweed and those dried for 12 and 24 h
491 (31.44, 32.47 and 34.77 mg/ml, respectively) had 100% inhibition against *L. monocytogenes*.
492 Any processing treatment which included boiling of *H. elongata* had weak activity against *L.*
493 *monocytogenes* (< 25% inhibition), however extract concentrations were lower most likely
494 due to leaching of phytochemicals during the boiling procedure. Extracts of steamed seaweed
495 had strong activity against *L. monocytogenes* at the highest dilution tested (96.34% inhibition
496 at 34 mg/ml). However when drying was used as a pre-treatment before steaming there was
497 less than half the inhibition against *L. monocytogenes*; 43.25% (31 mg/ml) and 43.85% (29.3
498 mg/ml) for 12 and 24 h dried samples, respectively. Microwaving at 450 and 900 W

499 produced extracts with strong inhibition against *L. monocytogenes* in the first dilution (97.24
500 and 97.26% inhibition at 37.56 and 43.37 mg/ml extract, respectively). As the yield of
501 microwaved *H. elongata* extract was higher than other treatments, this would suggest that the
502 extract is in fact slightly less potent than those of fresh seaweed. Extracts from fresh *H.*
503 *elongata* at the first concentration tested (31.44 mg/ml) were the most effective of the
504 processed seaweeds overall.
505 There was no significant difference ($p > 0.05$) between the first and second dilutions of fresh
506 *H. elongata* extract tested against *L. monocytogenes* (15.72 mg/ml) which again had 100%
507 inhibition in the second dilution. An inhibition activity of 89.88 to 92.99% was obtained by
508 dried extracts whereas extracts from boiled seaweeds had completely lost the antimicrobial
509 activity. Drying pre-treatment before boiling seemed to maintain weak antimicrobial activity
510 of the extracts in the range of 9.81 to 15.89% in the second dilution as compared to 0%.
511 Extracts from steamed seaweed at the second dilution of 17 mg/ml had strong activity against
512 *L. monocytogenes* giving 96.24% inhibition. Seaweeds which received a drying pre-treatment
513 (12 and 24 h) before steaming had significantly less antimicrobial activity against *L.*
514 *monocytogenes* in the second dilution with inhibition levels of 34.14 and 34.15% at 15.5 and
515 14.65 mg/ml, respectively ($p > 0.05$). Extracts from the second dilution of microwaved
516 seaweed had 82.9% (450 W) and 81.45% (900 W) inhibition against *L. monocytogenes* at
517 18.78 and 21.74 mg/ml extract concentrations, respectively. At the third and fourth dilutions;
518 although fresh seaweeds still had 98% activity, all processed seaweeds had significantly less
519 inhibition ($p < 0.05$).
520
521 3.6 Antimicrobial activity of processed *H. elongata* extracts against *S. abony*
522 *Salmonella* is a Gram-negative food-borne pathogenic bacterium and has become one of the
523 most important causes of acute enterocolitis throughout the world. Salmonellosis is caused by

524 any of over 2300 serovars of *Salmonella* (Wong *et al*., 2000; Lee *et al*., 2001). Fresh *H.*
525 *elongata* had the highest activity against *S. abony* as can be seen in Table 5. However,
526 overall, the processed extracts were least effective against *S. abony*, and no processing
527 treatment achieved 100% inhibition of the bacteria. A potential cause is that *S. abony* is
528 Gram-negative, as there are significant differences in the outer layers of Gram-negative and
529 Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique
530 periplasmic space which is not found in Gram-positive bacteria (Nikaido, 1996; Duffy and
531 Power, 2001). Antibacterial substances can easily destroy the bacterial cell wall and
532 cytoplasmic membrane and result in a leakage of the cytoplasm and its coagulation (Kalemba
533 and Kunicka, 2003).
534 Fresh *H. elongata* extracts had activity which ranged from 87.03 to 37.18% in the first to
535 fourth dilutions. Seaweed which had been dried for 12 and 24 h had moderate activity against
536 *S. abony* in the first dilution of extract but had weak activity from the second dilution
537 onwards (< 50% inhibition). Any processing treatment which included boiling had weak
538 activity against *S. abony* with a maximum of 22.11% inhibition. Extracts from steamed *H.*
539 *elongata* had strong activity in the first and second dilutions (> 90%) and moderate activity
540 thereafter. Drying of seaweed before steaming led to a significant reduction ($p < 0.05$) in
541 antimicrobial activity as compared to steaming alone against *S. abony* (44% inhibition) in the
542 first dilution and weak activity in subsequent dilutions. Activity of microwaved *H. elongata*
543 extracts at 450 and 900 W ranged from strong (≤ 94.4%) to weak (< 50%) against *S. abony*.
544
545 3.7 Antimicrobial activity of processed *H. elongata* extracts against *E. faecalis*
546 The Gram-positive bacterium *E. faecalis* is a natural member of the human and animal
547 gastrointestinal flora. This bacterium is an indicator of faecal contamination and has been
548 detected in food, milk and drinking water (Rincé *et al*., 2003). Antimicrobial activity of

549 processed *H. elongata* extracts against *E. faecalis* are outlined in Table 6. Fresh *H. elongata*
550 extracts had more than 99% antimicrobial activity until the second dilution and moderate
551 activity in the third and fourth dilutions (87.03 and 78.02%, respectively). Dried seaweed
552 extracts had very strong (100%) and strong (95.4%) activity in the first dilution for 12 and 24
553 h, respectively, however this level was halfed in the second dilution with 39.24% (12 h) and
554 53.01% inhibition (24 h). Extracts from boiled seaweeds had less than 10% activity in the
555 first two dilutions and was completely lost in lower dilutions. A drying pre-treatment before
556 boiling retained more of the bioactivity ($p < 0.05$) of the extract, for example extracts of *H.*
557 *elongata* processed by drying for 12 h followed by boiling at 80°C for 30 mins had 51.45%
558 inhibition. Extracts from steamed *H. elongata* had strong activity in the first and second
559 dilutions (≤ 95.21%) and moderate activity thereafter. Drying of seaweed before steaming led
560 to a significant reduction ($p < 0.05$) in antimicrobial activity as compared to steaming alone
561 against *E. faecalis* also (≤ 44.57% inhibition). Extracts of microwaved *H. elongata* had strong
562 activity in the first dilution (≤ 95.81%) while extracts from the second dilution onwards were
563 significantly less effective with activity below 53.07%.
564
565 3.8 Antimicrobial activity of processed *H. elongata* extracts against *P. aeruginosa*
566 *P. aeruginosa* is a ubiquitous Gram-negative food spoilage bacterium with great adaptability
567 and metabolic versatility. *P. aeruginosa* can attach onto a variety of surfaces and in a variety
568 of niches including the food processing environments by forming biofilms, which are more
569 resistant to environmental stresses, host-mediated responses, sanitizing agents, and
570 antimicrobial agents (Bremer *et al*., 2001). Antimicrobial activity of processed *H. elongata*
571 extracts against *P. aeruginosa* are presented in Table 7. Similar to Gram-negative *S. abony*,
572 there was also no processed seaweed extract which gave 100% inhibition against *P.*
573 *aeruginosa*. Antimicrobial activity of extracts from fresh *H. elongata* were strong in the first

574 dilution with 96.39% inhibition and moderate in the subsequent dilutions. Seaweed which
575 had been dried for 12 and 24 h achieved a maximum activity of 94.05%. Boiling led to a
576 significant reduction in antimicrobial activity of extracts against *P. aeruginosa.* Activity of
577 steamed *H. elongata* extracts ranged from 95.32 to 77.54% in the first to fourth dilutions.
578 Extracts from seaweed given a drying pre-treatment followed by steaming had less than half
579 the level of activity as compared to steaming alone. The antimicrobial activity of microwaved
580 extracts ranged from strong to weak (95.73 to 33.16%). In the present study, extracts from
581 fresh seaweeds had strong antimicrobial activity at a concentration as low as 3.93 mg/ml. In
582 the majority of reports on antimicrobial activities of seaweed extracts, bacterial growth
583 inhibiting activities were investigated using standard agar disc diffusion assays (Bansemir *et*
584 *al*., 2006; Kuda *et al*., 2007; Shanmughapriya *et al*., 2008). There have been few quantitative
585 reports on antimicrobial activity of seaweed extracts, however from those available; the
586 results of the present study have been shown to be more effective than reported by Dubber
587 and Harder (2008). These authors found extracts of *Ceramium rebrum* and *Laminaria*
588 *digitata* had strong activity at 10 and 31 mg/ml, respectively, which is less potent than those
589 of fresh seaweeds in the present study.
590
591 **4. Conclusion**
592 The findings of the present study indicate that the method of processing significantly
593 influences the concentrations of phytochemicals, antioxidant and antimicrobial parameters in
594 *H. elongata*. Consumption of *H. elongata* is dependant on some heat treatment in order to
595 achieve an edible texture. Since cooking invariably leads to a loss of antioxidant properties, a
596 compromise must be reached between palatability and nutrition. It was found that a
597 combination of drying followed by boiling reduced cooking time and led to less leaching of
598 phytochemicals. In terms of antioxidant activity of extract, a drying pre-treatment followed

by boiling enhanced the phenolic content per gram of extract and as a result less amount of the extract would be required to have a significant effect in food products. Processing significantly affects the antimicrobial activity of extracts from *H. elongata*. Extracts from fresh *H. elongata* had the highest antimicrobial activity against *L. monocytogenes*, *S. abony*, *E. faecalis* and *P. aeruginosa* with good inhibition as low as 4.16 mg/ml extract. A better knowledge of how these processing conditions affects the phytochemical compounds of interest is of pivotal importance. Reduction in the moisture content and cooking time also could have benefits in reducing transport and energy costs. Losses of health-related phytochemicals are thus likely to be a function of drying and cooking parameters such as time, temperature and degree of wounding stress to the plant during these processes.

Acknowledgements

The authors acknowledge funding from the Dublin Institute of Technology under the ABBEST Programme. The authors thank Denis Benson and Noel Grace from the Dublin Institute of Technology for their technical support and Manus Mc Gonagle for the supply of fresh seaweed.

624

625

626 **References**

627 Akindahunsi, A.A. and Oboh, G. 1999. Effect of some post-harvest treatments on the
628 bioavailability of zinc from some selected tropical vegetables. La Riv. Ital. Delle Sost. Gras.
629 285-287.

630 Akoh, C.C. and Min, B.D. 1997. Food Lipid Chemistry. In: Nutrition Biotechnology Marcel
631 Dekker Inc., New York.

632 Al-Azzawie, H.F. and Mohamed-Saiel, S.A. 2006. Hypoglycemic and antioxidant effect of
633 oleuropein in alloxan-diabetic rabbits. Life Sci. *78*, 1371-1377.

634 Amic, D., Davidovic-Amic, D. and Beslo, N. 2003. Trinajstic, Structure-radical scavenging
635 activity relationship of flavonoids. Croat. Chem. Acta. *76*, (1), 55-61.

636 Amin, I., Norazaidah Y. and Emmy Hainida, K. I. 2006. Antioxidant activity and phenolic
637 content of raw and boiled *Amaranthus* species. Food Chem. *94*, 47-52.

638 Aruoma, I.O. 1999. Antioxidant action of plant foods. Use of oxidative DNA damage, as a
639 tool for studying antioxidant efficacy. *Free Rad. Res. 30*, 419-427.

640 Bansemir, A., Blume, M., Schroder, S. and Lindequist, U. 2006. Screening of cultivated
641 seaweeds for antibacterial activity against fish pathogenic bacteria. Aquacult. *252*, 79-84.

642 Bremer, P.J., Monk, I. and Osborne, C.M. 2001. Survival of *Listeria monocytogenes* attached
643 to stainless steel surfaces in the presence or absence of Flavobacterium spp. J. Food Prot. *64*,
644 1369-1376.

645 Chandini, S.K., Ganesan, P. and Bhaskar, N. 2008. *In vitro* activities of three selected brown
646 seaweeds of India. Food Chem. *107*, 707-713.

647 Chiewchan, N., Praphraiphetch, C. and Devahastin, S. 2010. Effect of pretreatment on
648 surface topographical features of vegetables during drying. J. Food Eng. *101*, 41-48.

649 Chitlapilly-Dass, S., Abu-Ghannam, N., Antony-Babu, S. and Cummins, E.J. 2010. Ecology
650 and molecular typing of *L. monocytogenes* in a processing plant for cold-smoked salmon in
651 the Republic of Ireland. Food Res. Int. *43*, 1529–1536.
652 Cox, S., Gupta, S. and Abu-Ghannam, N. 2010. An assessment of the antioxidant and
653 antimicrobial properties of six species of edible Irish seaweeds. Int. Food Res. J. *17*, 205-220.
654 Duan, X.J., Zhang, W.W., Li, X.M. and Wang, B.G. 2006. Evaluation of antioxidant property
655 of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. Food Chem. *95*,
656 37-43.
657 Dubber, D. and Harder, T. 2008. Extracts of *Ceramium rubrum*, *Mastocarpus stellatus* and
658 *Laminaria digitata* inhibit growth of marine and fish pathogenic bacteria at ecologically
659 realistic concentrations. Aquacult. *274*, 196-200.
660 Duffy, C.F. and Power, R.F. 2001. Antioxidant and antimicrobial properties of some Chinese
661 plant extracts. Int. J. Antimicrob. Agents. *17*, 527-529.
662 Francisco, M., Velasco, P., Moreno, D.A., Garcia-Viguera, C. and Cartea, M.E. 2010.
663 Cooking methods of *Brassica rapa* affect the preservation of glucosinolates, phenolics and
664 vitamin C. Food Res. Int. *43*, 1455-1463.
665 Gahler, S., Otto K. and Bohm, V. 2003. Alterations of vitamin C, total phenolics, and
666 antioxidant capacity as affected by processing tomatoes to different products. J. Agricult.
667 Food Chem. *51*, 7962-7968.
668 Ismail, A., Marjan, Z.M. and Foong, C.W. 2004. Total antioxidant activity and phenolic
669 content in selected vegetables. Food Chem. *87*, 581-586.
670 Jormalainen, V. and Honkanen, T. 2004. Variation in natural selection for growth and
671 phlorotannins in the brown alga *Fucus vesiculosus*. Journal Evolution. Biol. *17* (4), 807-820.
672 Julkunen-Titto, R. 1985. Phenolic constituents in the leaves of northern willow: Methods for
673 the analysis of certain phenolics. J. Agricult. Food Chem. *33,* 213-217.

674 Kalemba, D. and Kunicka, A. 2003. Antibacterial and antifungal properties of essential oils.
675 Curr. Medic. Chem. *10*, 813-829.
676 Khandelwal, S., Udipi, S.A. and Ghugre, P. 2010. Polyphenols and tannins in Indian pulses:
677 Effect of soaking, germination and pressure cooking. Food Res. Int. *43*, 526-530.
678 Koivikko, R., Loponen, J., Pihlaja, K. and Jormalainen, V. 2007. High-performance liquid
679 chromatographic analysis of phlorotannins from the brown alga *Fucus vesiculosus*.
680 Phytochem. Anal. *18* (4), 326-332.
681 Kris-Etherton, P.M., Hecker, K.D., Bonanome, A. Coval, S.M., Binkoski, A.E. and Hilpert K.
682 F. 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease
683 and cancer. Amer. J. Med. *113*, 71-88.
684 Kuda, T., Kunii, T., Goto, H., Suzuki, T. and Yano, T. 2007. Varieties of antioxidant and
685 antibacterial properties of *Ecklonia stolonifera* and *Ecklonia kurome* products harvested and
686 processed in the Noto peninsula, Japan. Food Chem. *103*, 900-905.
687 Kuda, T., Tsunekawa, M., Goto, H. and Araki, Y. 2005. Antioxidant properties of four edible
688 algae harvested in the Noto Peninsula, Japan. J. Food. Comp. Anal. *18*, 7, 625-633.
689 Lee, W.C., Lee, M.J., Kim, J.S. and Park, S.Y. 2001. Foodborne illness outbreaks in Korea
690 and Japan studied retrospectively. J. Food Prot. *64* (6), 899-902.
691 Leskova, E. Kubikova, J., Kovacikova, E., Kosicka, M., Porubska, J. and Holcikova, K.
692 2006. Vitamin losses: Retention during heat treatment and continual changes expressed by
693 mathematical models. J. Food. Comp. Anal. *19* (4), 252-276.
694 Lim, Y.Y. and Murtijaya, J. 2007. Antioxidant properties of *Phyllanthus amarus* extracts as
695 affected by different drying methods. LWT- Food Sci. Technol. *40*, 1664-1669.
696 Matsukawa, R., Dubinsky, Z., Kishimoto, E., Masaki, K.F.Y. and Takeuchi, T. 1997. A
697 comparison of screening methods for antioxidant activity in seaweeds. J. Appl. Phycol. *9,* 29-
698 35.

699 Molyneux, P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for
700 estimating antioxidant activity. J. Sci. Technol. *26* (2), 211-219.
701 Nikaido, H. 1996. Outer membrane. In: Neidhardt, F.C. (Ed.), *Escherichia coli* and
702 *Salmonella typhimurium*: Cellular and Molecular Biology. Amer. S. Microbiol. Press.
703 Washington, D.C., pp. 29–47.
704 Nishinari, K. 2004. Rheology, food texture, and mastication. J. Text. Stud. *35*, 113-124.
705 Oboh, G. 2005. Effect of boiling on the antioxidant properties of some tropical green leafy
706 vegetables. LWT. *38*, 513-517.
707 Olivera, D.F., Vina, S.Z., Marani, C.M., Ferreyra, R.M., Mugridge, A., Chaves, A.R. and
708 Mascheroni, R.H. 2008. Effect of boiling on the quality of Brussels sprouts (*Brassica*
709 *oleracea* L. *gemmifera* DC) after frozen storage. J. Food Eng. *84*, 148-155.
710 Parada, J. and Aguilera, J.M. 2007. Food microstructure affects the bioavailability of several
711 nutrients. J. Food Sci. *72*, R21-R32.
712 Reaven, P.D. and Witzum, J.L. 1996. Oxidised LDL in atherogenesis. Role of dietary
713 modification. Ann. Rev. Nutri. *16*, 51-71.
714 Rincé, A. Le Breton, Y., Verneuil, N., Giard, J.C., Hartke, A. and Auffray, Y. 2003.
715 Physiological and molecular aspects of bile salt response in *Enterococcus faecalis*. Int. J.
716 Food Microbiol. *88*, 207-213
717 Rodrigues, A.S., Pérez-Gregorio, M.R., Garcia-Falcon, M.S. and Simal-Gándara, J. 2009.
718 Effect of curing and cooking on flavonols and anthocyanins in traditional varieties of onion
719 bulbs. Food Res. Int. *42*, 1331-1336.
720 Rungapamestry, V., Duncan, A.J., Fuller, Z. and Ratcliffe, B. 2007. Effect of cooking
721 *Brassica* vegetables on the subsequent hydrolysis and metabolic fate of glucosinolates. Proc.
722 Nutri. Soc. *66* (1), 69-81.

723 Sakihama, Y., Michael, F., Cohen, M.F., Stephen, C., Grace, S.C. and Yamasaki, H. 2002.
724 Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage
725 mediated by metals in plants. Toxicol, *177*, 67-80.
726 Shan, B., Cai, Y.Z., Brooks, J.D. and Corke, H. 2007. The *in vitro* antibacterial activity of
727 dietary spice and medicinal herb extracts. Int. J. Food Microbiol. *117*, 112-119.
728 Shanmughapriya, S., Manilal, A., Sujith, S., Selvin, J., Segal-Kiran, G. and
729 Natarajaseenivasan, K. 2008. Antimicrobial activity of seaweeds extracts against
730 multiresistant pathogens. Annals. Microbiol. *58* (3), 535-541.
731 Shon, M.Y., Kim, T.H. and Sung, N.J. 2003. Antioxidants and free radical scavenging
732 activity of *Phellinus baumii* (*Phellinus* of Hymenochaetaceae) extracts. Food Chem. *82*, 593-
733 597.
734 Shahidi, F. 2009. Neutraceuticals and functional foods: Whole versus processed foods.
735 Trends Food Sci Tech. *20* (9), 376-387.
736 Shukla, V.K.S, Wanasundara, P.K.J.P.D. and Shahidi, F. 1997. Natural antioxidants from
737 oilseeds. In: Shahidi, F. (Eds), Natural antioxidants: Chemistry, Health Effects, and
738 Applications. AOCS Press, Champaign, IL, (pp. 97-132).
739 Somsub, W., Kongkachuichai, R., Sungpuag, P. and Charoensiri, P. 2008. Effects of three
740 conventional cooking methods on vitamin C, tannin, myo-inositol phosphates contents in
741 selected Thai vegetables. J. Food Comp. Anal. *21*, 187-197.
742 Sultana, B., Anwar, F. and Iqbal, S. 2008. Effect of different cooking methods on the
743 antioxidant activity of some vegetables from Pakistan. Int. J. Food Sci. Technol. *43*, 560-567.
744 Taga, M.S., Miller, E.E. and Pratt, D.E. 1984. Chia seeds as a source of natural lipid
745 antioxidants. J. Amer. Oil Chem. Assoc. *61*, 928-931.
746 Turkmen, N., Sari, F. and Velioglu, Y.S. 2005. The effect of cooking methods on total
747 phenolics and antioxidant activity of selected green vegetables. Food Chem. *93*, 713-718.

748 Vairappan, C.S., Daitoh, M., Suzuki, M., Abe, T. and Masuda, M. 2001. Antibacterial
749 halogenated metabolites from the Malaysian *Laurencia* species. Phytochem. *58*, 291-297.
750 Volden, J., Borge, G.I.A., Hansen, M., Wicklund, T. and Bengtsson, G.B. 2009. Processing
751 (blanching, boiling, steaming) effects on the content of glucosinolates and antioxidant-related
752 parameters in cauliflower (*Brassica oleracea* L. ssp. *botrytis*). LWT- Food Sci. Technol. *42*,
753 63-73.
754 Watchtel-Galor, S., Wong, K.W. and Benzie, I.F.F. 2008. The effect of cooking on *Brassica*
755 vegetables. Food Chem. *110*, 706-710.
756 Wong, H.C., Liu, S.H., Ku, L.W., Lee, I.Y., Wang, T.K. and Lee, Y.S. 2000.
757 Characterization of *Vibrio parahaemolyticus* isolates obtained from foodborne illness
758 outbreaks during 1992 through 1995 in Taiwan. J. Food Prot. *63* (7), 900-906.
759 Xu, B. and Chang, S.K.C. 2008. Effect of soaking, boiling and steaming on total phenolic
760 content and antioxidant activities of cool season food legumes. Food Chem. 110, 1-13.
761 Yen, G.C. and Chen, H.Y. 1995. Antioxidant activity of various tea extracts in relation to
762 their antimutagenicity. J. Agricult. Food Chem. *43*, 27-37.
763 Zhang, D. and Hamauzu, Y. 2004. Phenolics, ascorbic acid, carotenoids and antioxidant
764 activity of broccoli and their changes during conventional and microwave cooking. Food
765 Chem. *88*, 503-509.
766 Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents
767 in mulberry and their scavenging affects on superoxide radicals. Food Chem. *64*, 555-559.
768
769
770
771
772

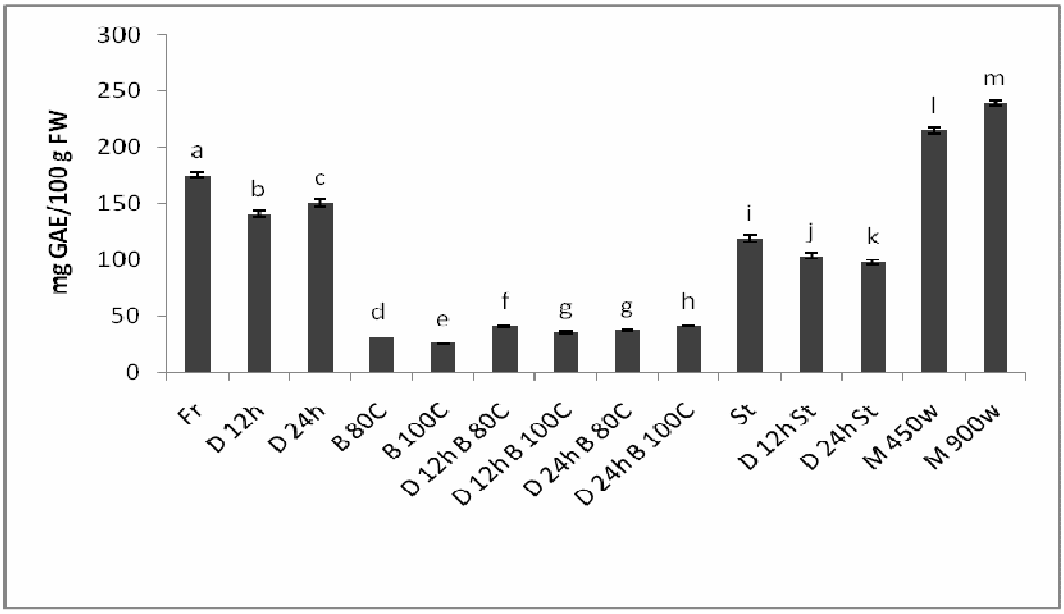
Tables and Figures

Table 1. Instrumental and sensory texture evaluation to determine edible texture level of cooked *H. elongata*

Cooking time (mins)	Instrumental texture (N/mm)	Sensory panel judgements*
0	45.33±1.30	Too hard and chewy
5	40.47±2.31	Too hard and chewy
10	37.38±1.93	Too hard and chewy
15	35.89±0.98	Too hard and chewy
20	34.62±1.61	Too hard and chewy
25	33.98±0.79	Too hard and chewy
30	32.40±0.15	Edible
35	32.11±0.74	Edible
40	30.42±1.05	Edible
45	30.22±0.95	Edible
50	30.09±1.02	Edible

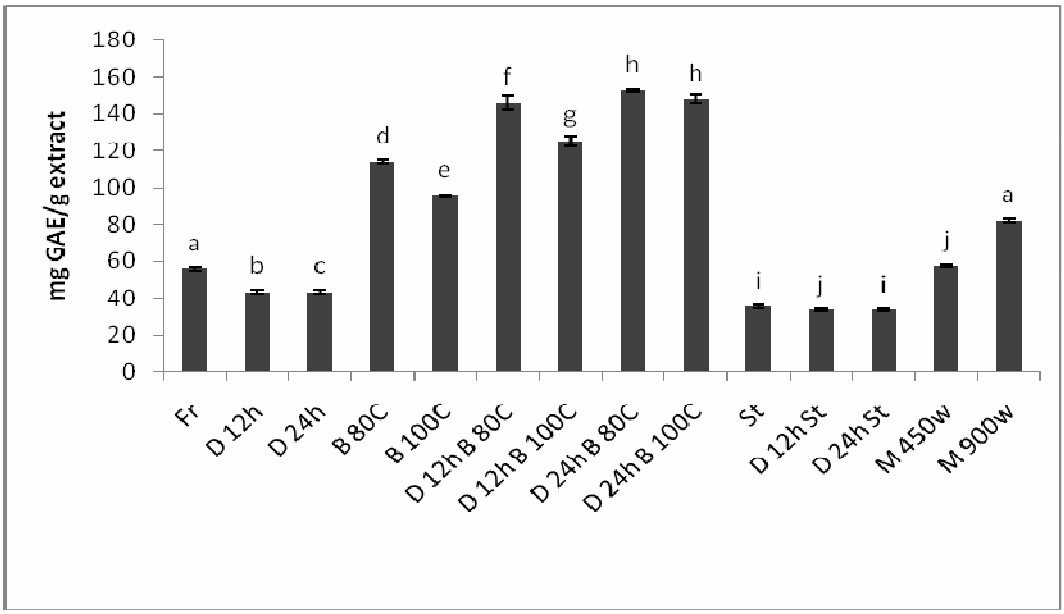
*Sensory panel judgements were based on the hardness and ease of chew of the seaweed.
Each value is presented as mean ± SD (n = 6).
Seaweeds were boiled at 100°C

Fig. 1 (A) Total phenolic content of processed *H. elongata* (mg gallic acid equivalents/100 g FW)



Each value is presented as mean ± SD (n = 6).
Means above each bar with different letters (a-m) differ significantly ($p < 0.05$)

Fig. 1 (B) Total phenolic content of processed *H. elongata* extract (mg gallic acid equivalents/g extract)



Each value is presented as mean ± SD (n = 6).
Means above each bar with different letters (a-j) differ significantly ($p < 0.05$)
Where each abbreviation is as follows. Fr: fresh; D 12h: Dried 12 hours; D 24h: Dried 24 hours; B 80C: Boiled 80°C; B 100C: Boiled 100°C; D 12h B 80C: Dried 12 hours and Boiled 80°C; D 12h B 100C: Dried 12 hours and Boiled 100°C; D 24h B 80C: Dried 24 hours and Boiled 80°C; D 24h B 100C: Dried 24 hours and Boiled 100°C; St: Steamed; D 12h St: Dried 12 hours and Steamed; D 24h St: Dried 24 hours and Steamed; M 450w: Microwaved at 450 Watts; M 900w: Microwaved at 900 Watts.

Table 2. Yield of total extract (as % w/w of seaweed on fresh weight basis), DPPH radical scavenging activity (%) of processed *H. elongata* extracts (concentration 100 µg/ml) and EC_{50} (µg/ml)* of each extract

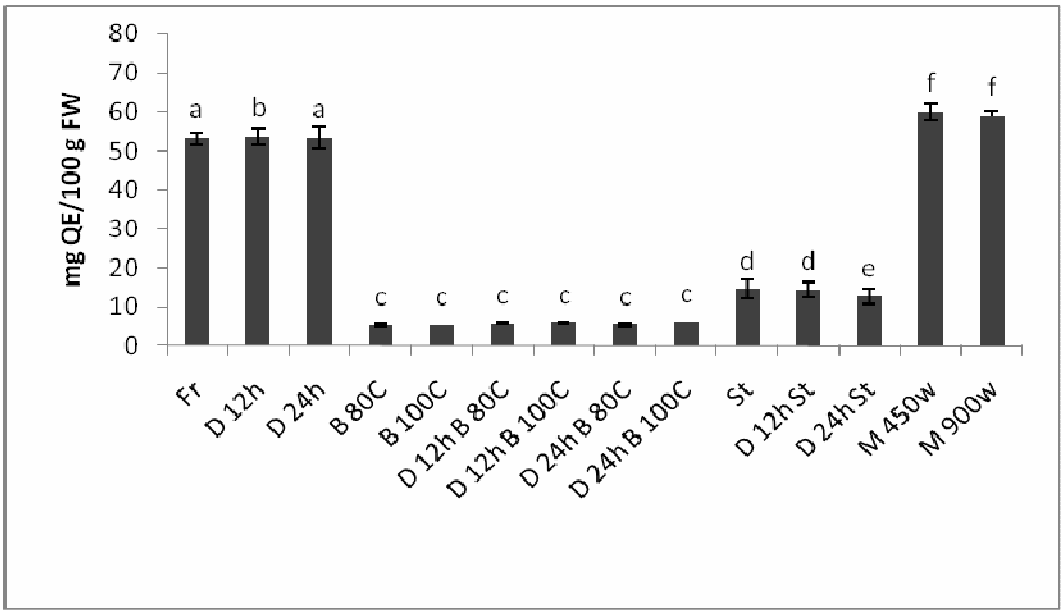
Processing treatment	Total methanol extract (%)	DPPH radical scavenging activity (%)	EC_{50} (µg/ml)
Fresh	3.14±0.19[a]	75.50±2.30[a]	25.00±1.81[a]
Dried 12h	3.25±0.20[a]	74.69±2.31[b]	50.00±3.31[b]
Dried 24h	3.48±0.29[ab]	67.87±1.05[c]	50.00±2.55[b]
Boiled 80ºC - 40 min	0.27±0.09[c]	92.96±1.55[d]	12.50±1.12[c]
Boiled 100ºC - 35 min	0.26±0.06[c]	93.54±1.24[e]	12.50±2.00[c]
Dried 12h and boiled 80ºC - 30 min	0.28±0.05[c]	95.33±0.80[f]	12.50±1.00[c]
Dried 12h and boiled 100ºC - 25 min	0.28±0.06[c]	100.00±0.00[g]	12.50±1.02[c]
Dried 24h and boiled 80ºC - 30 min	0.24±0.02[c]	88.50±1.24[h]	12.50±1.21[c]
Dried 24h and boiled 100ºC - 20 min	0.27±0.02[c]	89.04±1.85[i]	12.50±1.00[c]
Steamed - 45 min	3.37±0.21[a]	52.51±0.56[j]	100.00±0.98[d]
Dried 12h and steamed - 50 min	3.31±0.19[a]	53.79±1.88[k]	100.00±1.99[d]
Dried 24h and steamed - 50 min	3.30±0.20[a]	53.50±0.97[l]	100.00±0.96[d]
Microwaved 450w - 30 s	3.75±0.55[b]	76.29±1.57[m]	25.00±1.52[a]
Microwaved 900w - 20 s	4.34±0.56[d]	75.35±2.00[n]	25.00±1.88[a]

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-n) differ significantly ($p < 0.05$)

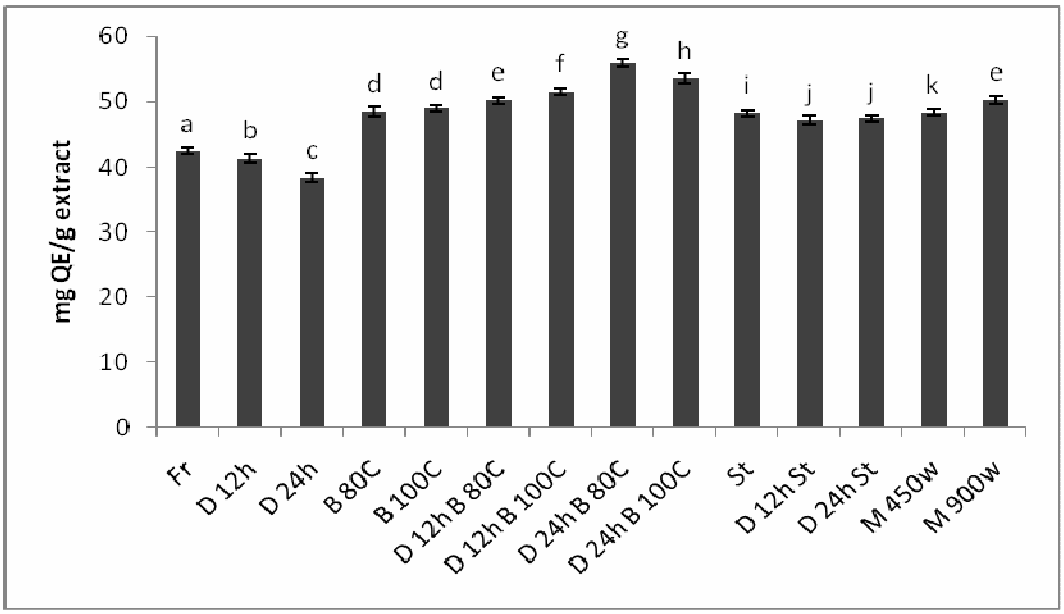
*EC_{50} value is defined as the amount of extract necessary to decrease the initial DPPH radical concentration by 50%.

Fig. 2 (A) Total flavanoid content of processed *H. elongata* (mg quercetin equivilants/100 g FW)



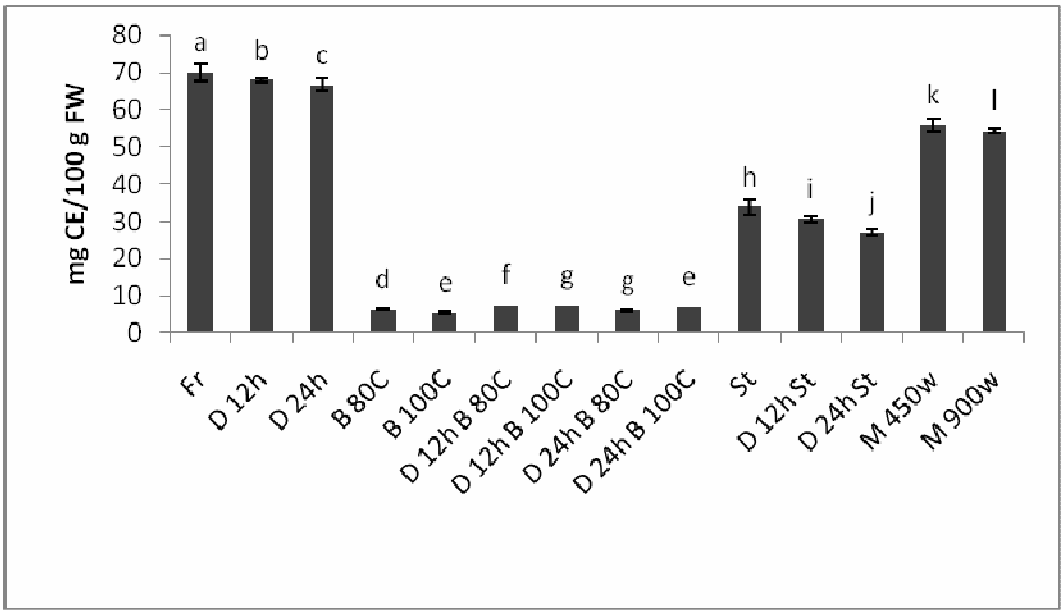
Each value is presented as mean ± SD (n = 6).
Means above each bar with different letters (a-f) differ significantly ($p < 0.05$)

Fig. 2 (B) Total flavanoid content of processed *H. elongata* extract (mg quercetin equivalents/g extract)



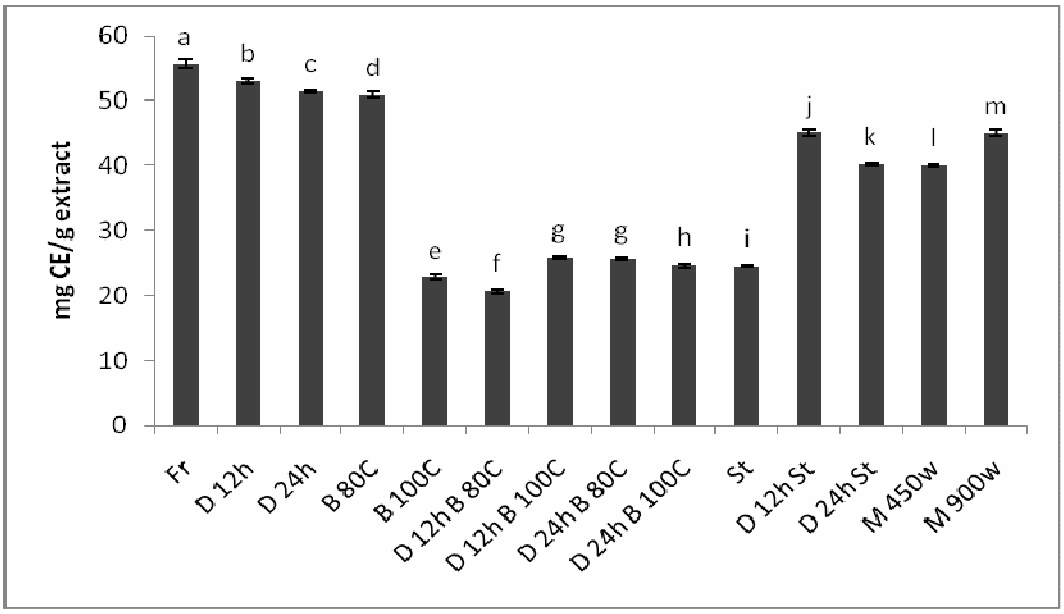
Each value is presented as mean ± SD (n = 6).
Means above each bar with different letters (a-k) differ significantly ($p < 0.05$)
Where each abbreviation is as follows. Fr: fresh; D 12h: Dried 12 hours; D 24h: Dried 24 hours; B 80C: Boiled 80°C; B 100C: Boiled 100°C; D 12h B 80C: Dried 12 hours and Boiled 80°C; D 12h B 100C: Dried 12 hours and Boiled 100°C; D 24h B 80C: Dried 24 hours and Boiled 80°C; D 24h B 100C: Dried 24 hours and Boiled 100°C; St: Steamed; D 12h St: Dried 12 hours and Steamed; D 24h St: Dried 24 hours and Steamed; M 450w: Microwaved at 450 Watts; M 900w: Microwaved at 900 Watts.

Fig. 3 (A) Total condensed tannin content of processed *H. elongata* (mg catechin equivilants/100 g FW)



Each value is presented as mean ± SD (n = 6).
Means above each bar with different letters (a-l) differ significantly ($p < 0.05$)

Fig. 3 (B) Total condensed tannin content of processed *H. elongata* extract (mg catechin equivalents/g extract)



Each value is presented as mean ± SD (n = 6).
Means above each bar with different letters (a-n) differ significantly ($p < 0.05$)
Where each abbreviation is as follows. Fr: fresh; D 12h: Dried 12 hours; D 24h: Dried 24 hours; B 80C: Boiled 80°C; B 100C: Boiled 100°C; D 12h B 80C: Dried 12 hours and Boiled 80°C; D 12h B 100C: Dried 12 hours and Boiled 100°C; D 24h B 80C: Dried 24 hours and Boiled 80°C; D 24h B 100C: Dried 24 hours and Boiled 100°C; St: Steamed; D 12h St: Dried 12 hours and Steamed; D 24h St: Dried 24 hours and Steamed; M 450w: Microwaved at 450 Watts; M 900w: Microwaved at 900 Watts.

881 **Table 3. Concentration of *H. elongata* extracts (mg/ml) from different processed**
882 **seaweeds (5 g original seaweed) for each dilution tested**
883

Processing treatment	Dilution 1 (mg/ml)	Dilution 2 (mg/ml)	Dilution 3 (mg/ml)	Dilution 4 (mg/ml)
Fresh	31.44	15.72	7.86	3.93
Dried 12h	32.47	16.23	8.12	4.06
Dried 24h	34.77	17.39	8.69	4.35
Boiled 80ºC - 40 min	2.73	1.36	0.68	0.34
Boiled 100ºC - 35 min	2.67	1.33	0.67	0.33
Dried 12h and boiled 80ºC - 30 min	2.80	1.40	0.70	0.35
Dried 12h and boiled 100ºC - 25 min	2.82	1.41	0.70	0.35
Dried 24h and boiled 80ºC - 30 min	2.42	1.21	0.61	0.30
Dried 24h and boiled 100ºC - 20 min	2.77	1.38	0.69	0.35
Steamed - 45 min	34.00	17.00	8.50	4.25
Dried 12h and steamed - 50 min	31.00	15.50	7.75	3.88
Dried 24h and steamed - 50 min	29.30	14.65	7.33	3.66
Microwaved 450w - 30 s	37.56	18.78	9.39	4.69
Microwaved 900w - 20 s	43.47	21.74	10.87	5.43

884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904

905 **Table 4. Percentage inhibition of methanolic extracts of *H. elongata* processed under**
906 **different conditions against *L. monocytogenes***
907

Processing treatment	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Fresh	100.00 ± 0.0^{aw}	100.00 ± 0.00^{aw}	99.37 ± 1.24^{ax}	98.09 ± 3.81^{ay}
Dried 12h	100.00 ± 0.0^{aw}	89.88 ± 1.70^{bx}	43.89 ± 1.43^{by}	31.68 ± 2.08^{bz}
Dried 24h	100.00 ± 0.0^{aw}	92.99 ± 8.16^{cx}	29.84 ± 2.19^{cy}	26.98 ± 6.72^{cz}
Boiled 80ºC - 40 min	27.05 ± 0.60^{bw}	0.00 ± 0.00^{dx}	0.00 ± 0.00^{dx}	0.00 ± 0.00^{dx}
Boiled 100ºC - 35 min	46.45 ± 3.42^{cw}	0.00 ± 0.00^{dx}	0.00 ± 0.00^{dx}	0.00 ± 0.00^{dx}
Dried 12h and boiled 80ºC - 30 min	24.95 ± 3.51^{dw}	10.00 ± 1.71^{ex}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 12h and boiled 100ºC - 25 min	21.02 ± 2.54^{ew}	14.00 ± 0.25^{fx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 24h and boiled 80ºC - 30 min	10.48 ± 3.23^{fw}	9.81 ± 1.15^{gx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 24h and boiled 100ºC - 20 min	23.20 ± 2.54^{gw}	15.89 ± 1.68^{hx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Steamed - 45 min	96.34 ± 3.64^{hw}	96.24 ± 1.56^{ix}	81.54 ± 5.56^{ey}	77.88 ± 5.54^{ez}
Dried 12h and steamed - 50 min	43.25 ± 3.23^{iw}	35.14 ± 0.25^{jx}	29.57 ± 1.77^{fy}	20.45 ± 0.78^{fz}
Dried 24h and steamed - 50 min	43.85 ± 0.23^{jw}	34.15 ± 1.12^{kx}	28.89 ± 1.89^{gy}	19.55 ± 0.98^{gz}
Microwaved 450w - 30 s	97.24 ± 0.05^{kw}	82.90 ± 5.90^{lx}	48.73 ± 1.57^{hy}	29.41 ± 5.45^{hz}
Microwaved 900w - 20 s	97.26 ± 1.25^{ew}	81.45 ± 3.07^{mx}	74.20 ± 1.84^{iy}	72.86 ± 3.10^{iz}

908 Each value is presented as mean ± SD (n = 6).
909 Means within each column with different letters (a-m) differ significantly ($p < 0.05$)
910 Means within each row with different letters (w-z) differ significantly ($p < 0.05$)
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928

Table 5. Percentage inhibition of methanolic extracts of *H. elongata* processed under different conditions against *S. abony*

Processing treatment	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Fresh	87.03±3.91^{aw}	78.80±1.41^{ax}	65.13±4.60^{ay}	37.18±5.71^{az}
Dried 12h	77.29±1.26^{bw}	39.24±2.27^{bx}	26.83±3.75^{by}	11.01±2.06^{bz}
Dried 24h	83.52±4.23^{cw}	49.01±3.08^{cx}	27.17±4.02^{cy}	10.99±1.07^{cz}
Boiled 80ºC - 40 min	10.37±3.97^{dw}	5.50±0.12^{dx}	0.00±0.00^{dy}	0.00±0.00^{dy}
Boiled 100ºC - 35 min	16.56±4.11^{ew}	0.00±0.00^{ex}	0.00±0.00^{dx}	0.00±0.00^{dx}
Dried 12h and boiled 80ºC - 30 min	13.73±1.09^{fw}	10.34±0.89^{fx}	0.00±0.00^{dy}	0.00±0.00^{dy}
Dried 12h and boiled 100ºC - 25 min	13.87±1.69^{gw}	6.01±1.29^{gx}	0.00±0.00^{dy}	0.00±0.00^{dy}
Dried 24h and boiled 80ºC - 30 min	22.11±2.98^{hw}	10.61±1.68^{gx}	0.00±0.00^{dy}	0.00±0.00^{dy}
Dried 24h and boiled 100ºC - 20 min	15.54±1.75^{iw}	11.02±4.87^{hx}	0.00±0.00^{dy}	0.00±0.00^{dy}
Steamed - 45 min	93.23±2.51^{jw}	93.04±2.51^{ix}	82.45±4.25^{ey}	77.12±4.45^{ez}
Dried 12h and steamed - 50 min	44.21±3.78^{kw}	35.78±0.29^{jx}	29.87±2.51^{fy}	20.12±2.58^{fz}
Dried 24h and steamed - 50 min	44.12±2.53^{kw}	36.15±1.25^{kx}	28.78±1.88^{gy}	19.70±1.80^{gz}
Microwaved 450w - 30 s	93.00±0.98^{lw}	58.65±1.29^{lx}	48.62±1.58^{hy}	30.64±1.35^{hz}
Microwaved 900w - 20 s	94.40±1.58^{mw}	64.09±2.26^{mx}	53.07±5.98^{iy}	31.28±3.78^{iz}

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-m) differ significantly ($p < 0.05$)

Means within each row with different letters (w-z) differ significantly ($p < 0.05$)

Table 6. Percentage inhibition of methanolic extracts of *H. elongata* processed under different conditions against *E. faecalis*

Processing treatment	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Fresh	100.00 ± 0.00^{aw}	99.61 ± 0.66^{ax}	87.03 ± 1.41^{ay}	78.02 ± 0.98^{az}
Dried 12h	100.00 ± 0.00^{aw}	39.24 ± 2.27^{bx}	36.30 ± 6.50^{by}	33.89 ± 5.10^{bz}
Dried 24h	95.40 ± 0.72^{bw}	53.01 ± 3.08^{cx}	42.28 ± 0.08^{cy}	29.96 ± 1.38^{cz}
Boiled 80ºC - 40 min	7.31 ± 1.25^{cw}	5.50 ± 0.12^{dx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Boiled 100ºC - 35 min	10.52 ± 2.54^{dw}	0.00 ± 0.00^{ex}	0.00 ± 0.00^{dx}	0.00 ± 0.00^{dx}
Dried 12h and boiled 80ºC - 30 min	49.45 ± 1.26^{ew}	10.34 ± 0.89^{fx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 12h and boiled 100ºC - 25 min	22.11 ± 4.32^{fw}	6.01 ± 1.29^{dx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 24h and boiled 80ºC - 30 min	16.67 ± 2.46^{gw}	10.61 ± 1.68^{gx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 24h and boiled 100ºC - 20 min	33.72 ± 3.92^{hw}	11.02 ± 4.87^{gx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Steamed - 45 min	95.21 ± 1.29^{iw}	93.54 ± 2.51^{hx}	81.47 ± 1.58^{ey}	76.42 ± 2.57^{ez}
Dried 12h and steamed - 50 min	42.32 ± 5.29^{jw}	35.78 ± 0.29^{ix}	28.74 ± 2.50^{fy}	19.99 ± 1.54^{fz}
Dried 24h and steamed - 50 min	44.57 ± 0.98^{kw}	36.15 ± 1.25^{jx}	29.87 ± 1.75^{gy}	18.78 ± 1.24^{gz}
Microwaved 450w - 30 s	90.12 ± 2.54^{lw}	48.62 ± 1.29^{kx}	31.13 ± 2.01^{hy}	23.48 ± 2.06^{hz}
Microwaved 900w - 20 s	95.81 ± 4.58^{mw}	53.07 ± 2.26^{cx}	43.61 ± 3.39^{iy}	24.42 ± 5.14^{iz}

Each value is presented as mean ± SD (n = 6).

Means within each column with different letters (a-m) differ significantly ($p < 0.05$)

Means within each row with different letters (w-z) differ significantly ($p < 0.05$)

977 **Table 7. Percentage inhibition of methanolic extracts of *H. elongata* processed under**
978 **different conditions against *P. aeruginosa***
979

Processing treatment	Dilution 1	Dilution 2	Dilution 3	Dilution 4
Fresh	96.39 ± 2.55^{aw}	89.11 ± 3.31^{ax}	74.63 ± 6.64^{ay}	51.11 ± 5.84^{az}
Dried 12h	72.80 ± 5.22^{bw}	48.77 ± 5.26^{bx}	17.71 ± 3.02^{by}	1.93 ± 0.72^{bz}
Dried 24h	94.05 ± 4.03^{cw}	41.19 ± 5.8^{cx}	22.88 ± 7.57^{cy}	12.16 ± 0.18^{cz}
Boiled 80ºC - 40 min	6.45 ± 2.00^{dw}	5.46 ± 1.23^{dx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Boiled 100ºC - 35 min	34.74 ± 1.52^{ew}	5.03 ± 5.27^{ex}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 12h and boiled 80ºC - 30 min	22.94 ± 2.34^{fw}	22.92 ± 1.56^{fx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 12h and boiled 100ºC - 25 min	23.09 ± 1.20^{gw}	12.19 ± 1.15^{gx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 24h and boiled 80ºC - 30 min	24.21 ± 1.60^{hw}	10.21 ± 1.07^{hx}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Dried 24h and boiled 100ºC - 20 min	34.12 ± 3.24^{iw}	20.98 ± 3.24^{ix}	0.00 ± 0.00^{dy}	0.00 ± 0.00^{dy}
Steamed - 45 min	95.32 ± 2.99^{jw}	85.66 ± 1.58^{jx}	83.45 ± 1.89^{ey}	77.54 ± 3.45^{ex}
Dried 12h and steamed - 50 min	44.14 ± 4.54^{kw}	34.21 ± 0.98^{kx}	27.89 ± 2.14^{fy}	19.78 ± 2.54^{fz}
Dried 24h and steamed - 50 min	43.25 ± 1.24^{lw}	36.47 ± 0.16^{lx}	28.77 ± 2.12^{gy}	19.10 ± 2.88^{gz}
Microwaved 450w - 30 s	93.44 ± 5.48^{mw}	70.25 ± 7.30^{mx}	46.73 ± 3.56^{hy}	33.16 ± 2.31^{hz}
Microwaved 900w - 20 s	95.73 ± 4.84^{nw}	70.21 ± 1.77^{mx}	46.85 ± 5.84^{iy}	37.73 ± 4.11^{iz}

980 Each value is presented as mean ± SD (n = 6).
981 Means within each column with different letters (a-n) differ significantly ($p < 0.05$)
982 Means within each row with different letters (w-z) differ significantly ($p < 0.05$)
983
984
985
986
987
988
989
990
991
992
993
994