Articles

2013-6

Enhancement of the Phytochemical and Fibre Content of Beef-Patties with Himanthalia Elongata Seaweed

Sabrina Cox
sabrina.cox@tudublin.ie

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

Recommended Citation

1 **Enhancement of the phytochemical and fibre content of beef-patties with**

2 *Himanthalia elongata* **seaweed**

3

4 Sabrina Cox, Nissreen Abu-Ghannam*

5

6 School of Food Science and Environmental Health,

7 College of Sciences and Health,

8 Dublin Institute of Technology, Cathal Brugha St.,

9 Dublin 1, Ireland.

10

11

12

13

14

15

16

17

18

19

20

21

22

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

24 **Tel:** +353-1-402-7570; **Fax:** +353-1-878-8978

25 **email:** nissreen.abughannam@dit.ie

Abstract

The effect of adding *Himanthalia elongata* seaweed (10 - 40% w/w) as a source of antioxidants and dietary fibre on physical, chemical, microbial and sensory traits of cooked beef patties was studied throughout chilled storage. Patties with seaweed showed reduced cooking losses and were nearly 50% more tender as compared to patties without seaweed. Microbiological counts and lipid oxidation were significantly lower in patties containing seaweed ($P < 0.05$), by day 30 of storage there was no bacterial growth in samples with $\geq 20\%$ seaweed and lipid oxidation levels were low (0.61 mg malondialdehyde/kg of sample). Seaweed incorporation significantly increased the dietary fibre (1.64 g per 100 g fw in 40% seaweed-patties), total phenolic content (up to 28.11 mg GAE/100 g fw) and DPPH radical scavenging activity (up to 52.32%) of patties compared to the control. Sensory analysis indicated that the seaweed-patties were accepted by consumers in terms of aroma, appearance, texture and taste. Patties containing 40% seaweed were rated highest in terms of overall acceptability, most likely due to improvement in texture and mouthfeel. Addition of seaweed in the formulation of beef patties leads to the enhancement of the nutritional and technological quality together with an acceptable sensory quality.

1. Introduction

Growing understanding of the relationship between diet and health is leading to new insights into the effect of food ingredients on physiological function and health,

50   inducing consumer demand for healthy, nutritious foods with additional health

51   promoting functions (Jiménez-Colmenero et al., 2010). Many new products have

52   been developed and marketed, offering increased health benefits and the potential to

53   reduce the risk of diseases. Sales of such "functional foods" in Europe have

54   increased significantly (Annunziata & Vecchio, 2011). Many components may be

55   added to meat, dairy, fish or vegetable-based products to make them ''functional'',

56   such as ω-3 fatty acids, prebiotics, probiotics and fibre (Jiménez-Colmenero, 2007).

57   Over the past few decades, meat products have come under increasing scrutiny by

58   medical, nutritional and consumer groups because of the associations established

59   between their consumption (or that of a number of their constituents, such as fat and

60   cholesterol) and the risk of some of the major degenerative and chronic diseases

61   (ischaemic heart disease, cancer, hypertension and obesity). Therefore meat-based

62   functional foods are being seen as an opportunity to improve the "image" of meat

63   and address consumer needs, and also to update the nutritional and dietary goals

64   (Jiménez-Colmenero, 2007). As meat is one of the most important commonly-

65   consumed fast foods, it offers an excellent way of promoting intake of functional

66   ingredients without any radical changes in eating habits (Cofrades et al., 2008). This

67   situation is prompting the emergence of new "healthier" meat products. Most

68   physiologically active substances come from plants, and when combined with other

69   foods such as meat, they can help provide a food with functional effects. The idea of

70   using plant products in the meat industry is not entirely new, as various types of

71   ingredients have been used for their technological, sensory, economic and nutritional

72   effects (Jiménez-Colmenero, 2010).

73   Meat is low in dietary fibre, therefore addition of ingredients containing fibre to

74   common meat products such as patties would be beneficial. Dietary fibre intake

75   provides many health benefits such as reducing the risk of developing diseases

76   including coronary heart disease, stroke, hypertension, diabetes, obesity and certain

77   gastrointestinal disorders. Furthermore, increased consumption of dietary fibre

78   improves serum lipid concentrations, lowers blood pressure, improves blood glucose

79   control in diabetes, promotes regularity, aids in weight loss and appears to improve

80   the immune function (Anderson et al., 2009).

81   Seaweeds are known to be a good source of dietary fibre (Cofrades et al., 2008).

82   Plant biomass or its derived bioactive compounds have been considered as possible

83   functional components in processed meat products for alleviation of the colourectal

84   cancer risk associated with the consumption of processed meats (Demeyer et al.,

85   2008). The introduction of functional ingredients such as botanicals, plant extracts

86   and seaweeds with probable biological activity into processed meat products is

87   receiving abundant attention (Calvo et al., 2008; Cofrades et al., 2008; Hayes et al.,

88   2005; Hernández-Hernández et al., 2009; Valencia et al., 2008). Seaweeds are also

89   high in phytochemicals such as phenolic compounds (Cox et al., 2011). Such natural

90   plant phytochemicals could therefore add further functional ingredients to meat

91   based convenience food products such as beefburgers. It has been reported that 34%

92   of men and 21.9% of women consume burgers in Ireland (Duffy et al., 2005),

93   therefore incorporation of seaweed into such beef patties would have potential as a

94   means of developing a healthier meat product.

95   The aim of this study was to investigate the addition of seaweed at varying

96   concentrations to beef burger patties in order to enhance the levels of fibre and

97   phytochemicals. The effect on sensory properties such as texture, colour and flavor

98   were investigated as were safety aspects such as bacterial enumeration and lipid

99   oxidation which are important principals of product development.

2. Materials and methods

Chemicals

1,1,3,3-tetramethoxyropane solution, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate (Na_2CO_3), Thiobarbituric Acid (TBA), total dietary fibre kit and tricholoroacetic acid (TCA) were purchased from Sigma Aldrich Chemie (Steinheim, Germany). Peptone water and plate count agar (PCA) were purchased from Sparks (Dublin, Ireland).

Seaweed material

Himanthalia elongata (H. elongata) was purchased from Quality Sea Veg., Co Donegal, Ireland. The seaweeds were collected in October 2011 and stored at 4 °C until further use.

Preparation of samples

H. elongata was washed thoroughly with tap water to remove epiphytes and salt, dried with absorbent paper and then cut into 3 cm long pieces before dehydration.

Dehydration and rehydration procedure

Dehydration was carried out as optimized in our previous studies (Gupta et al., 2011). Seaweed samples (5 g) were placed on a drying tray in a single layer. Drying of seaweed was carried out in a drier (Innova 42, Mason Technology, Ireland) at 40

121 °C air drying temperature over a period of 2 hours. Air velocity was 2.0 ± 0.1 m s^{-1}

122 measured with VWR Enviro-meter digital anemometer (VWR, Ireland). Dried

123 seaweed was rehydrated by immersion in 2 L of distilled water at 80.5 ± 0.05 °C for

124 20 ± 0.05 min as optimized in our previous studies (Cox et al., 2011). The seaweed

125 was then ground using a blender (Rotor, Germany) and stored at 4 °C until use.

126

Seaweed-patty preparation

128 Five different patty formulations were prepared containing 0, 10, 20, 30 and 40%

129 blanched seaweed. Lean beef ($\leq 5\%$ fat) was purchased from a local supermarket and

130 stored immediately in a refrigerator at 4 °C. Meat was cut into smaller pieces using a

131 sterile knife and ground in a meat grinder with a grind size of 4.5mm (Meteor

132 MATR, Ireland) which had been previously sterilised and chilled (4 °C). The

133 seaweed was added to each of the mixtures in sterile bowls and mixed by hand with

134 sterile utensils until the seaweed was homogenous throughout the meat. The final

135 temperature of the meat was < 12 °C in all cases and was formed with a manual

136 circular shaped mould. The patties were 1 cm thick and weighed 50 ± 0.05 g.

137 Samples were cooked in an oven (Rational Combi, Dämpfer, United Kingdom) at

138 200 °C for 15 min until the centre of the patties reached ≥ 70 °C for over 2 minutes

139 when tested with a temperature probe. The patties were then immediately cooled to 4

140 °C and placed in polyethylene bags (PA/PE, Brodericks Brothers Limited, Ireland)

141 and vacuum packed (La Minerva, Italy). The samples were stored at 4 °C throughout

142 the storage period for 30 days which is typical for a cooked beef product.

143

144

145

146 **Cooking yield**

147 Patties were weighed before cooking and after chilling at 4 °C. To estimate the

148 cooking yield, the patty weights were expressed as a percentage of the initial weight

149 using the following calculation:

150

151 $$Cooking\ yield\ (\%) = 100 \times \frac{cooked\ weight\ (g)}{raw\ weight\ (g)}$$ Eq. 1

152

153 **Total Dietary Fibre**

154 Total dietary fibre (TDF) was determined by Sigma analysis kit (Sigma-Aldrich,

155 Inc., USA) based on AOAC method 991.43. Samples (5 g) were cooked at 100 ºC

156 with heat stable α-amylase to initiate gelatinization, hydrolysis and depolymerisation

157 of starch. The samples were incubated at 60 ºC with protease (to solubilise and

158 depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to

159 glucose). The samples were then treated with four volumes of ethanol to precipitate

160 soluble fibre and remove depolymerised protein and glucose. The residue was

161 filtered, washed, dried and weighed. One duplicate was analysed for protein and the

162 other was incubated at 525 ºC to determine ash. The TDF was determined as the

163 weight of the filtered and dried residue less the weight of the protein and ash.

164

165 **Bacterial enumeration**

166 Samples were prepared in a vertical laminar-flow cabinet for the purposes of

167 microbial analysis. For each patty sample, 25 g was taken aseptically and placed in a

168 sterile stomacher bag with 225 ml of peptone water (Scharlau Chemie, Spain). After

169   2 min in a stomacher blender (Stomacher 400, Seward Medical, United Kingdom),

170   appropriate decimal dilutions were spread-plated (100 µl) onto Plate Count Agar

171   (PCA) (Scharlau Chemie, Spain) for total viable counts (TVC) and incubated at 37

172   °C for 24 h. The results were expressed as logarithms of colony forming units per

173   gram of sample (log CFU/g). Samples were taken on days 0, 7, 14, 21 and 30 for

174   analysis.

175

176   **pH measurement**

177   The pH of patties (10 g homogenised in 50 ml distilled water) was determined using

178   an Orion Model 520A pH metre (AGB Scientific Ltd) throughout the storage period.

179   Three readings were taken for each sample. Samples were taken on days 0, 7, 14, 21

180   and 30 for analysis.

181

182   **Lipid oxidation measurement**

183   Lipid oxidation was assessed on the basis of the amount of malondialdehyde formed

184   during storage. Malondialdehyde is the end-product of lipid peroxidation and was

185   evaluated using the TBARS assay with some modifications (Oussalah et al., 2006).

186   A 10 g portion of each meat sample was blended with 50 ml of distilled deionised

187   water and 10 ml of 15% tricholoroacetic acid (TCA) in a stomacher blender

188   (Stomacher 400, Seward Medical, England) for 2 min at 260 rpm. The homogenate

189   was centrifuged at 1500 gravity for 5 min and the supernatant fluid was filtered

190   through a Durapore 0.45 µm HV membrane filter (Millipore). A 2 ml aliquot of 60

191   mmol/L TBA reagent was added to 8 ml of the clear filtrate and vortexed for 15 s

192   and then heated in a boiling water bath for 10 min to develop a pink colour. After

193   cooling on ice to ambient temperature (~ 20 °C), the absorbance of the supernatant

194   was measured spectrophotometrically at 532 nm (Milton Roy Spectronic 1201). The

195   concentration of malondialdehyde in analysed samples was calculated on the basis of

196   a standard curve obtained using serial dilutions of 1,1,3,3-tetramethoxyropane

197   solution. The TBARS value was expressed as mg malondialdehyde/kg (mg

198   MDA/kg) of sample. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

199

200   **Extraction of phytochemicals**

201   Seaweed-patty samples (5 g) were powdered in liquid nitrogen using a mortar and

202   pestle, then extracted with 50ml of methanol (60%) under nitrogen atmosphere for 2

203   hours. The extraction was carried out at 40 °C at 100rpm in a shaker incubator

204   (Innova 42, Mason Technology, Ireland). Samples were filtered and centrifuged at

205   10,000 rpm for 15 min (Sigma 2K15, Mason Technology, Ireland). Resulting

206   extracts were evaporated to dryness using vacuum polyevaporator (Buchi Syncore

207   Polyvap, Mason Technology, Ireland) at 60 °C. A pressure gradient program was

208   designed for evaporation of the solvents with vacuum conditions of 337 and 72 mbar

209   for methanol and water, respectively.

210

211   **Total phenolic content**

212   The total phenolic concentration (TPC) was measured using the Folin-Ciocalteau

213   method (Taga et al., 1984). In this procedure, 100 µl aliquot of stock sample (extract

214   concentration 1000 µg/ml of water) was mixed with 2.0 ml of 2% Na_2CO_3 and

215   allowed to stand for 2 min at room temperature. Then 100 µl of 50% Folin-

216   Ciocalteau's phenol reagent was added. After incubation for 30 min at room

217   temperature in darkness, the absorbance was read at 720 nm using spectrophotometer

218   (Milton Roy Spectronic 1201). The total phenolic contents were expressed as mg

219   gallic acid equivalent per 100 gram fresh weight (fw) (mg GAE/100 g fw). Samples

220   were taken on days 0, 7, 14, 21 and 30 for analysis.

221

222   **DPPH radical scavenging activity**

223   Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl

224   (DPPH) according to the method of Yen & Chen (1995) with some modifications.

225   Samples were taken on days 0, 7, 14, 21 and 30 for analysis. Briefly, a 100 µl aliquot

226   of test sample (concentration 50 µg/ml) was placed in a 96-well microtitre plate and

227   100 µl of 0.16 mM DPPH methanolic solution was added. The mixture was shaken

228   and incubated for 30 min in darkness at 25 ºC. Changes in the absorbance of the

229   samples were measured at 517 nm using a microplate reader (Powerwave, Biotek,

230   VT, USA).

231

232   The ability to scavenge the DPPH radical was calculated using the following

233   equation given by Duan et al. (2006):

234    $\text{Scavenging effect (\%)} = \left[1 - \left(\frac{A_{sample} - A_{sampleblank}}{A_{control}} \right) \right] \times 100$ **Eq. 2**

235   Where: $A_{control}$ is the absorbance of the control (DPPH solution without sample),

236   A_{sample} is the absorbance of the test sample (DPPH solution plus test sample) and

237 $A_{sample\ blank}$ is the absorbance of the sample only (sample without any DPPH

238 solution).

239

240 **Texture evaluation**

241 Shear tests were performed using an Instron Universal Testing Machine (Model

242 4301, Canton MA, USA) supported with Bluehill 2 version 2.14 analysis software

243 for materials testing. A Warner Bratzler cutter was used in the shear tests. An

244 aluminum plate with dimensions of 10 x 6 cm^2, thickness of 1.3 cm and with an

245 opening of 3 mm in the centre was supported in the Instron base. Patty samples (5 g)

246 were sheared at a speed of 200 mm/min. The cutting implement was allowed to

247 travel the depth of the patty, cutting through the sample and hardness was defined as

248 the peak of force-deformation curve recorded in Newtons per mm (N/mm). Ten

249 replications of each sample were carried out. Samples were taken on days 0, 7, 14,

250 21 and 30 for analysis.

251

252 **Colour measurement**

253 Colour analysis was performed using a colourimeter (CIE Lab ColourQuest XE)

254 with D65 illuminant and 10 ° standard observer angle setting. Patty samples (5 g)

255 were taken on days 0, 7, 14, 21 and 30 for analysis. The colourimeter was calibrated

256 against a standard white reference tile (L* = 93.97; a* = -0.08 and b* = 1.21). The

257 colour values were represented on the CIE colour scales in terms of L*

258 (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). From

259 these values, total colour change from fresh (DE) was calculated according to the

260 following equation:

261 $$DE = \sqrt{(L*-L*_0)^2 + (a*-a*_0)^2 + (b*-b*_0)^2}$$ **Eq. 3**

262 Where; $L*_0$, $a*_0$ and $b*_0$ are the readings at time zero and $L*$, $a*$ and $b*$ are the

263 individual readings at each drying time.

264

265 **Sensory characteristics**

266 The sensory acceptance test was conducted in a standardised sensory test room (ISO

267 9599, 2007). Untrained panelists ($n = 20$) were recruited from staff and students of

268 the Dublin Institute of Technology using a five-point hedonic scale. Samples (25 g)

269 were served at the same time on white paper plates with random three-digit numbers,

270 and water at room temperature was provided for mouth-rinsing between samples.

271 The panelists were asked to assign scores for aroma (maximum of 5), appearance

272 (maximum of 5), texture (maximum of 5), flavour (maximum of 5) and overall

273 acceptability of the product (maximum of 5), where 5 was "like extremely" and 1

274 was "dislike extremely". The overall quality (maximum of 25) was computed by

275 combining scores of all five attributes.

276

277 **Statistical analysis**

278 All experiments were performed in triplicate and replicated twice. All statistical

279 analyses were carried out using STATGRAPHICS Centurion XV software (StatPoint

280 Technologies, Inc., Warrenton, VA). Statistical differences were determined using

281   ANOVA followed by Least Significant Difference (LSD) testing. Differences were

282   considered statistically significant when $p < 0.05$.

283

284   **3. Results and Discussion**

285   **Cooking yield and dietary fibre content of seaweed-patties**

286   Cooking loss was the highest in the control sample which had a 40.28% reduction in

287   yield. As seaweed levels were increased cooking losses declined. The processing

288   losses were 34.80, 34.32, 34.24 and 33.88% for 10, 20, 30 and 40% seaweed

289   concentrations, respectively. This demonstrated that adding seaweed had a

290   significant effect on retaining moisture as compared to control patties ($P < 0.05$).

291   Cofrades *et al*. (2008) and Fernández-Martín *et al*. (2009) also found that the

292   addition of *H. elongata* improved the water-binding properties of pork meat.

293   The use of dietary fibre in cooked meat products generally improves hydration

294   properties and fat holding capacity, reducing fat and water loss during cooking and

295   increasing emulsion stability (Thebaudin et al., 1997; Cofrades et al., 2000; Jiménez-

296   Colmenero et al., 2005). The objective of the current study was to incorporate

297   seaweed into beef patties in order to achieve healthier meat products while also

298   producing a product with good sensory attributes such as texture. Seaweeds contain

299   large amounts of dietary fibre and have a high water-holding capacity. The water-

300   holding capacity of seaweeds is closely related to the polysaccharide composition of

301   the dietary fibre fractions, and therefore the gelation process will depend on the type

302   and amount of their polysaccharides (Sánchez-Alonso et al., 2006).

303   Traditional beef patties are high in fat content (about 14%). Most of this fat is

304   saturated fatty acid (SFA) (about 60% of total fat), while the monounsaturated fatty

305   acid (MUFA) fraction accounts for about 36% of total fat, and the polyunstaturated

306   fatty acid (PUFA) fraction accounts for about 3% of total fat (Martínez et al., 2011).

307   There are often problems with reduction of fat in finely ground meat products, as it

308   can present a number of difficulties in terms of appearance, flavour and texture. This

309   can cause such products to be less accepted by the consumer (Keeton, 1994; García

310   et al., 2002; Tokusoglu & Ünal, 2003). Manufacturers have introduced several

311   modifications in an attempt to offset the detrimental effects of reducing the fat level.

312   These modifications include the use of non-meat ingredients that could help to

313   convey desirable texture and, more importantly, enhance water-holding capacity

314   (Ako, 1998; Keeton, 1994). In this regard, the incorporation of carbohydrates and

315   fibre have been successful in improving cooking yield, reducing formulation cost

316   and enhancing texture (Keeton, 1994; Jiménez-Colmenero, 1996; Mendoza et al.,

317   1998). There are strict food regulations within the EU in relation to labeling the

318   content of ingredients in food products. A product such as beef patties with seaweed

319   would be required to be labeled as such, and the percentage of both seaweed and

320   beef corresponding to the quantity of the ingredients would be required on the

321   product label (EU Directive 2000/13/EC, 2000).

322   In the current study, dietary fibre may have had an important effect on this

323   technological property because it holds water by adsorption and absorption

324   phenomena and some water is also retained outside the fibre matrix (free water)

325   (Sánchez-Zapata et al., 2010). The total dietary fibre content of the control patty and

326   seaweed-patty at a concentration of 40% can be seen in Fig. 1.

327   Rehydrated seaweed contained 4.02 g TDF per 100 g fw (4.02%) and when

328   incorporated into patties at 40%, the final product contained 1.64 g TDF per 100 g

329   fw (1.64%). These results are in line with Choi *et al*. (2012) who reported that pork

330   patties with dried *Laminaria japonica* incorporated at levels up to 5% contained 1.23

331   to 3.14% dietary fibre. López-López et al. (2010) reported the TDF in pork patties

332   containing dried seaweed (3%) to be 1.36% in the final product which is also lower

333   than that of the present study; however less seaweed was added as it was in dried

334   form. The recommended daily intake of dietary fibre is > 25 g per day (WHO/FAO,

335   2003). The addition of fibre to fast food product which is a commonly consumed and

336   low in fibre would help to increase the daily consumption of dietary fibre amongst

337   the population.

338

339   **Bacterial enumeration and pH of control and seaweed-patties during storage**

340   Microbial growth (log CFU/g) of the vacuum packed seaweed-patties over 30 days

341   of refrigerated storage can be seen in Table 1. There was no significant difference in

342   the total viable counts for all patties (control, 10, 20, 30 and 40% seaweed) within

343   the first 14 days of storage as there was no growth of bacteria in any of the samples

344   ($P > 0.05$). There was a significant difference ($P < 0.05$) between the control and the

345   seaweed-patties after 14 days as growth began in the control sample and reached

346   5.41 log CFU/g by day 30. Generally, the addition of seaweed did not affect the

347   spoilage of patties particularly in samples containing > 20% seaweed. A low level of

348   growth (1.09 log CFU/g) was seen in seaweed-patties by day 30, and only in patties

349   containing the lowest level of seaweed (10%). This level was however significantly

350   lower than the control samples ($P < 0.05$).

351   López-López et al. (2010) reported that the total viable counts of beef patties and

352   those with added seaweed ranged from 6 - 6.4 log CFU/g. Cofrades *et al*. (2011) also

353   reported that the TVC for restructured poultry steaks with added seaweed were in

354   excess of 6 log CFU/g, however the levels from both these studies are higher than

355   that of the present findings, most likely due to the fact that the patties were

356   uncooked. There are no guidelines specific to total viable counts in minced beef

357   intended to be eaten cooked apart from the requirement for *Salmonella* spp. to be

358   absent in 10 g of sample. Guidelines set out by the Food Safety Authority of Ireland

359   (FSAI) for Enterobacteriaceae numbers on raw meat samples stipulate that three of

360   five samples of raw meat must have counts of < 5 log CFU/g and no more than two

361   of five samples of raw meat can have counts between 5 and 7 log CFU/g. Meat

362   exceeding these limits is defined as unacceptable. The levels of TVC in the raw

363   patties before cooking in the present study was 2.09 log CFU/g which is well below

364   the FSAI limits and those established by The European Union Commission

365   Regulation (EC No. 2073/2005) on the microbiological criteria for foodstuffs. The

366   pH of the patties (Table 1) was also monitored throughout the shelf life as high

367   levels of microorganisms result in reductions in pH levels (Gómez-López et al.,

368   2007).

369   The initial pH values (day 0) of all patty samples were similar ranging from 6.01 to

370   6.05. These levels are in line with those observed for cooked pork patties with a pH

371   ranging from 6.06 - 6.13 as reported by Choi et al. (2012). Significant differences

372   between the control and seaweed-patties were observed after 14 days of storage. The

373   pH values of all seaweed-patties were 6.00, while that of the control was 5.96, which

374   is only slightly lower. By the end of the storage period (30 days) the pH of the

375   seaweed-patties still had not changed significantly ($P > 0.05$) and was in the range of

376   5.99 - 6.00 while the control had dropped to 5.82. These results are in agreement

377   with those of the bacterial enumeration as the acidity of the control had dropped and

378   was most and likely due to the increase in bacterial growth as compared to the

379   seaweed-patties.

380

381   **Lipid oxidation of control and seaweed-patties during storage**

382   Lipid oxidation generates a series of chemical reactions that can alter the physio-

383   chemical parameters, sensorial attributes (odour, colour and flavour) and shelf life in

384   meat and meat products (Liu et al., 1995). TBARS analysis measures the formation

385   of tertiary products of lipid oxidation, mainly malondialdehyde, which may

386   contribute off-flavour to oxidized fat (Lee et al., 2011). Lipid oxidation in precooked

387   products remains of concern to the meat industry due to the increased demand for

388   convenience foods. Undesirable flavour in precooked meats, commonly described as

389   warmed-over flavour, rapidly develops in cooked meat products during refrigerated

390   storage (Ahn et al., 2002). Precooked meats are likely to oxidize and produce

391   secondary compounds such as hexanal, pentanal, 2,4-decadienal, 2,3-oxtanedione,

392   and 2-octenal (Trout & Dale, 1990). Minced meat and meat products undergo

393   oxidative changes more quickly as grinding exposes lipid membranes to metal

394   oxidation catalysts (Lee et al., 2011).

395   Table 2 shows the effect of different seaweed concentrations on TBARS values of

396   cooked-patties during 30 days of storage. Initial TBARS levels (Day 0) of all

397   samples were similar ranging from 0.18 to 0.20 mg malondialdehyde/kg (mg

398   MDA/kg). TBARS values of all patties containing seaweed were significantly lower

399   ($P < 0.05$) than the control during storage. The TBARS levels began to increase at

400   day 14 of storage. This indicated that there was some protective effect of the

401   seaweed against lipid oxidation in cooked minced beef, potentially due to the

402   increase in phenolic compounds and DPPH activity as discussed. The reduction in

403   lipid oxidation could also be due to the reduction in meat content in the samples (10

404   - 40% less meat) which accordingly would have lower levels of fat present in the

405   samples thus reducing potential oxidation.

406   The differences in TBARS values of seaweed-patties ranged from 0.18 – 0.69 mg

407   MDA/kg from the beginning to end of storage. Therefore, the extent of this lipid

408   oxidation during refrigerated storage may be considered relatively low according to

409   Bhattacharya et al. (1988), Rojas & Brewer (2007) and López-López et al. (2010).

410   The results of the present study are in agreement with López-López et al. (2010) who

411   reported that the TBARS values of seaweed-patties ranged from 0.27 – 0.87 mg

412   MDA/kg during frozen storage.

413

414   **Total phenolic content of control and seaweed patties during storage**

415   The total phenolic content (TPC) of the seaweed-patties over the 30 days of storage

416   is shown in Fig. 2. Phenolic compounds exist as various structures, have different

417   molecular weights and are related to the innate flavour of food. They contain a

418   phenolic hydroxyl group, which has an antioxidative effect through interactions with

419   the phenol ring and has a resonance stabilization effect (Shahidi & Wanasundara,

420   1992). Differences in the TPC of all samples were significant ($P < 0.05$). The control

421   sample contained no detectable phenols at tested levels, while the TPC increased

422   significantly ($P < 0.05$) with increasing seaweed concentrations (10 - 40%). The

423   TPC ranged from 7.05 - 28.11 mg GAE/100 g fw and by day 30 these levels were

424   6.42 – 24.21 mg GAE/100 g fw.

DPPH radical scavenging activity of control and seaweed patties during storage

DPPH is a free radical widely used to determine the free radical-scavenging ability of various compounds (Amarowicz et al., 2004). The DPPH radical scavenging activity of the patties over 30 days of storage is presented in Fig. 3. The control sample contained no detectable phenols at tested levels. The initial levels of DPPH scavenging activity in all seaweed-patty samples were significantly different ($P <$ 0.05) and ranged from 30.23 - 52.34%. Throughout the storage period the DPPH activity declined significantly for each of the seaweed-patty samples ($P < 0.05$). By day 30, levels were in the range of 26.65 - 40.69% for the different concentrations of seaweeds.

Texture of control and seaweed patties during storage

The firmness/tenderness of the patty samples throughout storage is shown in Table 3. The initial tenderness of each of the patties (control, 10, 20, 30 and 40% seaweed) were all significatly different ($P < 0.05$) ranging from 17.50 - 19.06 N/mm. As seaweed levels increase, the patties become more tender. An addition of 40% seaweed represented a 46.98% difference in tenderness levels compared to that of the control. Dietary fibres from different sources have been studied for formulation of different meat products, with a view, among other things, to improve texture. It has generally been found that addition of such fibres to meat augmented firmness (Cofrades et al., 2008; Fernández-Martín et al., 2009; Sánchez-Zapata et al., 2010). However, while some authors have observed increases in firmness with the addition of fibres to meat, others have found no difference or the production of more tender products (Chun et al., 1999; Cofrades et al., 2000; Jiménez-Colmenero et al., 2005;

449   Selgas et al., 2005). López-López (2010) also reported that beef patties containing

450   seaweed were more tender than the control. The effect of seaweed addition on the

451   tenderness of the patties was most likely due to the role played by fibre. The texture

452   of all of the samples in the present study increased (became firmer) throughout

453   storage ($P < 0.05$). The firmness of the control samples was almost double that of

454   those containing 40% seaweed. By the end of the storage period (30 days) the

455   tenderness of the samples ranged from 21.33 – 40.23 N/mm, with the firmest being

456   the control and the most tender were those in patties containing the highest levels of

457   seaweed (40%). This is due to the retention of water in seaweed during the hydration

458   step and the reduction of levels of meat proteins due to its addition.

459

460   Colour of control and seaweed patties during storage

461   Colour was evaluated in order to detect the tendencies for seaweed addition to cause

462   changes in the beef-patties, given that colour is one of the main parameters

463   determining consumer acceptance of a product (Cofrades et al., 2008). Seaweed

464   addition had an immediate effect on colour parameters of patties in comparison to

465   the control (Table 4). At the initial stage (day 0), the L* values of the patty samples

466   with seaweed incorported were higher than that of the control (colour was lighter).

467   Seaweed concentrations (10 – 40%) also had a significant effect on the L* values as

468   the patties became lighter in colour with increasing seaweed levels ($P < 0.05$). It has

469   been reported that usually in meat products, the higher the moisture content, the

470   higher the lightness (L*) value (Pérez-Alvarez et al., 1999; Alesón-Carbonell et al.,

471   2005; Fernández-López et al., 2008). The higher L* values could therefore also be

472   due to the high moisture content of the seaweed and the moisture retention upon

473   cooking as compared to the control.

474   The a* values of the samples containing seaweed were significantly different (day 0)

475   as compared to the control ($P < 0.05$), with values ranging from 7.05 (10% seaweed)

476   to 8.39 (control). This parameter is a measure of the redness/greenness of a sample

477   with lower a* readings containing more green pigments. This would explain the

478   reduction in a* values as compared to the control as blanched *H. elongata* is bright

479   green in colour. The initial b* values (day 0) were significanly ($P < 0.05$) higher

480   than the control patties containing no seaweed. This parameter is a measure of the

481   yellowness/redness of the samples and the higher b* values of the seaweed-patties

482   indicate an increase in yellow colour.

483   With respect to colour during storage; L* values changed significantly for all

484   samples ($P < 0.05$). The L* values decreased by day 30, indicating a slight darkening

485   of the samples, with the exception of patties with 30 and 40% seaweed which

486   became slightly lighter in colour. There was a significant increase in a* values for all

487   samples (except 20 and 30% seaweed-patties) by day 30, which indicated that the

488   redness of the samples increased slightly, this indicated that there was a reduction in

489   the green colour of the blanched seaweed. There was also a significant increase in b*

490   values for all samples (except 10 and 20% seaweed-patties) by day 30. This indicates

491   that there was a reduction of the yellowness of the samples.

492   Although there were differences in the colour values throughout the storage period,

493   most of the colour parameters of the patty samples were basically steady (slightly

494   changed) which was also reported by Shan et al. (2009) who studied the effects of

495   adding spice and herb extracts to raw pork. Although the addition of seaweed

496   changed the colour of the patties as compared to the control, this is in line with meat

497   colour changes upon the addition of spice and herbs which are traditionally added to

498   meats. In order to determine the acceptability of the colour, this was taken into

499   account in the sensory analysis.

500

501   **Sensory analysis**

502   In order to determine if the seaweed-patties were acceptable in terms of aroma,

503   appearance, texture and taste, a preliminary consumer acceptability test was

504   undertaken. Table 5 summarises the sensory scores for aroma, appearance, texture,

505   taste and overall acceptability of control and seaweed-patties. The samples tested by

506   the sensory panel were the control (with no added seaweed), a mid-range seaweed-

507   patties (20% seaweed) and patties with 40% added seaweed which would have the

508   maximum level of antioxidants and TDF. Aroma, appearance, texture and taste of

509   the seaweed-patties were found to be significantly different to the control ($P < 0.05$).

510   The sensory scores for aroma ranged from 4.23 (20% seaweed) to 4.61 (control). The

511   fact that no strong seaweed aroma was detected could be attributed to blanching the

512   seaweed prior to adding to the meat.

513   The sensory score for appearance ranged from 4.23 to 4.84, with the score reducing

514   with increasing seaweed concentration. This showed that the patties without the

515   incorporation of seaweed were more visually appealing to the sensory panel,

516   however the mean score for all samples was still above 4, which is a positive result.

517   The scores for texture were significantly higher with increased levels of seaweed (P

518   < 0.05). Therefore the panel detected that seaweed altered the texture and possible

519   mouthfeel of the patties which was one of the objectives of the study. The addition of

520 blanched seaweeds over dried seaweeds in the present study offers exploitation of

521 the gelling properties of the seaweeds. This would also contribute to the

522 technological properties of the seaweed such as reducing cooking losses.

523 The seaweed-patties also had a significantly higher score for taste than the control

524 with 20% seaweed-patties ranking the highest ($P < 0.05$). The 40% seaweed-patty

525 ranked highest in the overall acceptability score ($P < 0.05$) with the control receiving

526 the lowest score. The results of the present study are promising particularly when

527 compared to those reported in literature. Piňero et al. (2008) found that the taste

528 scores for beef patties with added oat fibre to be lower than the control. Cofrades et

529 al. (2011) reported that while all restructured poultry steaks with added *H. elongata*

530 were judged acceptable by a sensory panel, the control received a higher score for

531 overall acceptability than those containing seaweed. On the other hand, Choi et al.

532 (2012) stated that sensory evaluations indicated that the greatest overall acceptability

533 in pork-patties was also attained in samples containing seaweed.

534

535 **4. Conclusion**

536 The addition of *H. elongata* to meat products in the development of functional foods

537 opens up new potential for seaweed utilisation. Incorporating such seaweeds is of

538 interest from a technological and functional point of view. The seaweed had a

539 positive effect on the cooking yield of the patties due to their hydrocolloid content

540 which reduce cooking losses. Total dietary fibre, polyphenolic content and

541 antioxidant activity were increased due to the incorporation of seaweed. Storage life

542 was enhanced in samples containing seaweed as compared to the control and lipid

543 oxidation was also greatly reduced due to the levels of phytochemicals present in the

544   seaweed. The seaweed also had a positive effect on the texture of the patties as they

545   were more tender than the control which was also confirmed in the sensory analysis

546   study. The seaweed-patties were found overall to be acceptable by a sensory panel,

547   particularly in terms of texture.

548

553

554   **References**

555   Ahn, I. U., & Grun, L. N. (2002). Fernando Antioxidant properties of natural plant

556   extracts containing polyphenolic compounds in cooked ground beef. *Journal of Food*

557   *Science*, 67 (4), 1364-1369.

558   Ako, C. (1998). Fat replacers. *Food Technology*, *52*, 47-53.

559   Alesón-Carbonell, L. J. Fernández-López, J. A. Pérez-Alvarez, & Kuri V. (2005).

560   Functional and sensory effects of fibre-rich ingredients on breakfast fresh sausages

561   manufacture. *Food Science and Technology International, 11,* 89-97.

562   Amarowicz, R., R. B., Pegg, P., Rahimi-Moghaddam, B., Barl, J., & Weil, A.

563   (2004). Free-radical scavenging capacity and antioxidant activity of selected plant

564   species from the Canadian prairies. *Food Chemistry, 84 (4),* 551-562.

565 Anderson, J. W., Baird, P., Davis, Jr., R. H., Ferreri, S., Knudtson, M., Koraym, A.,

566 Waters, V., & Williams, C. L. (2009). Health benefits of dietary fibre. *Nutrition*

567 *Reviews, 67 (4),* 188-205.

568 Annunziata, A., & Vecchio, R. (2011). Functional foods development in the

569 European market: A consumer perspective. *Journal of Functional Foods*, *3 (3),* 223-

570 228.

571 Bhattacharya, M., Hanna, M. A., & Mandigo, R. W. (1988). Effect of frozen storage-

572 conditions on yields, shear-strength and colour of ground-beef patties. *Journal of*

573 *Food Science*, *53 (3),* 696-700.

574 Calvo, M. Garcia, M., & Selgas, M. (2008). Dry fermented sausages enriched with

575 lycopene from tomato peel. *Meat Science*, *80,* 167-172.

576 Choi, Y. S., Choi, J. J., Han, D. J., Kim, H. Y., Kim, H. W., Lee, M. A., Chung, H.

577 J., & Kim, C. J. (2012). Effects of *Laminaria japonica* on the physico-chemical and

578 sensory characteristics of reduced-fat pork patties. *Meat Science*, *91*, 1-7.

579 Chun, S. S. Park, J. R Park, J. C., Suh, J. S., & Ahn, C. B. (1999). Quality

580 characteristics of hamburger patties added with seaweed powder. *Journal of the*

581 *Korean Society of Food Science and Nutrition*, *28 (1),* 140-144.

582 Cofrades, S., Guerra, M. A., Carballo, J., Fernández-Martín, F., & Jiménez-

583 Colmenero, F. (2000). Plasma protein and soy fibre content effect on bologna

584 sausage properties as influenced by fat level contents of processed edible seaweeds.

585 *Food Chemistry*, *85 (3),* 439-444.

586   Cofrades, S., López- López, I., Ruiz-Capillas, C., Triki, M., & Jiménex-Colmenero.

587   (2011). Quality characteristics of low-salt restructured poultry with microbial

588   transglutaminase and seaweed. *Meat Science, 87,* 373-380.

589   Cofrades, S., López-López, I., Solas, M. T., Bravo, L., & Jiménez-Colmenero, F.

590   (2008). Influence of different types and proportions of added edible seaweeds on

591   characteristics of low-salt gel/emustion meat systems. *Meat Science*, *79,* 767-776.

592   Cox, S., Abu-Ghannam, N., & Gupta, S. (2011). Effect of processing conditions on

593   phytochemical constituents of edible Irish seaweed *Himanthalia elongata. Journal of*

594   *Food Processing and Preservation,* in print.

595   Demeyer, D., Honikel, K., & De Smet, S. (2008). The World Cancer Research Fund

596   report 2007: a challenge for the meat processing industry. *Meat Science, 80*, 953-

597   959.

598   Duan, X. J., Zhang, W. W., Li, X. M., & Wang, B. G. (2006). Evaluation of

599   antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia*

600   *urceolata. Food Chemistry, 95*, 37-43.

601   Duffy, G., O Brien, S., Carney, E., Butler, F., Cummins, E., Nally, P., Mahon, D.,

602   Henchion, M., & Cowan, D. (2005). A quantitative risk assessment of E. coli 0157:

603   H7 in Irish minced beef. *Teagasc National Development Plan Report*, Project RMIS

604   No. 5035.

605   EC No. 2073/2005. (2005). The European Union Commission Regulation on the

606   microbiological criteria for foodstuffs. *Official Journal of the European Union*,

607   338/1-338-18.

608 EU Directive 2000/13/EC. (2000). Directive 2000/13/EC of the European Parliament

609 and of the Council (20 March 2000) on the approximation of the laws of the member

610 states relating to the labeling, presentation and advertising of foodstuffs. *Official*

611 *Journal of the European Union*, OJL109 6.5. 2000, 29.

612 Fernández-López, J., Sendra, E. Sayas-Barberá, E., Navarro, C., & Pérez-Alvarez, J.

613 A. (2008). Physico-chemical and microbiological profiles of "salchichón" (Spanish

614 dry-fermented sausage) enriched with orange fibre. *Meat Science*, *80*, 410-417.

615 Fernández-Martin, F., López-López, I., Cofrades, S., & Jiménez-Colmenero. (2009).

616 Influence of adding Sea Spaghetti seaweed and replacing the animal fat with olive

617 oil or konjac gel on pork meat batter gelation. Potential protein/alginate association.

618 *Meat Science, 83*, 209-217.

619 García, M.. Dominguez, R., Galvez, M., Casas, C., & Selgas, M. (2002). Utilization

620 of cereal and fruit fibres in low fat dry fermented sausages. *Meat Science*, *60 (3),*

621 227-236.

622 Gómez-López, V. M., Devlieghere, F., Ragaert, P., & Debevere, J. (2007). Shelf life

623 extension of minimally processed carrots by gaseous chlorine dioxide. *International*

624 *Journal of Food Microbiology*, *116 (2),* 10, 221-227.

625 Gupta, S., Cox, S., & Abu-Ghannam, N. (2011). Effect of different drying

626 temperatures on the moisture and phytochemical constituents of edible Irish brown

627 seaweed. *LWT - Food Science and Technology, 44 (5),* 1266-1272.

628 Hayes, J. E., Desmond, E. M., Troy, D. J. Buckley, D. J., & Mehra, R. (2005). The

629 effect of whey protein enriched fractions on the sensory properties of frankfurters.

630 *Meat Science*, *71*, 238-243.

631   Hernández-Hernández, E. Ponce-Alquicira, E. Jaramillo-Flores, M. E., & Guerrero

632   Legarreta, I. (2009). Antioxidant effect rosemary (*Rosmarinus officinalis* L.) and

633   oregano (*Origanum vulgare* L.) extracts on TBARS and colour of model raw pork

634   batters. *Meat Science, 81*, 410-417.

635   Jiménez-Colmenero, F. (1996). Technologies for developing low-fat meat products

636   *Trends in Food Science and Technology*, *7,* 41-48.

637   Jiménez-Colmenero, F., Ayo, M. J., & Carballo, J. (2005). Physicochemical

638   properties of low sodium frankfurter with added walnut: effect of transglutaminase

639   combined with caseinate, KCl and dietary fibre as salt replacers. *Meat Science*, *69*,

640   781-788.

641   Jiménez-Colmenero, F., Sánchez-Muniz, J., & Olmedilla-Alonso, B. (2010). Design

642   and development of meat-based functional foods with walnut: Technological,

643   nutritional and health impact. *Food Chemistry*, *123*, 959–967.

644   Jiménez-Colmenero, F. (2007). Healthier lipid formulation approaches in meat based

645   functional foods. Technological options for replacement of meat fats by non-meat

646   fats. *Trends in Food Science and Technology, 18,* 567-578.

647   Keeton, J. T. (1994). Low-fat meats products technological problems with

648   processing. *Meat Science, 36,* 261-276.

649   Lee., M. I., Choi, J. H., Choic, Y. S., Kim, H. Y., Kim, H. W., Hwang, K. E.,

650   Chung, H. K., & Kim, C. J. (2011). Effects of *kimchi* ethanolic extracts on oxidative

651   stability of refrigerated cooked pork. *Meat Science*, *89*, 405-411.

652   Liu, Q. M. C. Lanari, D., & Schaefer, M. (1995). A review of dietary vitamin E

653   supplementation for improvement of beef quality. *Journal of Animal Science*, *73,*

654   3131-3140.

655   López-López, I., Cofrades, S., Yakan, A., Solas. M. T., & Jiménez-Colmenero, F.

656   (2010). Frozen storage characteristics of low-salt and low-fat beef patties as affected

657   by wakame addition and replacing pork backfat with olive oil-in-water emulstion.

658   *Food Research International*, *43,* 1244-1254.

659   Martínez, B., Miranda, J. M., Vázquez, B. I., Fente, C. A., Franco, C. A., Rodríguez,

660   J. L., & Capeda, A. (2012). Development of a hamburger patty with healthier lipid

661   formulation and study of its nutritional, sensory and stability properties. *Food*

662   *Bioprocess Technology*, 5 (1), 200-208.

663   Mendoza, E., M. L. García, C. Casas, M. F., & Fernández, M. D. (1998). Selgas.

664   Utilización de proteínas como sustitutos de grasa en productos carnicos.

665   *Alimentación: Equipos y Tecnología, 7,* 87-92.

666   Oussalah, M., Caillet, S., Salmieri, S., Saucier, L., & Lacroix, M. (2004).

667   Antimicrobial and antioxidant effects of milk protein-based film containing essential

668   oils for the preservation of whole beef muscle. *Journal of Agricultural and Food*

669   *Chemistry, 52*, 5598-5605.

670   Pérez-Alvarez, J. A., Sayas-Barberá, E., Fernández-López, J., & Aranda-Catalá, V.

671   (1999). Physicochemical characteristics of Spanish-type dry-cured sausage. *Food*

672   *Research International*, *32,* 599-607.

673   Piňero, M. P., Parra, K., Huerta-Lindenez, N., Arenas de Moreo, L., Ferrer, M.,

674   Araujo, S., & Barboza, Y. (2008). Effects of oats soluble fibre (β-glucan) as a fat

675   replacer on physical, chemical, microbiological and sensory properties of low-fat

676   beef patties. *Meat Science*, *80*, 675-680.

677   Rojas, M. C., & Brewer, M. S. (2007). Effect of natural antioxidants on oxidative

678   stability of cooked, refrigerated beef and pork. *Journal of Food Science*, *72 (4),*

679   S282-S288.

680   Sahoo, J., & Kumar, N. (2005). Quality of vacuum packaged muscle foods stored

681   under frozen conditions: A review. *Journal of Food Science and Technology*, *42,*

682   209-213.

683   Sánchez-Alonso, I., Haji-Maleki, R., & Borderías, A. J. (2006). Effect of wheat fibre

684   in frozen stored fish muscular gels. *European Food Research and Technology*, *223*

685   *(4),* 571-576.

686   Sánchez-Zapata, E., Fernández-López, J., Peňaranda, M., Fuentes-Zaragoza, Sendra,

687   E., Sayas, E., & Pérez-Alverez, A. (2011) Technological properties of date paste

688   obtained from date by-products and its effect on the quality of a cooked meat

689   product. *Food Research International*, *44 (7),* 2401-2407.

690   Selgas, M. D. E., & Cáceres, M. L. (2005). García Long-chain soluble dietary fibre

691   as functional ingredient in cooked meat sausages. *Food Science and Technology*

692   *International*, *11 (1),* 41-47.

693   Shahidi, P. K. J., & Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical*

694   *Reviews in Food Science and Nutrition*, *32 (1),* 67-103.

695   Shan, B., Cai, Y. Z., Brooks, J. D., & Corke, H. (2009). Antibacterial and

696   antioxidant effects of five spice and herb extracts as natural preservatives of raw

697   pork. *Journal of the Science of Food and Agriculture, 80 (11),* 1879-1855.

698   Taga, M. S., Miller, E. E., & Pratt, D. E. (1984). Chia seeds as a source of natural

699   lipid antioxidants. *Journal of the American Oil Chemists Association*, *61,* 928-931.

700   Thebaudin, J. Y., Lefebvre, A. C., & Harrington, C.M. (1997). Bourgeois Dietary

701   fibres: Nutritional and technological interest. *Trends in Food Science and*

702   *Technology*, *8 (2),* 41-48.

703   Tokusoglu, Ö., & Ünal, K. (2003). Fat replacers in meat products. *Pakistan Journal*

704   *of Nutrition, 2 (3),* 196-203.

705   Trout, G. R., & Dale, S. (1990). Prevention of warmed-over flavour in cooked beef:

706   Effect of phosphate type, phosphate concentration, a lemon juice-phosphate blend,

707   and beef extract. *Journal of Agricultural and Food Chemistry, 38 (3),* 665-669.

708   Valencia, I., O'Grady, M. N., Ansorena, D., Astiasarán, I., & Kerry, J. P. (2008).

709   Enhancement of the nutritional status and quality of fresh pork sausages following

710   the addition of linseed oil, fish oil and natural antioxidants. *Meat Science*, *80,* 1046-

711   1054.

712   WHO. (2003). Diet, nutrition and the prevention of chronic diseases. Report of a

713   Joint WHO/FAO Expert Consultation. WHO Technical Report Series 916, Geneva.

714   Yen, G. C., & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in

715   relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry, 43*,

716   27-32.

717

718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751

752

753

754

755

756

757

758

759

760 **Legends to Figures**

761 **Fig. 1. Total dietary fibre content of control and seaweed patties**

762 **Fig. 2. Total phenolic content of control and seaweed patties during storage (■:**

763 **10%; ▲: 20%; –: 30%; ●: 40% seaweed)**

764 **Fig. 3. DPPH radical scavenging activity of control and seaweed patties during**

765 **storage (■: 10%; ▲: 20%; –: 30%; ●: 40% seaweed)**

766

767

768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
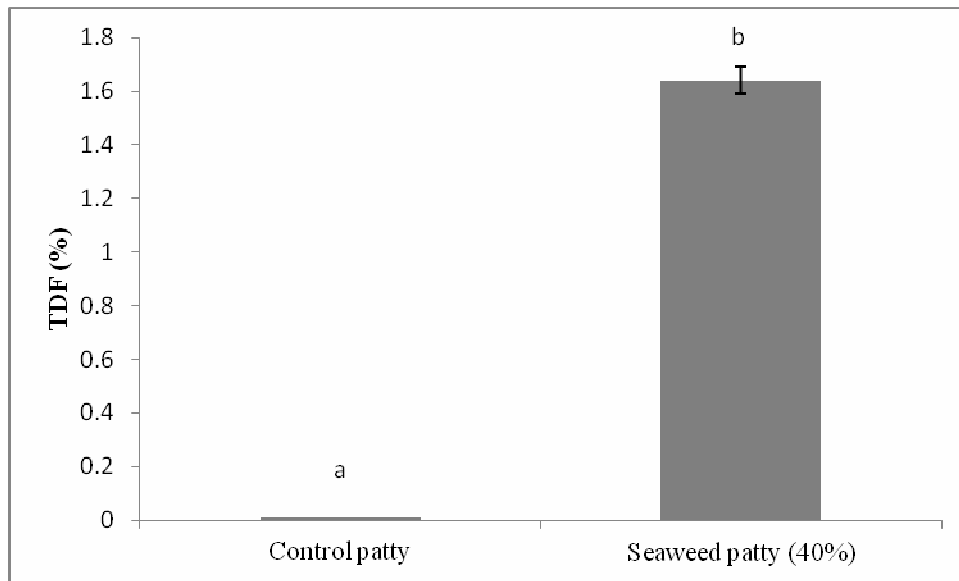789
790

791

Fig. 1. Total dietary fibre content of control and seaweed patties
Each value is presented as mean ± SD (n = 3).

792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820

821 **Table 1. Bacterial enumeration and pH of control and seaweed patties during**

822 **storage**

Patty	Control (0%)	10% seaweed	20% seaweed	30% seaweed	40% seaweed
Bacterial enumeration (log CFU/g)					
Days					
0	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az
7	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az
14	1.10±0.01by	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az
21	3.05±0.03cy	0.00±0.00az	0.00±0.00az	0.00±0.00az	0.00±0.00az
30	5.41±0.02dx	1.09±0.01by	0.00±0.00az	0.00±0.00az	0.00±0.00az
pH					
Days					
0	6.05±0.03ay	6.04±0.02ay	6.03±0.02az	6.01±0.02az	6.02±0.02az
7	6.00±0.01az	6.01±0.02az	6.00±0.03az	6.00±0.02az	6.01±0.03az
14	5.96±0.01by	6.00±0.01az	6.00±0.02az	6.00±0.02az	6.00±0.03az
21	5.95±0.02by	6.00±0.02az	6.00±0.01az	5.99±0.02az	5.99±0.02az
30	5.82±0.01cy	5.99±0.02bz	5.99±0.02bz	6.00±0.03az	6.00±0.03az

823 **Each value is presented as mean ± SD (n = 6, bacterial enumeration; n = 3, pH).**
824 **Means within each column with different letters (a – e) differ significantly ($P < 0.05$).**
825 **Means within each row with different letters (v – z) differ significantly ($P < 0.05$).**
826

827

828

829

830

831

832

833

834 **Table 2. Lipid oxidation of control and seaweed patties during storage (mg**

835 **malondialdehyde/kg)**

Day	Control (0%)	10% seaweed	20% seaweed	30% seaweed	40% seaweed
0	0.19±0.03ax	0.20±0.01ay	0.18±0.02az	0.19±0.01ax	0.19±0.04ax
7	0.45±0.05bv	0.25±0.03bw	0.27±0.03bx	0.22±0.01by	0.24±0.06bz
14	0.77±0.05cv	0.40±0.06cw	0.38±0.01cx	0.39±0.03cy	0.45±0.06cz
21	0.89±0.04dv	0.61±0.05dw	0.55±0.05dx	0.57±0.04dy	0.56±0.02dz
30	1.12±0.02ew	0.69±0.02ex	0.69±0.06ex	0.66±0.02ey	0.61±0.02ez

836 **Each value is presented as mean ± SD (n = 6).**
837 **Means within each column with different letters (a – e) differ significantly ($P < 0.05$).**
838 **Means within each row with different letters (v – z) differ significantly ($P < 0.05$).**
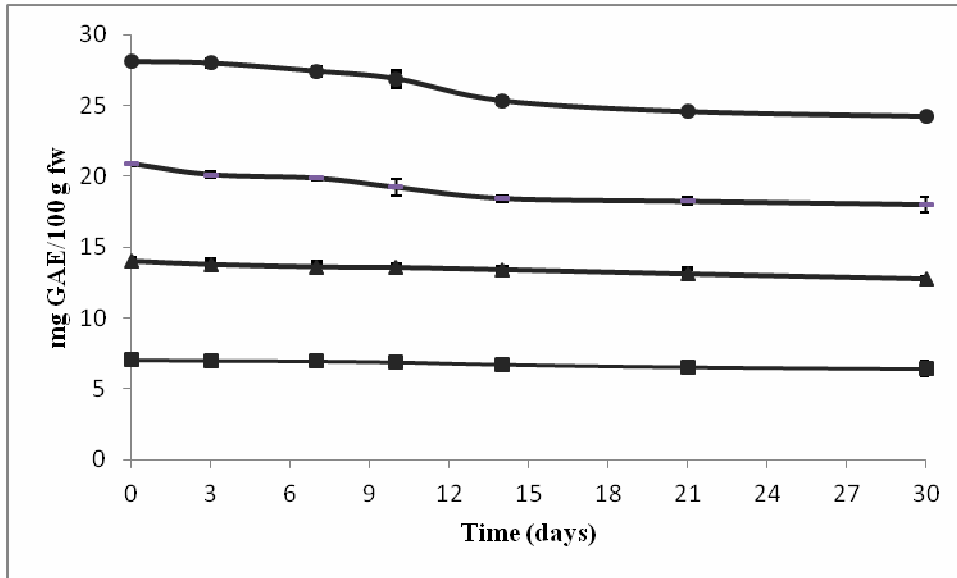839

840

841

842

843

844

845

846

847

848

849

850

Fig. 2. Total phenolic content of control and seaweed patties during storage (■: 10%; ▲: 20%; –: 30%; ●: 40% seaweed)
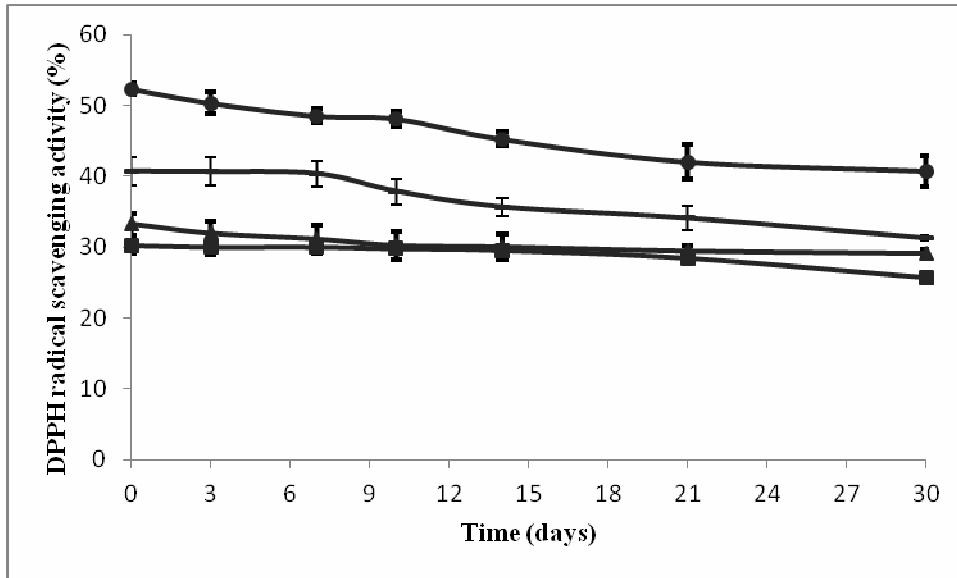Each value is presented as mean ± SD (n = 6).

851
852
853
854
855
856
857
858
859
860
861
862
863
864
865

866

Fig. 3. DPPH radical scavenging activity of control and seaweed patties during storage (■: 10%; ▲: 20%; –: 30%; ●: 40% seaweed)
Each value is presented as mean ± SD (n = 6).

867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883

884 **Table 3. Texture of control and seaweed patties during storage (N/mm)**

Day	Control (0%)	10% seaweed	20% seaweed	30% seaweed	40% seaweed
0	18.06±1.68av	19.06±1.16aw	17.63±1.35ax	17.50±1.10ay	17.77±1.34az
7	25.33±2.31bv	21.25±1.55bw	19.82±1.94bx	18.88±2.30by	18.54±1.25bz
14	32.76±3.30cv	25.11±3.32cw	23.42±2.30cx	22.38±2.38cy	20.11±3.33cz
21	38.22±1.98dv	26.77±2.33dw	24.02±1.34dx	22.78±2.87dy	20.87±2.10dz
30	40.23±1.76ev	28.44±3.54ew	24.54±2.04ex	23.98±2.12ey	21.33±3.45ez

885 **Each value is presented as mean ± SD (n = 6).**
886 **Means within each column with different letters (a – e) differ significantly ($P < 0.05$).**
887 **Means within each row with different letters (v – z) differ significantly ($P < 0.05$).**
888
889

890

891

892

893

894

895

896

897

898

899

900

901

902

903

904

Table 4. Colour of control and seaweed patties during storage (Hunter L*, a*, b*)

Coordinate	Day	Control (0% seaweed)	10% seaweed	20% seaweed	30% seaweed	40% seaweed
L*	0	36.63±0.22aw	39.06±0.08ax	39.08±0.16ax	40.12±0.03ay	40.25±0.11az
	7	35.89±0.56bv	37.08±1.23bw	37.89±0.23bx	40.15±0.80by	41.58±1.12bz
	14	34.63±0.11cv	37.99±0.47cw	37.66±0.29cx	41.25±0.88cy	40.99±0.87cz
	21	34.39±1.18dv	37.39±0.85dw	37.56±0.10dx	41.72±1.02dy	40.12±0.17dz
	30	35.49±1.12ev	37.45±0.52ew	38.12±0.23ex	41.56±1.6ey	40.32±1.07ez
a*	0	8.39±0.04av	7.05±0.33aw	7.96±0.24ax	7.99±0.12ay	8.32±0.09az
	7	8.73±0.09bv	7.12±0.44bw	8.23±0.20bx	8.01±0.39by	8.33±0.56az
	14	9.70±0.56cv	6.96±0.56cw	7.99±0.34cx	8.22±0.23cy	8.87±0.41bz
	21	9.37±0.45dv	6.98±0.25dw	7.58±0.03dx	7.97±0.25dy	8.12±0.57cz
	30	8.91±0.78ev	7.88±0.23ew	7.77±0.87ex	7.87±0.33ey	8.56±0.41dz
b*	0	14.22±0.12av	16.67±0.11aw	16.00±0.02ax	16.54±0.14ay	16.66±0.13az
	7	15.51±0.54bw	16.69±0.14ax	15.97±0.25by	16.99±0.10bz	16.67±0.66az
	14	15.82±0.12cv	16.61±0.45bw	16.04±0.30cx	17.11±0.03cy	17.25±0.49bz

21	15.21±0.13dv	16.55±0.78cw	15.97±0.24dx	17.10±0.65cy	17.32±0.23cz
30	15.74±0.45ev	16.56±1.10dw	15.93±0.55ex	16.67±0.70dy	17.22±0.87dz

Each value is presented as mean ± SD (n = 6).
Means within each column with different letters (a – e) differ significantly ($P < 0.05$).

Table 5. Mean scores for aroma, appearance, texture and taste of the control and seaweed patties

Patty	Sensory attributes				
	Aroma	Appearance	Texture	Taste	Overall acceptability
Control	4.61±0.66a	4.84±0.37a	3.00±0.95a	3.76±0.61a	3.75±1.64a
20% seaweed	4.23±0.83b	4.30±0.48b	3.07±0.44b	4.23±0.83b	4.09±0.88b
40% seaweed	4.38±0.77c	4.23±0.59c	3.69±0.49c	4.15±0.80c	4.25±0.78c

Each value is presented as mean ± SD (n = 20).
Means within each column with different letters differ significantly ($P < 0.05$).