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Enhancement of the phytochemical and fibre content of beef-patties with *Himanthalia elongata* seaweed

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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 ≥ 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.

Keywords: Functional foods; seaweeds; antioxidants; fibre; product development.

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, inducing consumer demand for healthy, nutritious foods with additional health promoting functions (Jiménez-Colmenero et al., 2010). Many new products have been developed and marketed, offering increased health benefits and the potential to reduce the risk of diseases. Sales of such “functional foods” in Europe have increased significantly (Annunziata & Vecchio, 2011). Many components may be added to meat, dairy, fish or vegetable-based products to make them ‘‘functional”, such as ω-3 fatty acids, prebiotics, probiotics and fibre (Jiménez-Colmenero, 2007).

Over the past few decades, meat products have come under increasing scrutiny by medical, nutritional and consumer groups because of the associations established between their consumption (or that of a number of their constituents, such as fat and cholesterol) and the risk of some of the major degenerative and chronic diseases (ischaemic heart disease, cancer, hypertension and obesity). Therefore meat-based functional foods are being seen as an opportunity to improve the “image” of meat and address consumer needs, and also to update the nutritional and dietary goals (Jiménez-Colmenero, 2007). As meat is one of the most important commonly-consumed fast foods, it offers an excellent way of promoting intake of functional ingredients without any radical changes in eating habits (Cofrades et al., 2008). This situation is prompting the emergence of new “healthier” meat products. Most physiologically active substances come from plants, and when combined with other foods such as meat, they can help provide a food with functional effects. The idea of using plant products in the meat industry is not entirely new, as various types of ingredients have been used for their technological, sensory, economic and nutritional effects (Jiménez-Colmenero, 2010).

Meat is low in dietary fibre, therefore addition of ingredients containing fibre to common meat products such as patties would be beneficial. Dietary fibre intake provides many health benefits such as reducing the risk of developing diseases including coronary heart disease, stroke, hypertension, diabetes, obesity and certain gastrointestinal disorders. Furthermore, increased consumption of dietary fibre improves serum lipid concentrations, lowers blood pressure, improves blood glucose control in diabetes, promotes regularity, aids in weight loss and appears to improve the immune function (Anderson et al., 2009).

Seaweeds are known to be a good source of dietary fibre (Cofrades et al., 2008). Plant biomass or its derived bioactive compounds have been considered as possible functional components in processed meat products for alleviation of the colourectal cancer risk associated with the consumption of processed meats (Demeyer et al., 2008). The introduction of functional ingredients such as botanicals, plant extracts and seaweeds with probable biological activity into processed meat products is receiving abundant attention (Calvo et al., 2008; Cofrades et al., 2008; Hayes et al., 2005; Hernández-Hernández et al., 2009; Valencia et al., 2008). Seaweeds are also high in phytochemicals such as phenolic compounds (Cox et al., 2011). Such natural plant phytochemicals could therefore add further functional ingredients to meat based convenience food products such as beefburgers. It has been reported that 34% of men and 21.9% of women consume burgers in Ireland (Duffy et al., 2005), therefore incorporation of seaweed into such beef patties would have potential as a means of developing a healthier meat product.

The aim of this study was to investigate the addition of seaweed at varying concentrations to beef burger patties in order to enhance the levels of fibre and phytochemicals. The effect on sensory properties such as texture, colour and flavor were investigated as were safety aspects such as bacterial enumeration and lipid 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

°C air drying temperature over a period of 2 hours. Air velocity was 2.0 ± 0.1 m s^{-1} measured with VWR Enviro-meter digital anemometer (VWR, Ireland). Dried seaweed was rehydrated by immersion in 2 L of distilled water at 80.5 ± 0.05 °C for 20 ± 0.05 min as optimized in our previous studies (Cox et al., 2011). The seaweed was then ground using a blender (Rotor, Germany) and stored at 4 °C until use.

Seaweed-patty preparation

Five different patty formulations were prepared containing 0, 10, 20, 30 and 40% blanched seaweed. Lean beef (≤ 5% fat) was purchased from a local supermarket and stored immediately in a refrigerator at 4 °C. Meat was cut into smaller pieces using a sterile knife and ground in a meat grinder with a grind size of 4.5mm (Meteor MATR, Ireland) which had been previously sterilised and chilled (4 °C). The seaweed was added to each of the mixtures in sterile bowls and mixed by hand with sterile utensils until the seaweed was homogenous throughout the meat. The final temperature of the meat was < 12 °C in all cases and was formed with a manual circular shaped mould. The patties were 1 cm thick and weighed 50 ± 0.05 g. Samples were cooked in an oven (Rational Combi, Dämpfer, United Kingdom) at 200 °C for 15 min until the centre of the patties reached ≥ 70 °C for over 2 minutes when tested with a temperature probe. The patties were then immediately cooled to 4 °C and placed in polyethylene bags (PA/PE, Brodericks Brothers Limited, Ireland) and vacuum packed (La Minerva, Italy). The samples were stored at 4 °C throughout the storage period for 30 days which is typical for a cooked beef product.

Cooking yield

Patties were weighed before cooking and after chilling at 4 °C. To estimate the cooking yield, the patty weights were expressed as a percentage of the initial weight using the following calculation:

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

Total Dietary Fibre

Total dietary fibre (TDF) was determined by Sigma analysis kit (Sigma-Aldrich, Inc., USA) based on AOAC method 991.43. Samples (5 g) were cooked at 100 ºC with heat stable α-amylase to initiate gelatinization, hydrolysis and depolymerisation of starch. The samples were incubated at 60 ºC with protease (to solubilise and depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to glucose). The samples were then treated with four volumes of ethanol to precipitate soluble fibre and remove depolymerised protein and glucose. The residue was filtered, washed, dried and weighed. One duplicate was analysed for protein and the other was incubated at 525 ºC to determine ash. The TDF was determined as the weight of the filtered and dried residue less the weight of the protein and ash.

Bacterial enumeration

Samples were prepared in a vertical laminar-flow cabinet for the purposes of microbial analysis. For each patty sample, 25 g was taken aseptically and placed in a sterile stomacher bag with 225 ml of peptone water (Scharlau Chemie, Spain). After

2 min in a stomacher blender (Stomacher 400, Seward Medical, United Kingdom), appropriate decimal dilutions were spread-plated (100 µl) onto Plate Count Agar (PCA) (Scharlau Chemie, Spain) for total viable counts (TVC) and incubated at 37 °C for 24 h. The results were expressed as logarithms of colony forming units per gram of sample (log CFU/g). Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

pH measurement

The pH of patties (10 g homogenised in 50 ml distilled water) was determined using an Orion Model 520A pH metre (AGB Scientific Ltd) throughout the storage period. Three readings were taken for each sample. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

Lipid oxidation measurement

Lipid oxidation was assessed on the basis of the amount of malondialdehyde formed during storage. Malondialdehyde is the end-product of lipid peroxidation and was evaluated using the TBARS assay with some modifications (Oussalah et al., 2006). A 10 g portion of each meat sample was blended with 50 ml of distilled deionised water and 10 ml of 15% tricholoroacetic acid (TCA) in a stomacher blender (Stomacher 400, Seward Medical, England) for 2 min at 260 rpm. The homogenate was centrifuged at 1500 gravity for 5 min and the supernatant fluid was filtered through a Durapore 0.45 µm HV membrane filter (Millipore). A 2 ml aliquot of 60 mmol/L TBA reagent was added to 8 ml of the clear filtrate and vortexed for 15 s and then heated in a boiling water bath for 10 min to develop a pink colour. After cooling on ice to ambient temperature (~ 20 °C), the absorbance of the supernatant was measured spectrophotometrically at 532 nm (Milton Roy Spectronic 1201). The concentration of malondialdehyde in analysed samples was calculated on the basis of a standard curve obtained using serial dilutions of 1,1,3,3-tetramethoxyropane solution. The TBARS value was expressed as mg malondialdehyde/kg (mg MDA/kg) of sample. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

Extraction of phytochemicals

Seaweed-patty samples (5 g) were powdered in liquid nitrogen using a mortar and pestle, then extracted with 50ml of methanol (60%) under nitrogen atmosphere for 2 hours. The extraction was carried out at 40 °C at 100rpm in a shaker incubator (Innova 42, Mason Technology, Ireland). Samples were filtered and centrifuged at 10,000 rpm for 15 min (Sigma 2K15, Mason Technology, Ireland). Resulting extracts were evaporated to dryness using vacuum polyevaporator (Buchi Syncore Polyvap, Mason Technology, Ireland) at 60 °C. A pressure gradient program was designed for evaporation of the solvents with vacuum conditions of 337 and 72 mbar for methanol and water, respectively.

Total phenolic content

The total phenolic concentration (TPC) was measured using the Folin-Ciocalteau method (Taga et al., 1984). In this procedure, 100 µl aliquot of stock sample (extract concentration 1000 µg/ml of water) was mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. Then 100 µl of 50% Folin-

Ciocalteau's phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720 nm using spectrophotometer (Milton Roy Spectronic 1201). The total phenolic contents were expressed as mg gallic acid equivalent per 100 gram fresh weight (fw) (mg GAE/100 g fw). Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) according to the method of Yen & Chen (1995) with some modifications. Samples were taken on days 0, 7, 14, 21 and 30 for analysis. Briefly, a 100 µl aliquot of test sample (concentration 50 µg/ml) was placed in a 96-well microtitre plate and 100 µl of 0.16 mM DPPH methanolic solution was added. The mixture was shaken and incubated for 30 min in darkness at 25 ºC. Changes in the absorbance of the samples were measured at 517 nm using a microplate reader (Powerwave, Biotek, VT, USA).

The ability to scavenge the DPPH radical was calculated using the following equation given by Duan et al. (2006):

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

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

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

Texture evaluation

Shear tests were performed using an Instron Universal Testing Machine (Model 4301, Canton MA, USA) supported with Bluehill 2 version 2.14 analysis software for materials testing. A Warner Bratzler cutter was used in the shear tests. An aluminum plate with dimensions of 10 x 6 cm^2, thickness of 1.3 cm and with an opening of 3 mm in the centre was supported in the Instron base. Patty samples (5 g) were sheared at a speed of 200 mm/min. The cutting implement was allowed to travel the depth of the patty, cutting through the sample and hardness was defined as the peak of force-deformation curve recorded in Newtons per mm (N/mm). Ten replications of each sample were carried out. Samples were taken on days 0, 7, 14, 21 and 30 for analysis.

Colour measurement

Colour analysis was performed using a colourimeter (CIE Lab ColourQuest XE) with D65 illuminant and 10 ° standard observer angle setting. Patty samples (5 g) were taken on days 0, 7, 14, 21 and 30 for analysis. The colourimeter was calibrated against a standard white reference tile (L* = 93.97; a* = -0.08 and b* = 1.21). The colour values were represented on the CIE colour scales in terms of L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). From these values, total colour change from fresh (DE) was calculated according to the following equation:

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

Where; L^*_0, a^*_0 and b^*_0 are the readings at time zero and L*, a* and b* are the individual readings at each drying time.

Sensory characteristics

The sensory acceptance test was conducted in a standardised sensory test room (ISO 9599, 2007). Untrained panelists (*n* = 20) were recruited from staff and students of the Dublin Institute of Technology using a five-point hedonic scale. Samples (25 g) were served at the same time on white paper plates with random three-digit numbers, and water at room temperature was provided for mouth-rinsing between samples. The panelists were asked to assign scores for aroma (maximum of 5), appearance (maximum of 5), texture (maximum of 5), flavour (maximum of 5) and overall acceptability of the product (maximum of 5), where 5 was "like extremely" and 1 was "dislike extremely". The overall quality (maximum of 25) was computed by combining scores of all five attributes.

Statistical analysis

All experiments were performed in triplicate and replicated twice. All statistical analyses were carried out using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA). Statistical differences were determined using

ANOVA followed by Least Significant Difference (LSD) testing. Differences were considered statistically significant when $p < 0.05$.

3. Results and Discussion

Cooking yield and dietary fibre content of seaweed-patties

Cooking loss was the highest in the control sample which had a 40.28% reduction in yield. As seaweed levels were increased cooking losses declined. The processing losses were 34.80, 34.32, 34.24 and 33.88% for 10, 20, 30 and 40% seaweed concentrations, respectively. This demonstrated that adding seaweed had a significant effect on retaining moisture as compared to control patties ($P < 0.05$). Cofrades *et al*. (2008) and Fernández-Martín *et al*. (2009) also found that the addition of *H. elongata* improved the water-binding properties of pork meat.

The use of dietary fibre in cooked meat products generally improves hydration properties and fat holding capacity, reducing fat and water loss during cooking and increasing emulsion stability (Thebaudin et al., 1997; Cofrades et al., 2000; Jiménez-Colmenero et al., 2005). The objective of the current study was to incorporate seaweed into beef patties in order to achieve healthier meat products while also producing a product with good sensory attributes such as texture. Seaweeds contain large amounts of dietary fibre and have a high water-holding capacity. The water-holding capacity of seaweeds is closely related to the polysaccharide composition of the dietary fibre fractions, and therefore the gelation process will depend on the type and amount of their polysaccharides (Sánchez-Alonso et al., 2006).

Traditional beef patties are high in fat content (about 14%). Most of this fat is saturated fatty acid (SFA) (about 60% of total fat), while the monounsaturated fatty acid (MUFA) fraction accounts for about 36% of total fat, and the polyunsaturated fatty acid (PUFA) fraction accounts for about 3% of total fat (Martínez et al., 2011). There are often problems with reduction of fat in finely ground meat products, as it can present a number of difficulties in terms of appearance, flavour and texture. This can cause such products to be less accepted by the consumer (Keeton, 1994; García et al., 2002; Tokusoglu & Ünal, 2003). Manufacturers have introduced several modifications in an attempt to offset the detrimental effects of reducing the fat level. These modifications include the use of non-meat ingredients that could help to convey desirable texture and, more importantly, enhance water-holding capacity (Ako, 1998; Keeton, 1994). In this regard, the incorporation of carbohydrates and fibre have been successful in improving cooking yield, reducing formulation cost and enhancing texture (Keeton, 1994; Jiménez-Colmenero, 1996; Mendoza et al., 1998). There are strict food regulations within the EU in relation to labeling the content of ingredients in food products. A product such as beef patties with seaweed would be required to be labeled as such, and the percentage of both seaweed and beef corresponding to the quantity of the ingredients would be required on the product label (EU Directive 2000/13/EC, 2000).

In the current study, dietary fibre may have had an important effect on this technological property because it holds water by adsorption and absorption phenomena and some water is also retained outside the fibre matrix (free water) (Sánchez-Zapata et al., 2010). The total dietary fibre content of the control patty and seaweed-patty at a concentration of 40% can be seen in Fig. 1.

Rehydrated seaweed contained 4.02 g TDF per 100 g fw (4.02%) and when incorporated into patties at 40%, the final product contained 1.64 g TDF per 100 g fw (1.64%). These results are in line with Choi *et al*. (2012) who reported that pork patties with dried *Laminaria japonica* incorporated at levels up to 5% contained 1.23 to 3.14% dietary fibre. López-López et al. (2010) reported the TDF in pork patties containing dried seaweed (3%) to be 1.36% in the final product which is also lower than that of the present study; however less seaweed was added as it was in dried form. The recommended daily intake of dietary fibre is > 25 g per day (WHO/FAO, 2003). The addition of fibre to fast food product which is a commonly consumed and low in fibre would help to increase the daily consumption of dietary fibre amongst the population.

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

Microbial growth (log CFU/g) of the vacuum packed seaweed-patties over 30 days of refrigerated storage can be seen in Table 1. There was no significant difference in the total viable counts for all patties (control, 10, 20, 30 and 40% seaweed) within the first 14 days of storage as there was no growth of bacteria in any of the samples ($P > 0.05$). There was a significant difference ($P < 0.05$) between the control and the seaweed-patties after 14 days as growth began in the control sample and reached 5.41 log CFU/g by day 30. Generally, the addition of seaweed did not affect the spoilage of patties particularly in samples containing > 20% seaweed. A low level of growth (1.09 log CFU/g) was seen in seaweed-patties by day 30, and only in patties containing the lowest level of seaweed (10%). This level was however significantly lower than the control samples ($P < 0.05$).

López-López et al. (2010) reported that the total viable counts of beef patties and those with added seaweed ranged from 6 - 6.4 log CFU/g. Cofrades *et al*. (2011) also reported that the TVC for restructured poultry steaks with added seaweed were in excess of 6 log CFU/g, however the levels from both these studies are higher than that of the present findings, most likely due to the fact that the patties were uncooked. There are no guidelines specific to total viable counts in minced beef intended to be eaten cooked apart from the requirement for *Salmonella* spp. to be absent in 10 g of sample. Guidelines set out by the Food Safety Authority of Ireland (FSAI) for Enterobacteriaceae numbers on raw meat samples stipulate that three of five samples of raw meat must have counts of < 5 log CFU/g and no more than two of five samples of raw meat can have counts between 5 and 7 log CFU/g. Meat exceeding these limits is defined as unacceptable. The levels of TVC in the raw patties before cooking in the present study was 2.09 log CFU/g which is well below the FSAI limits and those established by The European Union Commission Regulation (EC No. 2073/2005) on the microbiological criteria for foodstuffs. The pH of the patties (Table 1) was also monitored throughout the shelf life as high levels of microorganisms result in reductions in pH levels (Gómez-López et al., 2007).

The initial pH values (day 0) of all patty samples were similar ranging from 6.01 to 6.05. These levels are in line with those observed for cooked pork patties with a pH ranging from 6.06 - 6.13 as reported by Choi et al. (2012). Significant differences between the control and seaweed-patties were observed after 14 days of storage. The pH values of all seaweed-patties were 6.00, while that of the control was 5.96, which is only slightly lower. By the end of the storage period (30 days) the pH of the seaweed-patties still had not changed significantly ($P > 0.05$) and was in the range of 5.99 - 6.00 while the control had dropped to 5.82. These results are in agreement with those of the bacterial enumeration as the acidity of the control had dropped and was most and likely due to the increase in bacterial growth as compared to the seaweed-patties.

Lipid oxidation of control and seaweed-patties during storage

Lipid oxidation generates a series of chemical reactions that can alter the physio-chemical parameters, sensorial attributes (odour, colour and flavour) and shelf life in meat and meat products (Liu et al., 1995). TBARS analysis measures the formation of tertiary products of lipid oxidation, mainly malondialdehyde, which may contribute off-flavour to oxidized fat (Lee et al., 2011). Lipid oxidation in precooked products remains of concern to the meat industry due to the increased demand for convenience foods. Undesirable flavour in precooked meats, commonly described as warmed-over flavour, rapidly develops in cooked meat products during refrigerated storage (Ahn et al., 2002). Precooked meats are likely to oxidize and produce secondary compounds such as hexanal, pentanal, 2,4-decadienal, 2,3-oxtanedione, and 2-octenal (Trout & Dale, 1990). Minced meat and meat products undergo oxidative changes more quickly as grinding exposes lipid membranes to metal oxidation catalysts (Lee et al., 2011).

Table 2 shows the effect of different seaweed concentrations on TBARS values of cooked-patties during 30 days of storage. Initial TBARS levels (Day 0) of all samples were similar ranging from 0.18 to 0.20 mg malondialdehyde/kg (mg MDA/kg). TBARS values of all patties containing seaweed were significantly lower ($P < 0.05$) than the control during storage. The TBARS levels began to increase at day 14 of storage. This indicated that there was some protective effect of the seaweed against lipid oxidation in cooked minced beef, potentially due to the increase in phenolic compounds and DPPH activity as discussed. The reduction in lipid oxidation could also be due to the reduction in meat content in the samples (10 - 40% less meat) which accordingly would have lower levels of fat present in the samples thus reducing potential oxidation.

The differences in TBARS values of seaweed-patties ranged from 0.18 – 0.69 mg MDA/kg from the beginning to end of storage. Therefore, the extent of this lipid oxidation during refrigerated storage may be considered relatively low according to Bhattacharya et al. (1988), Rojas & Brewer (2007) and López-López et al. (2010). The results of the present study are in agreement with López-López et al. (2010) who reported that the TBARS values of seaweed-patties ranged from 0.27 – 0.87 mg MDA/kg during frozen storage.

Total phenolic content of control and seaweed patties during storage

The total phenolic content (TPC) of the seaweed-patties over the 30 days of storage is shown in Fig. 2. Phenolic compounds exist as various structures, have different molecular weights and are related to the innate flavour of food. They contain a phenolic hydroxyl group, which has an antioxidative effect through interactions with the phenol ring and has a resonance stabilization effect (Shahidi & Wanasundara, 1992). Differences in the TPC of all samples were significant ($P < 0.05$). The control sample contained no detectable phenols at tested levels, while the TPC increased significantly ($P < 0.05$) with increasing seaweed concentrations (10 - 40%). The TPC ranged from 7.05 - 28.11 mg GAE/100 g fw and by day 30 these levels were 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;

Selgas et al., 2005). López-López (2010) also reported that beef patties containing seaweed were more tender than the control. The effect of seaweed addition on the tenderness of the patties was most likely due to the role played by fibre. The texture of all of the samples in the present study increased (became firmer) throughout storage ($P < 0.05$). The firmness of the control samples was almost double that of those containing 40% seaweed. By the end of the storage period (30 days) the tenderness of the samples ranged from 21.33 – 40.23 N/mm, with the firmest being the control and the most tender were those in patties containing the highest levels of seaweed (40%). This is due to the retention of water in seaweed during the hydration step and the reduction of levels of meat proteins due to its addition.

Colour of control and seaweed patties during storage

Colour was evaluated in order to detect the tendencies for seaweed addition to cause changes in the beef-patties, given that colour is one of the main parameters determining consumer acceptance of a product (Cofrades et al., 2008). Seaweed addition had an immediate effect on colour parameters of patties in comparison to the control (Table 4). At the initial stage (day 0), the L* values of the patty samples with seaweed incorporated were higher than that of the control (colour was lighter). Seaweed concentrations (10 – 40%) also had a significant effect on the L* values as the patties became lighter in colour with increasing seaweed levels ($P < 0.05$). It has been reported that usually in meat products, the higher the moisture content, the higher the lightness (L*) value (Pérez-Alvarez et al., 1999; Alesón-Carbonell et al., 2005; Fernández-López et al., 2008). The higher L* values could therefore also be due to the high moisture content of the seaweed and the moisture retention upon cooking as compared to the control.

The a* values of the samples containing seaweed were significantly different (day 0) as compared to the control ($P < 0.05$), with values ranging from 7.05 (10% seaweed) to 8.39 (control). This parameter is a measure of the redness/greenness of a sample with lower a* readings containing more green pigments. This would explain the reduction in a* values as compared to the control as blanched *H. elongata* is bright green in colour. The initial b* values (day 0) were significicanly ($P < 0.05$) higher than the control patties containing no seaweed. This parameter is a measure of the yellowness/redness of the samples and the higher b* values of the seaweed-patties indicate an increase in yellow colour.

With respect to colour during storage; L* values changed significantly for all samples ($P < 0.05$). The L* values decreased by day 30, indicating a slight darkening of the samples, with the exception of patties with 30 and 40% seaweed which became slightly lighter in colour. There was a significant increase in a* values for all samples (except 20 and 30% seaweed-patties) by day 30, which indicated that the redness of the samples increased slightly, this indicated that there was a reduction in the green colour of the blanched seaweed. There was also a significant increase in b* values for all samples (except 10 and 20% seaweed-patties) by day 30. This indicates that there was a reduction of the yellowness of the samples.

Although there were differences in the colour values throughout the storage period, most of the colour parameters of the patty samples were basically steady (slightly changed) which was also reported by Shan et al. (2009) who studied the effects of adding spice and herb extracts to raw pork. Although the addition of seaweed changed the colour of the patties as compared to the control, this is in line with meat colour changes upon the addition of spice and herbs which are traditionally added to meats. In order to determine the acceptability of the colour, this was taken into account in the sensory analysis.

Sensory analysis

In order to determine if the seaweed-patties were acceptable in terms of aroma, appearance, texture and taste, a preliminary consumer acceptability test was undertaken. Table 5 summarises the sensory scores for aroma, appearance, texture, taste and overall acceptability of control and seaweed-patties. The samples tested by the sensory panel were the control (with no added seaweed), a mid-range seaweed-patties (20% seaweed) and patties with 40% added seaweed which would have the maximum level of antioxidants and TDF. Aroma, appearance, texture and taste of the seaweed-patties were found to be significantly different to the control ($P < 0.05$). The sensory scores for aroma ranged from 4.23 (20% seaweed) to 4.61 (control). The fact that no strong seaweed aroma was detected could be attributed to blanching the seaweed prior to adding to the meat.

The sensory score for appearance ranged from 4.23 to 4.84, with the score reducing with increasing seaweed concentration. This showed that the patties without the incorporation of seaweed were more visually appealing to the sensory panel, however the mean score for all samples was still above 4, which is a positive result. The scores for texture were significantly higher with increased levels of seaweed ($P < 0.05$). Therefore the panel detected that seaweed altered the texture and possible mouthfeel of the patties which was one of the objectives of the study. The addition of blanched seaweeds over dried seaweeds in the present study offers exploitation of the gelling properties of the seaweeds. This would also contribute to the technological properties of the seaweed such as reducing cooking losses.

The seaweed-patties also had a significantly higher score for taste than the control with 20% seaweed-patties ranking the highest ($P < 0.05$). The 40% seaweed-patty ranked highest in the overall acceptability score ($P < 0.05$) with the control receiving the lowest score. The results of the present study are promising particularly when compared to those reported in literature. Piňero et al. (2008) found that the taste scores for beef patties with added oat fibre to be lower than the control. Cofrades et al. (2011) reported that while all restructured poultry steaks with added *H. elongata* were judged acceptable by a sensory panel, the control received a higher score for overall acceptability than those containing seaweed. On the other hand, Choi et al. (2012) stated that sensory evaluations indicated that the greatest overall acceptability in pork-patties was also attained in samples containing seaweed.

4. Conclusion

The addition of *H. elongata* to meat products in the development of functional foods opens up new potential for seaweed utilisation. Incorporating such seaweeds is of interest from a technological and functional point of view. The seaweed had a positive effect on the cooking yield of the patties due to their hydrocolloid content which reduce cooking losses. Total dietary fibre, polyphenolic content and antioxidant activity were increased due to the incorporation of seaweed. Storage life was enhanced in samples containing seaweed as compared to the control and lipid oxidation was also greatly reduced due to the levels of phytochemicals present in the seaweed. The seaweed also had a positive effect on the texture of the patties as they were more tender than the control which was also confirmed in the sensory analysis study. The seaweed-patties were found overall to be acceptable by a sensory panel, particularly in terms of texture.

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Legends to Figures

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

Fig. 2. Total phenolic content of control and seaweed patties during storage (■: 10%; ▲: 20%; –: 30%; ●: 40% seaweed)

Fig. 3. DPPH radical scavenging activity of control and seaweed patties during storage (■: 10%; ▲: 20%; –: 30%; ●: 40% seaweed)

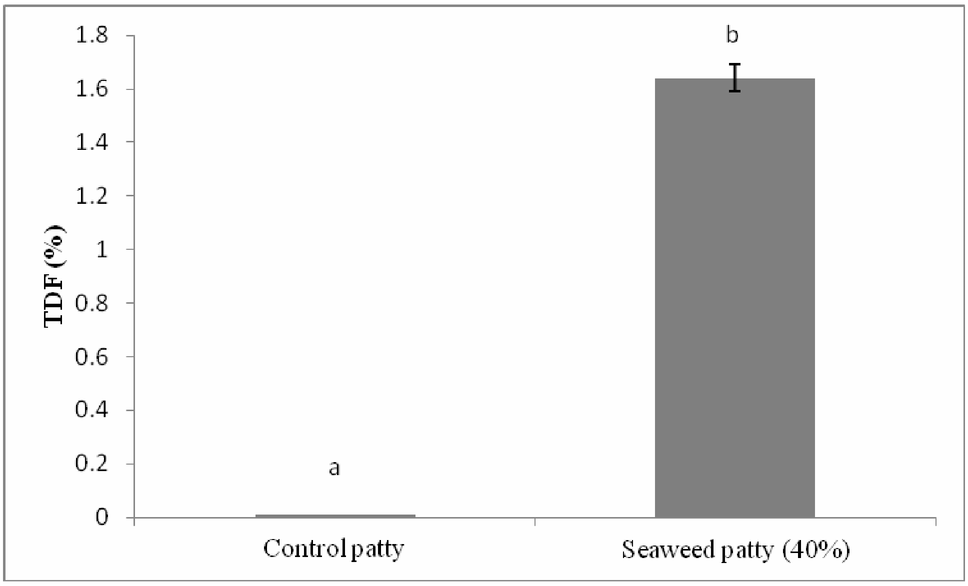

Fig. 1. Total dietary fibre content of control and seaweed patties

Each value is presented as mean ± SD (n = 3).

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

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

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

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

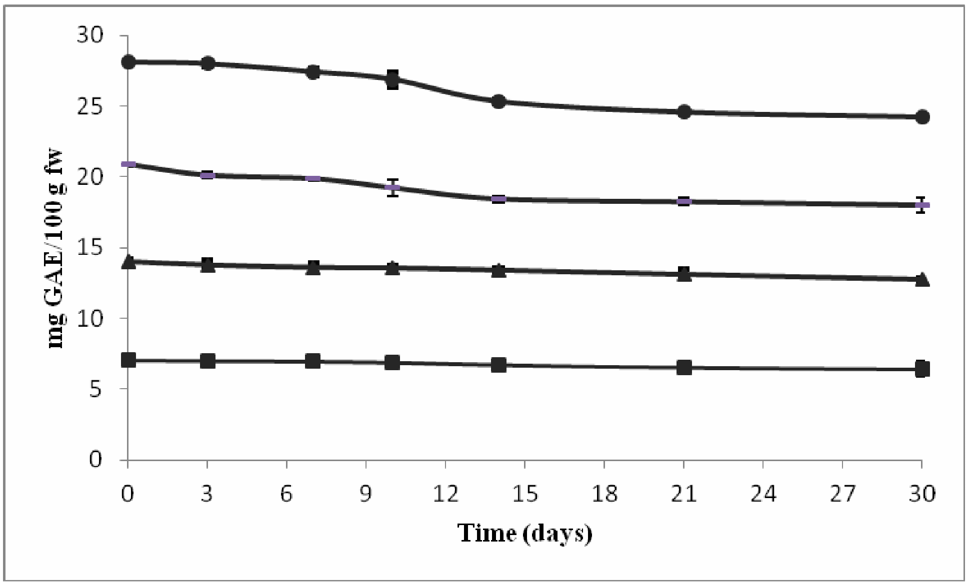

Fig. 2. Total phenolic content of control and seaweed patties during storage (■: 10%; ▲: 20%; –: 30%; ●: 40% seaweed)
Each value is presented as mean ± SD (n = 6).

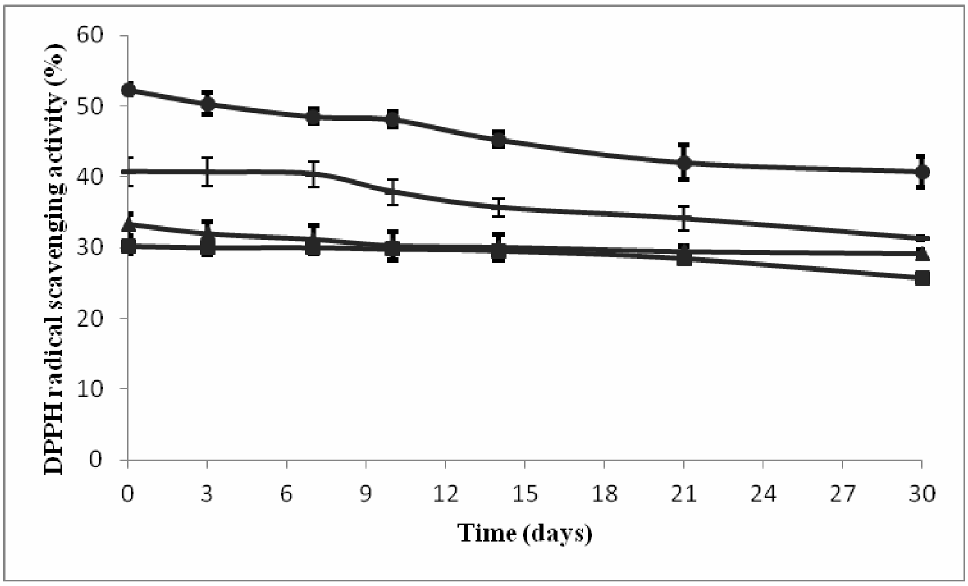

Fig. 3. DPPH radical scavenging activity of control and seaweed patties during storage (■: 10%; ▲: 20%; –: 30%; ●: 40% seaweed)
Each value is presented as mean ± SD (n = 6).

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

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

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 ($P < 0.05$).