2010-12-22

Growth Inhibition of Common Food Spoilage and Pathogenic Microorganisms in the Presence of Brown Seaweed Extracts

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

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

Gaurav Rajauria
Technological University Dublin, gaurav.rajauria@tudublin.ie

See next page for additional authors

Recommended Citation

Gupta, S., Cox, N., Rajauria, G., Jaiswal, A.K. & Abu-Ghannam, N. (2010). Modelling the growth inhibition of common food spoilage and pathogenic micro-organisms in the presence of brown seaweed extracts. *Food and Bioprocess Technology,* DOI: 10.1007/s11947-010-0502-6

Funder: Irish Government under the Technological Sector Research Scheme (Strand III) of the National Development Plan

Food Bioprocess Technol
DOI 10.1007/s11947-010-0502-6

ORIGINAL PAPER

Growth Inhibition of Common Food Spoilage and Pathogenic Microorganisms in the Presence of Brown Seaweed Extracts

Shilpi Gupta · Sabrina Cox · Gaurav Rajauria · Amit Kumar Jaiswal · Nissreen Abu-Ghannam

Received: 18 August 2010 / Accepted: 22 December 2010

Abstract The possibility of using extracts from brown seaweed, *Himanthalia elongata*, as a natural antimicrobial agent for food preservation is presented. The effect of different concentrations of seaweed extract on the growth kinetics of four common food spoilage (*Pseudomonas aeruginosa* and *Enterococcus faecalis*) and food pathogenic microorganisms (*Listeria monocytogenes* and *Salmonella abony*) was examined. Seaweed extract at a concentration of 6% inhibited the growth of all four of the studied organisms. Lower concentrations of seaweed extract prolonged the lag phase and reduced both the exponential growth rate and final population densities of the culture. Suitability of three kinetic models, Baranyi–Roberts, modified Gompertz and logistic, for describing the growth/survival of organisms in the presence of different concentrations of the extract, was evaluated. Root mean square error (RMSE) and correlation coefficient (R^2) were used to evaluate the model performance. The R^2 value was greater than 0.95 for most of the cases indicating that the models could provide a good fitting to the experimental data. The RMSE and residual sum of squares were very low for all the three models, and no significant difference was observed in the goodness of fit between the three models as indicated by the *F* test.

Keywords Seaweed · Non-thermal methods · Food preservation · Baranyi–Roberts · Modified Gompertz · Logistic

S. Gupta · S. Cox · G. Rajauria · A. K. Jaiswal · N. Abu-Ghannam (✉)
School of Food Science and Environmental Health,
Dublin Institute of Technology,
Cathal Brugha St,
Dublin 1, Ireland
e-mail: nissreen.abughannam@dit.ie

Nomenclature

A	The lower asymptotic line of the growth curve as t decreases to zero
B	The relative maximum specific growth rate (per hour) at time M
C	The difference between the upper asymptotic line of the growth curve (maximum population level N_{max}) minus the lower asymptotic line ($N_{max}-N_0$) (log colony-forming unit (CFU) per millilitre)
M	The time at which the specific growth rate is maximum (hours)
N_0	Initial population level at time t=0 (log CFU per millilitre)
N_t	The cell number at any time t (log CFU per millilitre)
N_{max}	Maximum population level
R^2	The coefficient of determination
RSS	Residual sum of squares
RMSE	Root mean square error

Greek Letters

γ	Log_{10} maximum population density for Baranyi–Roberts model (log CFU per millilitre)
λ	Lag time (hours)
μ	Exponential specific growth rate for Baranyi–Roberts model (per hour)
μ_{max}	Maximum specific growth rate (per hour)

Introduction

Preservatives are required to maintain the quality, extend shelf life and ensure safety of fresh and processed food products. Although chemical preservatives form an essential part in food preservation, legislation has restricted their

use in different foods (Brul and Coote 1999). At the same time, consumer preferences are moving towards foods that contain lower levels of chemical preservatives, exhibit characteristics of fresh or natural products and are microbiologically safe as well.

Non-thermal methods such as the addition of naturally occurring compounds having antibacterial activity (Hayes et al. 2010), high pressure carbon dioxide (Garcia-Gonzalez et al. 2009), high intensity pulsed electric field (Mosqueda-Melgar et al. 2008), irradiation (Alighourchi et al. 2008) or ultrasound (Schenk et al. 2008; Salleh-Mack and Roberts 2007) are increasingly gaining attention for preservation of minimally processed foods. In recent years, the use of naturally occurring antimicrobial agents to inhibit pathogen growth and prevent food spoilage has received special attention (Hayes et al. 2010). Useful antimicrobial phytochemicals can be divided into several categories such as phenolics and polyphenols; quinines; flavones, flavonoids and flavonols; tannins; alkaloids and lectins; coumarins and polypeptides. Nowadays, minimal preservation processes based on the combined factors technology are also gaining importance for food preservation. Char et al. (2010) studied the response of *Listeria innocua* to combined treatments involving moderate temperatures and the addition of different levels of citral to obtain a minimally processed orange juice.

Seaweeds are considered a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterised by a broad spectrum of biological activities. Although seaweeds grow in a harsh environment, they seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cells have some protective compounds and mechanisms (Matsukawa et al. 1997). Since seaweeds are a good source of antimicrobial compounds, ω3 fatty acids, antioxidants and other bioactive compounds, there is an interest to utilize these products as nutraceuticals and in functional foods (Yuan 2008). Compounds, such as polyphenols, flavonoids and polysaccharides, having antioxidant and antimicrobial activities have been detected in brown, red and green algae (Cox et al. 2009; Zaragoza et al. 2008). Ara et al. (2002) reported brown algae to be active against a number of Gram-positive and Gram-negative organisms. Nagayama et al. (2002) reported that phlorotannins, brown-algal phenolic compounds, such as eckol and eckol-related compounds, from *Ecklonia kurome*, have strong bactericidal activity. A series of polyphenolic compounds such as catechins, flavonols and flavonol glycosides have been identified from methanol extracts of red and brown algae (Hosokawa et al. 2006) and found to have antioxidant and antimicrobial activity. Horie et al. (2008) isolated sargaquinoic acid derivatives from the brown alga *Sargassum sagamianum* having antibacterial properties.

Traditional microbial enumeration techniques are time-consuming, and therefore, mathematical microbial models are used to assess the potential for growth of micro-organisms in foods during processing and storage (Bovil et al. 2001). Empirical sigmoidal type models such as the modified Gompertz and logistic models or the semi-mechanistic model of Baranyi–Roberts have been used for fitting bacterial growth (Xiong et al. 1999). However, data on the use of actual plant extract for inhibiting microbial growth and modelling the resulting kinetics are scarce. Most of the studies done till date use either thermal treatments or purified compounds having antimicrobial activity for studying growth inhibition.

This study was conducted to determine the effect of different concentrations of brown seaweed (*Himanthalia elongata*) extract against *Listeria monocytogenes*, *Salmonella abony*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. There are some reports available wherein the antimicrobial effect of seaweed extract has been studied on different organisms (Taskin et al. 2010; Cox et al. 2009; Ely et al. 2004; Nagayama et al. 2002), but no studies are available where the growth inhibition has been modelled. Hence, the present study investigates the utilization of methanolic extract from brown seaweed as a natural antimicrobial agent for food preservation by examining its effects on the growth kinetics of four common food spoilage and food pathogenic microorganisms. In order to describe growth inhibition in the presence of seaweed extract, performance of three commonly used primary models, namely the Baranyi–Roberts, modified Gompertz and logistic models, was evaluated.

Materials and Methods

Seaweed Material

Brown seaweed *H. elongata* (Pheophyta) was purchased from Quality Sea Veg., Co. Donegal, Ireland. Samples were received in September 2009 and washed thoroughly with freshwater to remove epiphytes and salt.

Preparation of Seaweed Extracts

The extraction of seaweed was carried out with 60% methanol under nitrogen atmosphere at 40 °C and 100 rpm in a shaker incubator (Innova 42, Mason Technology, Ireland). Samples were filtered and centrifuged at 10,000 rpm (8,720×*g*) 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 programme was designed for evaporation of the

solvents with vacuum conditions of 33,700 and 7,200 Pa for methanol and water, respectively.

Antimicrobial Activity

Microbial Culture

Two species of common food pathogenic and food spoilage bacteria selected for this study were *L. monocytogenes* (ATCC 19115), *S. abony* (NCTC 6017), *E. faecalis* (ATCC 7080) and *P. aeruginosa* (ATCC 27853), respectively (Medical Supply Company, Dublin, Ireland). All cultures were maintained at −70 °C in 20% glycerol stocks and grown in Tryptic Soy Broth (TSB; Scharlau Chemie, Barcelona, Spain) at 37 °C, except for *P. aeruginosa* which was incubated at 30 °C, to obtain sub-cultures. A final cell concentration of 1×10^6 colony-forming units (CFU)/ml was used for the experiments.

Relation Between Turbidity and Viable Count

Before the kinetics study, a relationship between optical density at 600 nm and viable count was determined for all of the bacteria studied. A volume of 200 µl of bacterial suspension containing 6 log CFU/ml was dispensed into 50 wells of the 96-well microtitre plate (Sarstedt Ltd., UK). Every hour, the optical density (OD) of the microtitre plate was read. At the same time, an aliquot of 100 µl from one well was transferred into 900 µl of maximum recovery diluent (Scharlau Chemie, Barcelona, Spain) to determine the viable cell count. Spreading was carried out on Tryptic soy agar (Scharlau Chemie, Barcelona, Spain) plates by taking 100 µl of relevant dilution. Plates were incubated at 37 °C, with the exception of *P. aeruginosa* (30 °C), for 24 h before determining the CFU per millilitre. A standard curve (OD_{600nm} vs. log CFU per millilitre) was drawn from the results obtained. This curve was later used for conversion of the OD values to log CFU per millilitre for respective bacteria in the presence of seaweed extract.

Antimicrobial Activity Assay

The influence of varying concentrations of extract on efficacy was assessed against the four organisms using 96-well microtitre plates. Extract (300 mg) obtained from 5 g fresh seaweed was dissolved in TSB (2.5 ml), and 200 µl was added to the first row of each plate. All other wells were filled with 100 µl of TSB, and 100 µl from the first well was serial diluted into 2-fold along each column. Finally, 100 µl of bacterial suspension containing 6 log CFU/ml was added to the wells. The last row was used for bacterium and media controls. Sample blanks were also prepared for all of the extracts. The plate was incubated in the microtitre reader for 24 h at respective temperature for each organism. Microbial growth was recorded every 2 h on a Powerwave microplate spectrophotometer (Powerwave, Biotek) driven by Gen5 reader control and data analysis software. Turbidity was measured as absorbance at 600 nm, with 20 s agitation before each OD measurement. The OD values were converted to log CFU per millilitre by the standard curve as described in "Relation Between Turbidity and Viable Count" section.

Growth Curve

To describe the inhibition of bacterial growth in the presence of seaweed extract, three primary growth models, namely modified Gompertz, logistic and Baranyi–Roberts model, were fitted to the data, and their performance was comparatively evaluated. Growth curves were plotted to evaluate the antibacterial activities of the seaweed extract.

Baranyi–Roberts Model

A programme implemented in Microsoft Excel (DM-Fit; Institute of Food Research, Norwich, UK) was used to fit the equation of Baranyi and Roberts (1994) to the growth data. To evaluate the effect of different extract concentrations main kinetic parameters such as exponential specific growth rate (μ), Log_{10} maximum population density (γ), lag time (λ) and the coefficient of determination (R^2) were calculated.

Modified Gompertz Model

The modified Gompertz model (Gibson et al. 1987) is given by Eq. 1,

$$N_t = A + C \times \exp\left[-\exp\left\{-B \times (t-M)\right\}\right] \quad (1)$$

where N_t is the cell number (log CFU per millilitre) at any time t, A is the lower asymptotic line of the growth curve as t decreases to zero (that is N_0: initial population level at time t=0 (log CFU per millilitre)), C is the difference between the upper asymptotic line of the growth curve (maximum population level, N_{max}) minus the lower asymptotic line (for example, $N_{max}-N_0$ (log CFU per millilitre)), B is the relative maximum specific growth rate (per hour) at time M and M is the time at which the specific growth rate is maximum. Equations 2, 3 and 4 can then be used for the calculation of maximum specific growth rate (μ_{max} (per hour)), lag phase duration (λ, hours) and maximum cell population (N_{max}), respectively:

$$\mu_{max} = \frac{B \times C}{e} \quad (2)$$

where e=2.7182

$$\lambda = M - \frac{1}{B} \tag{3}$$

$$N_{max} = A + C \tag{4}$$

Logistic Model

The logistic model used for defining bacterial growth as a function of time at constant environmental conditions, such as temperature, pH, water activity etc. is given by Eq. 5 (Gibson et al. 1987):

$$N_t = A + \frac{C}{1 + \exp[-B \times (t - M)]} \tag{5}$$

where N_t, A, B, M and C have the same meaning as given for the modified Gompertz equation. The μ_{max} and λ parameters can be calculated by Eqs. 6 and 7, respectively, as follows:

$$\mu_{\max} = \frac{B \times C}{4} \tag{6}$$

$$\lambda = M - \frac{2}{B} \tag{7}$$

Model Comparison

Root Mean Square Error The smaller the root mean square error (RMSE) values, the better the fit of the model to the data.

$$\text{RMSE} = \sqrt{\frac{\sum (\text{predicted} - \text{observed})^2}{n - p}} \tag{8}$$

where n is the number of observations and p the number of parameters to be estimated.

Curve Fitting

A plot of microbial count versus time for each extract concentration was used to derive the starting values for the parameters, N_0 and $N_{\max}$, for all three models evaluated. The lag time was obtained from the raw data by noting the time when exponential growth started. The experimental data were fitted to equations described above by nonlinear regression with a Marquardt algorithm using the software Statgraphics Centurion XV (StatPoint Technologies, Inc., Warrenton, VA, USA). The aim of the fitting procedure was to find each model's parameters that best described the data by minimizing the sum of the squares of the differences between the model simulated and experimental values.

Statistical Analysis

All experiments were performed in duplicate and replicated at least three times. All statistical analyses were carried out using STATGRAPHICS Centurion XV. Statistical differences between extract activities were determined using ANOVA followed by least significant difference testing. Differences were considered statistically significant when $p<0.05$.

Results and Discussion

Antimicrobial Effect of Different Concentrations of *H. elongata* Extracts

In our previous study (Cox et al. 2009), we had reported the antioxidant capacity of six species of Irish seaweeds and found the methanolic extracts from *H. elongata* to be the richest in terms of antioxidant properties. The selection of the pathogenic microbes (*L. monocytogenes* and *S. abony*) was made after discussions with the Food Safety Authority of Ireland as these were found to be the most challenging organisms for the safety of food products in Ireland. The other two (*E. faecalis* and *P. aeruginosa*) are the most widespread food spoilage microorganisms. Since the yield of the extract obtained was only 60 mg/g seaweed, growth inhibition was checked by measuring the OD by a microtitre plate-based assay rather than by the conventional spread plate method. As expected, control samples showed a rapid and prolific growth, as the populations were 9.6, 10, 14.1 and 17.2 log CFU/ml after 24 h for *S. abony*, *L. monocytogenes*, *P. aeruginosa* and *E. faecalis*, respectively (Fig. 1). The incorporation of seaweed extract resulted in variable levels of inhibition in the growth of the different organisms. Resistance to extract was not correlated with taxonomy, since *E. faecalis* (Gram positive) and *P. aeruginosa* (Gram negative) were the most sensitive to all of the different concentrations of the extract followed by *S. abony* and *L. monocytogenes*. Figure 1 shows the influence of the different concentrations of crude extracts obtained from *H. elongata* against the four studied organisms. The extract had a strong antagonizing effect on the food spoilage and pathogens studied, showing a remarkable dose–response relationship with an increase of the lag phase duration and decrease of the exponential growth rate. In addition, a reduction in the maximum number attained or a complete suppression of growth was observed. The addition of *H. elongata* extracts resulted in complete

Q1

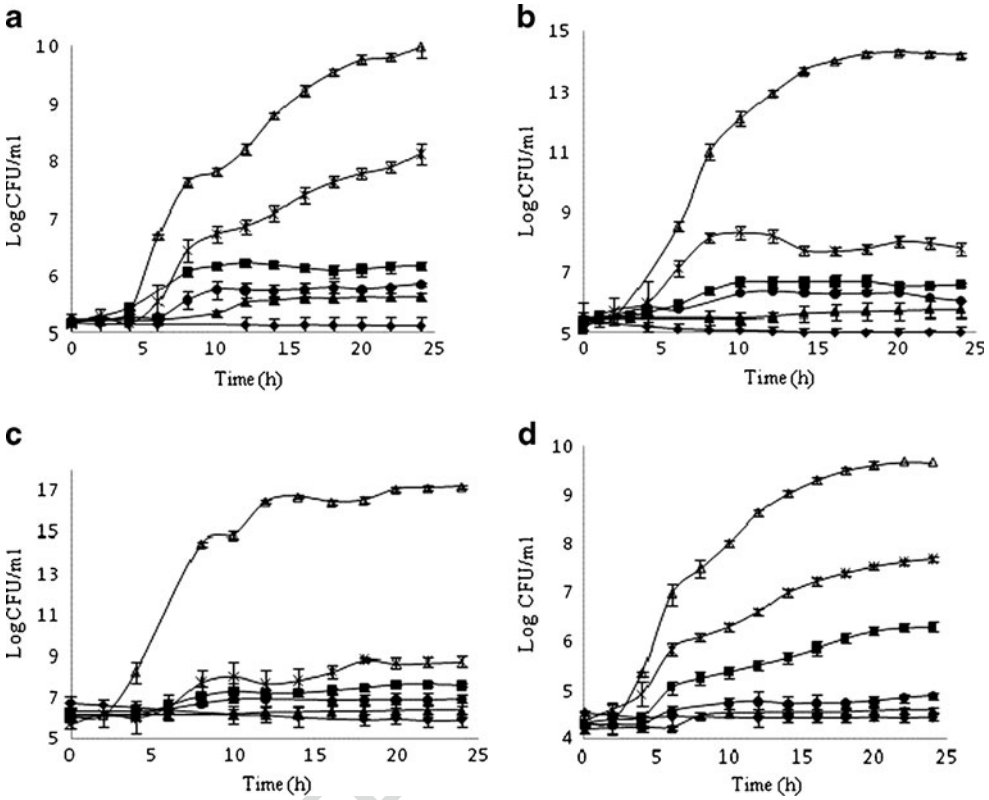

Fig. 1 Growth kinetics of food spoilage and pathogenic bacteria in presence of different concentrations of seaweed extract: **a** *L. monocytogenes*, **b** *P. aeruginosa*, **c** *E. faecalis* and **d** *S. abony*. Different concentrations of extract used: *diamond* 6%, *black triangle* 3%, *circle* 1.5%, *square* 0.75%, *asterisk* 0.375% and *white triangle* 0%

growth inhibition of all the studied organisms at the highest extract concentration (6%) used (Fig. 1). The bactericidal activity can be attributed due to the presence of phenolic compounds such as bromophenols and phlorotannins, produced by brown algae (Nagayama et al. 2002). Phenolic compounds from other plant sources have also been reported to inhibit various foodborne pathogens (Plaza et al. 2010; Kim et al. 2005; Prashanth et al. 2001). Polyphenols, such as tannins and flavonoids, are important antibacterial substances. Halogen-containing terpenoids, acetylenes and phenols have also been identified in several seaweed species as biologically active compounds having antibacterial and anti-tumoural activities (Cardozo et al. 2007; Vairappan et al. 2001; Carvalho and Roque 2000). Plaza et al. (2010) identified volatile compounds like fatty acids, alkanes, phenols and compounds such as phytol (2-hexadecen-1-ol, 3,7,11,15-tretramethyl) and neophytadiene in the ethanol extracts from *Synechocystis* sp. and *H. elongata*. These compounds have been already proposed to have antimicrobial activity (Alagić et al. 2006).

The cell density in the presence of 6% extracts upon completion of the assay (24 h) was lower for all four bacteria than the initial bacterial density. Similar effect was seen in earlier studies on the effect of seaweed extracts on growth of marine and fish pathogenic extracts (Dubber and Harder 2008) where it had been anticipated the reason for this frequently observed result could be associated with the complete disappearance of the bacterial DNA upon incubation with algal extract components. Therefore, the extracts presumably evoked not only a bacteriostatic but also bacteriolytic mode of action. Studies by Ceylan et al. (1998) revealed that addition of 1% spice (garlic, clove and cinnamon) to salami mixed with starter culture and *Escherichia coli* O157:H7 resulted in slight reduction of the pathogen; however, the addition of 7.5% garlic and clove killed 99% of the pathogen. Similar results were obtained in the present study as well wherein addition of 6% extracts resulted in growth inhibition and extract concentrations lesser than that caused a reduction in the cell numbers.

As the extract concentration was serially diluted, the inactivation effect was reduced. Although the addition of extracts at a concentration of 3% did not result in a complete inactivation of bacteria, the growth kinetics was highly altered. An increase in the cell number, after 24 h of incubation with 3% extract, for each of the four bacteria was in the range of 0.21–0.43 log CFU/ml. A lag phase much longer than the control was observed, and the specific growth rate was significantly reduced for all the organisms. At the same time, a reduction of 98% (*E. faecalis*), 97% (*P. aeruginosa*), 93% (*S. abony*) and 91% (*L. monocytogenes*) in the stationary level growth was observed as compared to the control. There was a significant difference ($p<0.05$) in the stationary phase growth (24 h) of *P. aeruginosa*, *E. faecalis*, *L. monocytogenes* and *S. abony* upon the addition of 3%, 1.5% and 0.75% extract. The increase in the cell

number upon the addition of 1.5% extract, after 24 h incubation, was in the range of 0.6–0.8 log CFU/ml. Thus, it can be said that addition of these concentrations of extracts resulted in an extended lag phase. Despite the fact that reducing the extract concentration to 0.75% resulted in a lag phase similar to that of the control, the specific growth rate was highly suppressed. Hence, an increase of 1.5-, 1.6-, 2.2- and 2.3-fold in the stationary phase growth of *S. abony*, *L. monocytogenes*, *P. aeruginosa* and *E. faecalis*, respectively, was seen in the control (0% extract) as compared to samples containing 0.75% extract. Thus, the bacteria started to grow at almost similar times, but the presence of the extract suppressed the maximum cell number attained. This was further evident from a slight increase of 1–1.9 log CFU/ml for each of the four bacteria after 24 h growth. Reducing the extract concentration further to 0.35% and 0.18% resulted in growth patterns very similar to the control in case of *L. monocytogenes* and *S. abony*.

The present study utilized methanol as a solvent for extraction of compounds responsible for the observed effect. Studies are also available wherein different solvents have been utilized for the extraction of biologically active compounds from seaweeds. Earlier reports on the effectiveness of extraction methods evidenced that methanol extraction yielded higher antimicrobial activity than other solvents such as *n*-hexane and ethyl acetate (Sastry and Rao 1994; Paul and Puglisi 2004). It is well documented that using organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activities as compared to water-based methods (Masuda et al. 1997; Lima-Filho et al. 2002).

A significant finding of the present study was the potency of the extract against Gram-negative bacteria (*P. aeruginosa*). Gram-negative bacteria are more resistant pathogens compared to the Gram-positive bacteria. They have an additional lipopolysaccharide layer on the outer surface which prevents certain drugs and antibiotics from penetrating the cell thus accounting for the high resistance of these bacteria to antibiotics (Dowling 2004). Therefore, the present study brings out a new insight towards the development of antimicrobial agents against Gram-negative bacteria from seaweeds. In recent years, the use of non-thermal techniques for preservation of food has been gaining importance. The use of ozone, irradiation or

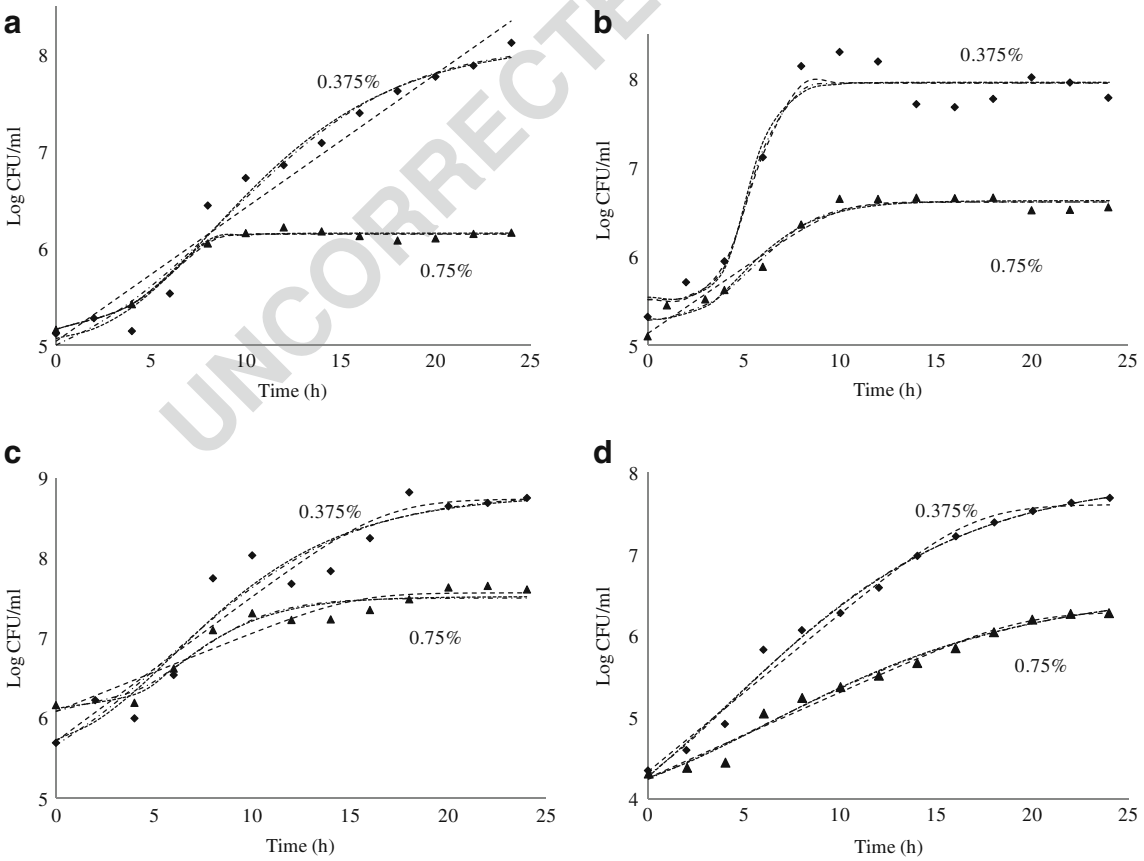

Fig. 2 Fitting of the three models to the inactivation of the four organisms by extract at a concentration of 0.75% and 0.375%. **a** *L. monocytogenes*, **b** *P. aeruginosa*, **c** *E. faecalis* and **d** *S. abony*. Different concentrations of extract used: *triangle* 0.75% and *diamond* 0.375%. Different models: *dotted line* modified Gompertz model equation, *dashed line* Baranyi–Roberts model equation and *dash-dotted line* logistic model equation. Points represent experimental data

ultrafiltration is better than thermal sterilization but can have a detrimental impact on the bioactive compounds present in foods if used at higher levels (Tiwari et al. 2009; Alighourchi et al. 2008; Zárate-Rodríguez et al. 2000). In this regard, the use of natural antimicrobials in foods to prevent spoilage might also provide the additional benefit of preserving the bioactive properties of foods. Thus, the addition of extracts from seaweeds can not only impart microbiological safety to food products as they are rich in bioactives (Cox et al. 2009) but can also provide foods with antioxidants in order to prevent oxidative spoilage.

Comparison of Kinetic Models

Generally, the models that can be used for describing the kinetics of survival curves are either empirical or based on biological assumptions. Three primary growth models (modified Gompertz (empirical), logistic (empirical) and Baranyi–Roberts model (semi-mechanistic model)) were used to analyse the delay or inhibition of growth against the four different organisms. In most of the cases, the R^2 values for all the models were greater than 0.9 (except when extract at a concentration of 1.5% was added to *P. aeruginosa*), indicating a good fit to the experimental data. Examples of the fit of the three models to the inactivation of the four organisms at an extract concentration of 0.75% and 0.375% are depicted in Fig. 2. All the parameters obtained for the three mathematical models were directly related to the extract concentration. Analyses of variance indicated that the maximum specific growth rate, μ_{max}, was significantly reduced ($p<0.05$) with increasing extract concentration suggesting that the cells became more sensitive (Table 1). The estimated values for the lag phase for all the three models tended to increase as the extract concentration increased. In individual model analysis, it was found that all the three models were capable of fitting the experimental data very reasonably and produced almost similar curves; however, no model could produce consistently best fit to all the growth curves analysed. RMSE (Table 2) was used as a statistical measure for comparison of the experimental and model simulated values. There was no significant difference between the RMSE ($p>0.05$) for the three models. One way to discriminate the goodness of fit among different models is to compare them statistically

Table 1 Estimations of the kinetic parameters using the logistic, modified Gompertz and the Baranyi-Roberts models

		Logistic		Baranyi–Roberts			Gompertz		
	Conc. (%)	μ	Lag	μ	Lag	A	μ	Lag	A
L. monocytogenes	6	−0.0062	–	−0.0032	–	–	−0.007	–	4.94
	3	0.101	8.75	0.104	8.83	5.62	0.109	8.83	5.63
	1.5	0.195	6.24	0.18	6.106	5.77	0.179	6.02	5.77
	0.75	0.212	2.8	0.173	2.56	6.15	0.256	2.99	6.15
	0.375	0.191	–	0.137	–	–	0.209	2.77	8.13
	0	0.336	–	0.287	–	9.89	0.364	1.74	10.12
S. abony	6	−0.163	1.73	−0.026	–	4.43	−0.177	1.75	4.43
	3	0.169	6.4	0.154	6.24	4.55	0.144	6.02	4.55
	1.5	0.223	0.47	0.191	4.49	4.76	0.148	4.05	4.77
	0.75	0.116	–	0.105	–	6.31	0.12	–	6.57
	0.375	0.22	–	0.193	–	7.61	0.226	–	7.95
	0	0.471	–	0.399	–	9.56	0.515	1.1	9.71
P. aeruginosa	6	−0.005	–	−0.0415	–	5.03	−0.053	–	5.02
	3	0.035	10.3	0.0275	9.59	5.74	0.035	10.43	5.74
	1.5	0.129	2.5	0.102	–	6.25	0.141	0.74	6.25
	0.75	0.213	–	0.148	–	6.61	0.218	2.59	6.62
	0.375	0.764	3.74	0.669	3.54	7.94	0.808	3.65	7.95
	0	0.991	2.06	0.8127	1.49	14.118	1.09	3.1	14.25
E. faecalis	6	−0.055	–	−0.049	–	5.92	−0.057	–	5.85
	3	0.017	6.4	0.0104	–	–	0.019	7.38	6.37
	1.5	0.167	5.55	0.147	5.33	6.88	0.173	5.47	6.88
	0.75	0.171	2.51	0.098	–	7.56	0.183	3.22	7.52
	0.375	0.216	–	0.18	–	8.73	0.235	0.985	8.79
	0	1.54	2.19	1.29	1.87	16.78	1.63	2.58	16.9

t2.1 **Table 2** Values of the statistical indices, RMSE and RSS, for the three models against four different bacteria at six different extract concentrations

		RMSE			RSS			
	Conc. (%)	A	B	C	A	B	C	
L. monocytogenes	6	0.01	0.0	0.01	0.0	0.0	0.0	t2.4
	3	0.015	0.018	0.012	0.0018	0.0027	0.0012	t2.5
	1.5	0.035	0.036	0.035	0.0113	0.012	0.0113	t2.6
	0.75	0.044	0.043	0.046	0.014	0.013	0.0146	t2.7
	0.375	0.197	0.27	0.181	0.351	0.659	0.295	t2.8
	0	0.273	0.310	0.251	0.673	0.865	0.567	t2.9
S. abony	6	0.01	0.010	0.01	0.0	0.0	0.0	t2.10
	3	0.023	0.023	0.021	0.004	0.004	0.004	t2.11
	1.5	0.057	0.057	0.056	0.026	0.026	0.025	t2.12
	0.75	0.109	0.116	0.108	0.108	0.121	0.104	t2.13
	0.375	0.126	0.156	0.13	0.147	0.219	0.153	t2.14
	0	0.238	0.306	0.206	0.51	0.842	0.388	t2.15
P. aeruginosa	6	0.01	0.026	0.01	0.0	0.01	0.0	t2.16
	3	0.019	0.021	0.015	0.0024	0.003	0.001	t2.17
	1.5	0.146	0.133	0.149	0.148	0.124	0.155	t2.18
	0.75	0.106	0.106	0.121	0.112	0.112	0.147	t2.19
	0.375	0.332	0.233	0.257	0.994	0.488	0.595	t2.20
	0	0.235	0.337	0.15	0.44	0.909	0.181	t2.21
E. faecalis	6	0.027	0.031	0.027	0.004	0.006	0.004	t2.22
	3	0.019	0.026	0.017	0.002	0.005	0.002	t2.23
	1.5	0.021	0.017	0.027	0.003	0.002	0.005	t2.24
	0.75	0.155	0.185	0.139	0.192	0.272	0.156	t2.25
	0.375	0.337	0.357	0.332	1.026	1.147	0.994	t2.26
	0	0.455	0.559	0.374	1.65	2.51	1.12	t2.27

A logistic, *B* Baranyi–Roberts, *C* modified Gompertz equation

Zwietering et al. (1990). The models were statistically validated with the use of *F* test. The calculated *F* values were lower than the *F* table values, indicating that there was no significant difference in the goodness of fit between the three models, except for 6% extract concentration against *P. aeruginosa*. In this case, fitting by Gompertz and logistic were found to be better than Baranyi–Roberts model (data not shown). Although the performance of the modified Gompertz model was better than the logistic and the Baranyi–Roberts model, the use of one primary model or the other in case of inactivation curves should be guided by specific requirements (Geeraerd et al. 1997). However, based on the RMSE and residual sum of squares (RSS) values, it can be said that all the three model equations were effective for describing sigmoidal curves as previously reported by Xiong et al. (1999), who modelled the thermal inactivation of *L. monocytogenes*. The model kinetic parameters such as the λ, μ and N_{max} estimated using the experimental data with the modified Gompertz, logistic and Baranyi–Roberts model are summarized in Table 2. As there was no difference in the goodness of fit of the three models, the parameters of modified Gompertz were further analysed, as an example, to study the effect of extract concentration on the bacterial growth. The most prominent effect of the extract was an increase in the lag phase duration. The increase in lag phase due to the addition of extract at a concentration of 3% was more than 3-fold for *L. monocytogenes* and *P. aeruginosa* as compared to the control. A delay in, or inhibition of, microbial growth is particularly useful in terms of food safety. The extension of the lag phase is probably the most widely used parameter to describe the inhibitory effects of antimicrobial compounds, and a slight delay in the lag phase may have an important

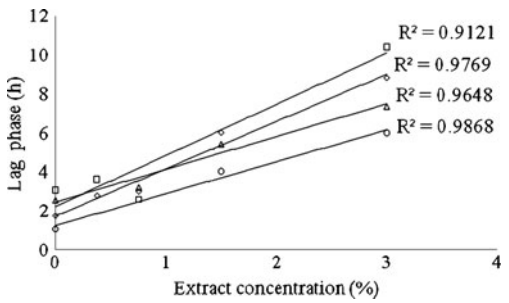

Fig. 3 Relation between lag phase and concentration of extract against the four different organisms (*diamond—L. monocytogenes*, *circle—S. abony*, *square—P. aeruginosa* and *triangle—E. faecalis*)

influence on the shelf life of food products. Thus, the prolonging of lag phase could be used as an appropriate index for evaluation of the activity of the antimicrobial compounds. In the present study, a linear positive correlation (R^2 ranging between 0.912 and 0.986) was obtained between the lag phase and the concentration of seaweed extract for the different organisms (Fig. 3). The seaweed extracts were potent even at an extract concentration as low as 0.75% resulting in 42% and 20% increase in the lag phase of *L. monocytogenes* and *E. faecalis*, respectively, as compared to the control. Extract concentration of 3% increased the lag phase in a range of 65% to 81% for all of the organisms. In an early report on microbial growth modelling of fresh filled pasta stored at different temperatures by Giannuzzi (1998), it was observed that the ratio of specific growth rate to generation time was nearly constant which suggests a linear relationship between lag phase and the reciprocal of the maximum specific growth rate. Similar observations were confirmed in the present study during the inactivation of the four organisms at different extract concentrations.

The concentration of extract also had a strong effect on the maximum specific growth rate. A reduction of 99% and 96.8% was observed in the maximum specific growth rate, as compared to control at 3% extract concentration (*E. faecalis* and *P. aeruginosa*, respectively).

It has been reported in the literature that flavonoids, polysaccharides, sesquiterpenes and phlorotannins can be obtained from seaweeds. These active ingredients produce varied pharmacological effects such as anti-angiogenic, anti-inflammation, disinfection and anti-tumour. The application of the extracts of *H. elongata* in food industry may contribute to such pharmacological activities as food antioxidation, health care and in addition as food nutrient. Therefore, these extracts could be applied as natural additives with extensive market prospect.

Conclusion

H. elongata can be considered as a promising marine plant in the development of bioactive ingredients for functional foods, nutraceuticals and other applications. The extracts showed an evident antimicrobial effect against the microorganisms used in the present study in a dose-dependent manner. Complete growth inactivation of all of the studied organisms was observed at a concentration of 6%. Addition of extracts at a concentration less than that resulted in an extension of the lag phase and significantly reduced maximum specific growth rate. A reduction of 91–98% in the stationary level growth as compared to the control was also observed. The findings suggest that seaweed extracts have a good potential as natural antibacterial substances in food preservation. It might be possible that high concentrations of these extracts may adversely affect the organoleptic properties of food; however, lower concentrations may be sufficient for food safety in situations where bacterial load is low.

Acknowledgement The authors would like to acknowledge funding from the Irish Government under the Technological Sector Research Scheme (Strand III) of the National Development Plan.

References

Alagić, S., Stančić, I., Palić, R., Stojanović, G., & Lepojević, Ž. (2006). Chemical composition of the supercritical CO_2 extracts of the Y*aka, Prilep* and *Otlja* tobaccos. *J Essent Oil Res, 18*(2), 185–188.

Alighourchi, H., Barzegar, M., & Abbasi, S. (2008). Effect of gamma irradiation on the stability of anthocyanins and shelf-life of various pomegranate juices. *Food Chem, 110*(4), 1036–1040.

Ara, J., Sultana, V., Ehteshamul-Haque, S., Athar, M., & Qasim, R. (2002). Antibacterial activity of marine macroalgae from Karachi coast. *Bull Pol Acad Sci, 50*, 199–206.

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *Int J Food Microbiol, 23* (3–4), 277–294.

Bovil, R. A., Bew, J., & Baranyi, J. (2001). Measurements and predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating temperature. II. Rapidly changing temperatures. *Int J Food Microbiol, 67*(1–2), 131–137.

Brul, S., & Coote, P. (1999). Preservative agents in foods—mode of action and microbial resistance mechanisms. *Int J Food Microbiol, 50*(1–2), 1–17.

Cardozo, K. H. M., Guaratini, T., Barros, M. P., Falcão, V. R., Tonon, A. P., Lopes, N. P., et al. (2007). Metabolites from algae with economical impact. *Comp Biochem Physiol C, 146* (1–2), 60–78.

Carvalho, L. R., & Roque, N. F. (2000). Halogenated and/or sulphated phenols from marine macroalgae. *Quim Nova, 23*(6), 757–765.

Ceylan, E., Kang, D., Daniel, Y. C. F. (1998). Spices may reduce *Escherichia coli* O157:H7 in meat. Available at http://genetics.miningco.com/library/blpressecoli.htm. Accessed on 5 Aug 2010.

Char, C. D., Guerrero, S. N., & Alzamora, S. M. (2010). Mild thermal process combined with vanillin plus citral to help shorten the inactivation time for *Listeria innocua* in orange juice. *Food Bioprocess Technol, 3*(5), 752–761.

Cox, S., Abu-Ghannam, N., & Gupta, S. (2009). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *Int Food Res J, 17*(1), 205–220.

Dowling, P. M. (2004). Antimicrobial therapy. In H. Bertone (Ed.), *Equine clinical pharmacology* (pp. 13–48). London: Saunders.

Dubber, D., & Harder, T. (2008). Extracts of *Ceramium rubrum*, *Mastocarpus stellatus* and *Laminaria digitata* inhibit growth of marine and fish pathogenic bacteria at ecologically realistic concentrations. *Aquaculture, 274*(2–4), 196–200.

Ely, R., Supriya, T., & Naik, C. G. (2004). Antimicrobial activity of marine organisms collected off the coast of South East India. *J Exp Mar Biol Ecol, 309*(1), 121–127.

Garcia-Gonzalez, L., Geeraerd, A. H., Elst, K., Van Ginneken, L., Van Impe, J. F., & Devlieghere, F. (2009). Influence of type of microorganism, food ingredients and food properties on high-pressure carbon dioxide inactivation of microorganisms. *Int J Food Microbiol, 129*(3), 253–263.

Geeraerd, A., Herremans, C., Van Impe, J. (1997). Structural model requirements to describe microbial inactivation. In: *Proceedings Science and Technology (International Institute of Refrigeration). Predictive microbiology applied to chilled food preservation* (pp 280–287). Quimper: European Commission, C2.

Gibson, A. M., Bratchell, H., & Roberts, T. A. (1987). The effect of sodium chloride and temperature on rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *J Appl Bacteriol, 62*(6), 479–490.

Giannuzzi, L. (1998). Mathematical modelling of microbial growth in fresh filled pasta stored at different temperatures. *J Food Process Preserv, 22*(6), 433–447.

Hayes, J. E., Stepanyan, V., Allen, P., O'Grady, M. N., & Kerry, J. P. (2010). Effect of lutein, sesamol, ellagic acid and olive leaf extract on the quality and shelf-life stability of packaged raw minced beef patties. *Meat Sci, 84*(4), 613–620.

Horie, S., Tsutsumi, S., Takada, Y., & Kimura, J. (2008). Antibacterial quinone metabolites from the brown alga, *Sargassum sagamianum*. *Bull Chem Soc Jpn, 81*(9), 1125–1130.

Hosokawa, M., Bhaskar, N., Sashima, T., & Miyashita, K. (2006). Fucoxanthin as a bioactive and nutritionally beneficial marine carotenoid: a review. *Carotenoid Sci, 10*, 15–28.

Kim, Y. S., Hwang, C. S., & Shin, D. H. (2005). Volatile constituents from the leaves of *Polygonum cuspidatum* S. et Z. and their antibacterial activities. *Food Microbiol, 22*(1), 139–144.

Lima-Filho, J. V. M., Carvalho, A. F. F. U., & Freitas, S. M. (2002). Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian coast. *Braz J Microbiol, 33*(4), 311–333.

Masuda, M., Abe, T., Sato, S., Suzuki, T., & Suziki, M. (1997). Diversity of halogenated secondary metabolites in the red algae *Laurencia nipponica* (Rhodomelaceae Ceramiales). *J Phycol, 33*(2), 196–208.

Matsukawa, R., Dubinsky, Z., Kishimoto, E., Masaki, K., Masuda, Y., & Takeuchi, T. (1997). A comparison of screening methods for antioxidant activity in seaweeds. *J Appl Phycol, 9*(1), 29–35.

Mosqueda-Melgar, J., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2008). Non-thermal pasteurization of fruit juices by combining high-intensity pulsed electric fields with natural antimicrobials. *Innovative Food Sci Emerg Technol, 9*(3), 328–340.

Nagayama, K., Iwamura, Y., Shibata, Y., Hirayama, I., & Nakamura, T. (2002). Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. *J Antimicrob Chemother, 50*(6), 889–893.

Paul, V. J., & Puglisi, M. P. (2004). Chemical mediation of interactions among marine organisms. *Nat Prod Rep, 21*, 189–209.

Plaza, M., Santoyo, S., Jaime, L., García-Blairsy Reina, G., Herrero, M., S noráns, F. J., et al. (2010). Screening for bioactive compounds from algae. *J Pharm Biomed Anal, 51*(2), 450–455.

Prashanth, D., Asha, M. K., & Amit, A. (2001). Antibacterial activity of *Punica granatum*. *Fitoterapia, 72*(2), 171–173.

Salleh-Mack, S. Z., & Roberts, J. S. (2007). Ultrasound pasteurization: The effects of temperature, soluble solids, organic acids and pH on the inactivation of *Escherichia coli* ATCC 25922. *Ultrason Sonochem, 14*(3), 323–329.

Sastry, V. M. V. S., & Rao, G. R. K. (1994). Antibacterial substances from marine algae: Successive extraction using benzene, chloroform and methanol. *Bot Mar, 37*(4), 357–360.

Schenk, M., Guerrero, S., & Alzamora, S. M. (2008). Response of some microorganisms to ultraviolet treatment on fresh-cut pear. *Food Bioprocess Technol, 1*(4), 384–392.

Taskin, E., Caki, Z., Ozturk, M., & Taskin, E. (2010). Assessment of *in vitro* antitumoral and antimicrobial activities of marine algae harvested from the eastern Mediterranean Sea. *Afr J Biotechnol, 9*(27), 4272–4277.

Tiwari, B. K., O'Donnell, C. P., & Cullen, P. J. (2009). Effect of non thermal processing technologies on the anthocyanin content of fruit juices. *Trends Food Sci Technol, 20*(3–4), 137–145.

Vairappan, C. C., Daitoh, M., Suzuki, M., Abe, T., & Masuda, M. (2001). Antibacterial halogenated metabolites from the Malaysian *Laurencia* sp. *Phytochemistry, 58*(2), 291–297.

Xiong, R., Xie, G., Edmondson, A. S., Linton, R. H., & Sheard, M. A. (1999). Comparison of the Baranyi model with the modified Gompertz equation for modelling thermal inactivation of *Listeria monocytogenes* Scott A. *Food Microbiol, 16*(3), 269–279.

Yuan, Y. V. (2008). Marine algal constituents. In C. Barrow & F. Shahidi (Eds.), *Marine nutraceuticals and functional foods* (pp. 259–296). Boca Raton: CRC.

Zaragoza, M. C., Lopez, D., Saiz, M. P., Poquet, M., Perez, J., Puig-Parellada, P., et al. (2008). Toxicity and antioxidant activity in vitro and in vivo of two *Fucus vesiculosus* extracts. *J Agr Food Chem, 56*(17), 7773–7780.

Zárate-Rodríguez, E., Ortega-Rivas, E., & Barbosa-Cánovas, G. V. (2000). Quality changes in apple juice as related to nonthermal processing. *J Food Qual, 23*(3), 337–349.

Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & Van'T Riet, K. (1990). Modelling of the bacterial growth curve. *Appl Environ Microbiol, 56*(6), 1875–1881.

AUTHOR QUERY

AUTHOR PLEASE ANSWER QUERY.

Q1. Figures 1 and 3 has poor quality with pixelated image. Please provide better quality of the said figures.