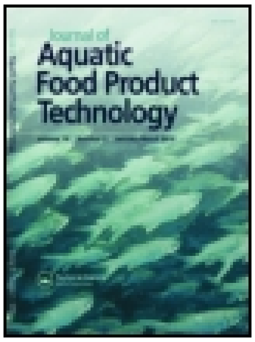

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Optimisation of Concentration of *Undaria pinnarifida* (Wakame) and *Himathalia elongate* (Sea Spaghetti) Varieties to Effect Digestibility, Texture and Consumer Attribute Preference

Jonathan D. Wilkin[a], Katrina Ross[a], Tiffany Alric[b], Matthew Hooper[a], John V. Grigor[a], and Boon-Seang Chu[a]

[a]Division of Engineering and Food Science, School of Applied Sciences, Abertay University, Dundee, Scotland; [b]Veterinary and Agronomy School, VetAgro Sup, Lempdes, France

ABSTRACT

In this study, seaweed concertation in a model system (cracker) was investigated to better understand addition of seaweed on starch modulation, texture, and consumer attribute detection. Temporal dominance of sensations (TDS) showed that seaweed concentrations over 15% had a dominant attribute of fishy for both Wakame and Sea Spaghetti varieties, whereas for both, Crunchy was observed for the 5% and 10% crackers. This corralled well with the texture analysis where crackers fortified with both seaweeds at 5% and 10% have a harder texture, with lower fracturability compared to higher concentrated seaweed crackers. The fracturability levels within the 15% and 20% crackers were comparable to the no substitution controls. In vitro digestion indicated that glucose was liberated immediately after initiation, with both seaweed systems reaching plateau by 4 minutes. In this study it was observed that seaweed variety affected starch digestibility, with wakame seaweed inhibiting starch digestion.

KEYWORDS

Seaweed; wakame; sea spaghetti; new product development; digestibility; texture

Introduction

The seaweed industry is growing; in 2018, the total world production was 32.4 million tonnes for wild-collected and cultivated aquatic algae (FAO 2020). Due to consumers becoming aware of Asian diets, particularly the Japanese diet, seaweeds are an attractive food product within the UK and other European countries (Birch et al. 2019; Lucas et al. 2019).

Seaweeds can be sun-dried by spreading over a net or tarpaulin or dried conventionally with warm air through rotary dryers. Drying affects the nutritional composition of brown seaweeds (Chan et al. 1997), and freeze-drying can give greater extractability and quality of proteins than oven-drying, but not with essential fatty acids (Wong and Cheung 2001.). Seaweeds are then milled to specific lengths depending on the product and functionality of the ingredient. Some studies have shown that drying reduced the total phenolic content (TPC) and total flavonoid content of seaweeds by 29% and 30%, respectively. However, studies have shown that incomplete drying (50% moisture loss) increased the TPC, where a 40% increase in TPC was observed when compared with complete drying of seaweeds (Gager et al. 2020; Gupta et al. 2011).

Research has shown that seaweed consumption can be linked to certain health benefits due to their anticancer activities (Fedorov et al. 2013; Sanjeewa et al. 2017), anti-inflammatory properties (Jaswir and Monsur 2011; Ramberg et al. 2010; Wells et al. 2017); high antioxidant activity (Pinteus et al. 2017; Sanjeewa et al. 2017); antimicrobial properties (Águila-Ramírez et al. 2017), and sustainable films and composites for food and pharmaceuticals (Khalil et al. 2017). In addition, owing to their high content

CONTACT Jonathan D. Wilkin j.wilkin@abertay.ac.uk Division of Engineering and Food Science, School of Applied Sciences, Abertay University, Bell Street, Dundee, Scotland.

of soluble dietary fibers, such as alginate, carrageenans, and agar, seaweeds form viscous solutions or gels that have the ability to interfere with food digestion (Edwards 1995). Human trials have demonstrated that intervention of Nori seaweed before eating a standard meal could decease postprandial glycemic response in healthy adults (Goñi et al. 2000). Meanwhile, chondrus and wakame seaweeds have been found to modify the starch digestion of white bread in *in vitro* systems (Goñi et al. 2002). Research in modulation of starch digestion to control postprandial blood glucose levels has attracted a lot of interest recently due to its potential to help manage diabetes and hyperglycemia.

Findings in relation to health benefits of seaweeds have offered new opportunities for developing seaweeds as a fortifying ingredient of functional foods. However, proving the health benefits of the fortifying ingredient is only half the story, the resulting fortified food needs to be at least as acceptable as the unfortified equivalent. Unfortunately, fortification can dramatically change the taste, appearance, and overall acceptability of food. For example, Hutchings et al. (2017), using a random-effects meta-analysis, reported that as little as 2 g per 100 g fortification of seaweed to cereal products caused a moderate to large reduction in overall acceptability. Therefore, developing a new product requires understanding of consumer preferences of the product and then assessing different attributes within the product (MacFie 2007). Understanding how these ingredients affect the overall product attributes is key to developing novel foods (Fernqvist and Ekelund 2014).

This study aimed to investigate consumer perception of taste and texture of wheat cracker as a food model that is separately fortified with two varieties of seaweed, wakame and sea spaghetti, at different concentrations. A sensory tool called Temporal Dominance of Sensations (TDS) was used to understand consumer perception of texture and taste of the crackers. Although serving size is standardized, differences in the masticatory parameters, breakdown paths, and bolus formation can still be observed through TDS (Young et al. 2016). Another aim of this study was to determine if the presence of seaweed is sufficient to modulate starch digestion kinetics of the crackers by pancreatic enzymes in simulated *in vitro* experiments. A slower starch digestion rate would be beneficial for controlling postprandial blood glucose levels. As a growing consumer trend, consumers are searching for healthier and sustainable diets. This project provided the opportunity to better understand consumer perception of taste and texture through TDS, effects of texture on the food product, and ultimately digestibility of seaweed species as a fortification product into simple food products (crackers). Seaweeds can be added to food products, but understanding the correct concentration with perceived health benefits will provide a clear pathway for industrial adoption.

Materials and methods

Materials

Seaweed species *Undaria pinnarifida* (wakame) and *Himathalia elongate* (sea spaghetti) were sourced from New Wave Foods Ltd. (Wick, Scotland). The fresh seaweed was responsibly harvested by hand, washed, and dried at low temperatures (20°C) for 24 hours. Water activity (a_w) was measured to ensure consistency of final product (<0.6 a_w) and then milled before passing through a 1 mm^2 mesh-sieve. The seaweed samples were stored in a dark, dry, cool place prior to use.

Wholemeal wheat flour, refined rapeseed oil, and white sugar were purchased from a local supermarket. All cracker samples were formulated using ingredients with the same batch code. Lyophilized porcine pancreatin (8 x USP), bis-tris buffer, and 3,5-dinitrosalicylic acid, along with all other chemicals were purchased from Sigma-Aldrich (Dorset, UK). The chemicals purchased were of analytical grade.

Cracker development

Pilot experiments were conducted to determine flour varieties and maximum levels of dried seaweed that could be added without impinging the cracker in terms of texture and flavor. The cracker dough was found to be too tough with seaweed concentrations higher than 20%, with this limit deemed suitable through small kitchen panel testing. The control cracker recipe was wholemeal flour (65.0 g), water (42.75 g), rapeseed oil (5.0 g), and sugar (2.5 g).

Using Minitab (Version 17), a factorial design was set up to incorporate two different seaweeds into a standard cracker mixture. The wholemeal flour constituent was replaced with seaweed at the following percentages: 5, 10, 15, and 20 w/w%. A total of 30 experimental runs of different cracker recipes including control with no seaweed were generated (Table 1). The other ingredients were kept constant.

The crackers were mixed using a Kenwood Chef Titanium XL KVL8300S, rolled out (2 mm thick), cut to 2 cm^2 squares, and placed on a baking sheet in the center of an oven at 180 °C for 14 min. The crackers were cooled and placed in airtight containers and used within 2 days.

Panelist selection for TDS sessions

Panelists were recruited from within Abertay University's societal network, through poster and notification media methods. In total, fifteen panelists (9 females; 6 males) were selected from within the School of Applied Science at the university using the following inclusion criteria: interest in healthy eating; a wish to try innovative food products; aged between 20 and 40 years old; non-smokers; have good oral and general health; can distinguish between sensory attributes from a questionnaire; fluent

Table 1. Factorial design comprised of 30 experiment runs with different seaweed-to-flour ratios (w/w) in the cracker recipe. The amount of water (42.75 g), rapeseed oil (5.0 g), and sugar (2.5 g) in all samples was fixed. For the control, the amount of flour was 65.0 g.

Seaweed Concentration (ratio)	Wholemeal Flour (ratio)	Seaweed Species
0.05	0.95	Wakame
0.10	0.90	Wakame
0.00	1.00	Control
0.15	0.85	Wakame
0.00	1.00	Control
0.10	0.90	Sea Spaghetti
0.20	0.80	Sea Spaghetti
0.05	0.95	Sea Spaghetti
0.20	0.80	Wakame
0.10	0.90	Wakame
0.20	0.80	Wakame
0.15	0.85	Wakame
0.20	0.80	Sea Spaghetti
0.00	1.00	Control
0.00	1.00	Control
0.10	0.90	Sea Spaghetti
0.00	1.00	Control
0.20	0.80	Sea Spaghetti
0.05	0.95	Sea Spaghetti
0.05	0.95	Sea Spaghetti
0.10	0.90	Wakame
0.05	0.95	Wakame
0.00	1.00	Control
0.15	0.85	Sea Spaghetti
0.15	0.85	Sea Spaghetti
0.15	0.85	Wakame
0.10	0.90	Sea Spaghetti
0.05	0.95	Wakame
0.15	0.85	Sea Spaghetti
0.20	0.80	Wakame
\		

in English; and used computers frequently. Testing was conducted at Abertay University's Food Sensory Consumer labs (ISO8589:2007).

Sensory attribute generation for TDS

Feedback from a small preliminary consumer trial involving different panelists to the TDS panel was used to construct an attribute list for the seaweed crackers. Sensory attributes were discussed describing cracker samples leading to a final list of 10 attributes (Pineau et al. 2009). The attribute list has a mix of types, which included texture and taste attributes. These were salty, crunchy, bitter, sweet, sticky, fishy, chewy, hard, gritty, and soft. The attributes were order balanced to avoid positional bias.

Warm-up and preparation process

Panelists undertook a 5-minute warmup before the sensory session. During this warmup period, the concept of TDS and a definition of the attributes were explained. Panelists were given a trial run through the whole TDS test to ensure that they were ready to undergo the experiment. Panelists were encouraged to ask questions to clarify any instructions during this warmup period.

Experimental process

Each sensory session (5 sessions in total, with 5/6 samples per session) lasted approximately 20 minutes, with a 30-second break between samples. Samples were arranged in randomized balanced order generated by Compusense Software (Compusense, Ontario, Canada). Participants attended every session, where they sampled randomly 5/6 samples of seaweed crackers. Each participant tested every seaweed cracker sample but not all in the same session.

Data collection utilized a similar protocol outlined by Vázquez-Araújo et al. (2013), but with amendments. Panelists were seated in individual booths with green light illumination to mask the color of the seaweed samples, which removed the visual bias and ability to identify seaweed variety by appearance. Table 1 shows the fortification and controls of crackers prepared prior to panelists completing this study.

Panelists were instructed to place the sample in their mouth, then immediately press the "Start" button on the computer screen. Panelists were then presented with attributes on the screen before selecting the perceived dominant attribute at any given time point. Panelists were instructed during the warmup that not all attributes needed to be selected and that the same attribute could be chosen several times. Panelists were asked to chew the sample until it felt natural to swallow, at which point the panelists were instructed to stop the TDS by clicking the "Stop" button, before being given a further 40 seconds to complete the TDS evaluation. It was deemed that 40 seconds post swallow was adequate time to provide the participants with an aftertaste. Panelists were asked to rinse their mouth with water and await the next sample.

Generation of response rate and significance line TDS graphs

The number of selected attributes at each time point within 40 seconds determined the response rate. In each individual dominance rate graph, a "significance level" was included, and it was set at 10% (Ps) (Pineau et al. 2009), using the below calculation:

$$Ps = \ Po \ + \ 1.282 * (\sqrt{\ } (Po(1 - Po)/n)$$

Po = chance level and n = number of subjects x replicates

A higher dominance rate reflects increased agreement amongst panelists as to which attribute dominates at a particular time point. The TDS data was processed by calculating the dominance rates against standardized time according to Pineau et al. (2009) using Compusense. The total number of attributes selected were divided by the total number of subjects and repetitions, which gave the percentage of dominance. As the participants could only select one attribute at a time, the y-axis is presented as a percentage of the population (of participants). The lines were smoothed via Bezier-based smoothing, and this generated the TDS curves. The significant line was added to the TDS curves representing the probability that an attribute is chosen that is significantly higher than chance ($p > .05$).

Texture analysis

Seaweed and control cracker samples underwent texture analysis using the TA.XTX2 texture analyzer (Stable Microsystems Surrey, UK). All analysis was carried out at room temperature. The sample was analyzed using a Three Point Bend Rig (small), with the sample placed between the holder and probe before being compressed using a 5 kg load cell. Breakpoint (N) was recorded using the force-in-compression tests. The analyzer was set to a return-to-start cycle with calibrated probe height, and a pre-test and test speed of 1 mm/s, and a post-test speed of 10 mm/sec. Trigger force was 20 g, and the probe distance was set at 20 mm (Abdel-Samie et al. 2010).

In vitro digestion experiment

Porcine pancreatin was used to test the digestibility of the seaweed crackers. *In vitro* digestion was investigated as a function of seaweed content (5, 10, 15, and 20% w/w) in crackers. Aliquots of pancreatin and calcium chloride were added to bis-tris buffer (0.05 M, pH 6.8) in a 50 mL conical flask to final concentrations of 1.5 mg/mL and 40 mg/mL, respectively. The 25 ml mixture was incubated at 37 °C for 5 min in an agitating water bath shaker (50 rpm). Digestion was started by adding 0.30 g pre-ground cracker sample, using mortar and pestle. The mixture continued to be incubated in the water bath. Then, 100 μL of solution was withdrawn at different time intervals into Eppendorf tubes, with the digestion stopped by immersion in an ice bath. The extent of starch digestion was analyzed by measuring liberated glucose concentration.

Added to this mixture was 100 μL of 3,5-dinitrosalicylic (DNS) acid, before heating in boiling water for 10 min to allow color to develop. The reaction was arrested by cooling the mixture in an ice bath for 20 min before the absorbance of the sample was read at 540 nm using a UV-Vis spectrophotometer (Genesys Model 6, ThermoFisher Scientific, Waltham, MA, USA). Plain crackers without seaweed were used as control sample. The concentration of liberated glucose was determined from a calibration curve ($R^2 = 0.9917$) plotted using glucose solutions with concentrations ranging from 0.01 to 0.50 mg/ml.

Statistical analysis

All experiments were conducted in triplicate, and, where applicable, the results were analyzed with an analysis of variance (ANOVA) test at the 95% significant level ($P < .05$) using SPSS (version 22.0, SPSS Inc., Chicago, IL, USA).

Results

Temporal dominance of sensations

Figure 1 shows a typical representation of the control cracker without the addition of seaweed (Figure 1). Crunchy dominates all replicates of the control samples, followed by sweetness from 8 to 24 seconds. No further attributes were identified after 24 seconds in the control samples.

Figure 2 shows a representation of a set of typical TDS figures for the crackers with wakame at different concentrations (5–20%). The 5% wakame cracker had high dominance for the crunchy

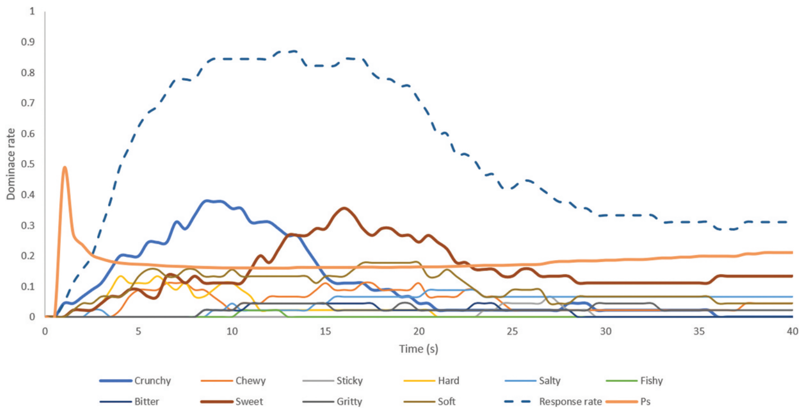

Figure 1. A representation of the control, which had no added seaweed.

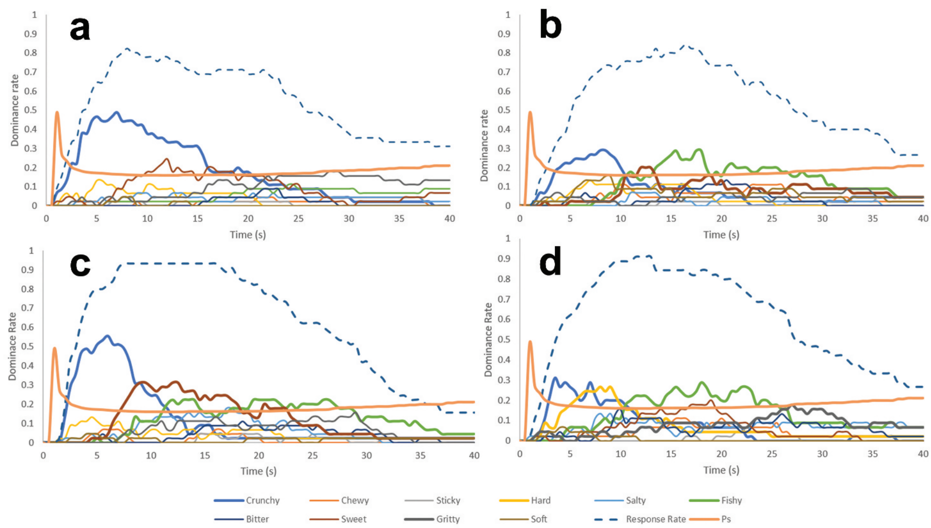

Figure 2. A representative figure for each seaweed cracker with differing percentage of Wakame, (a) 5%, (b) 10%, (c) 15% and (d) 20%.

attribute at the initial eating process, and then the attribute for sweetness took over roughly halfway through consumption. The crunchy attribute was noted as the dominant attribute at the initial stage of oral processing for all percentages of wakame added to the crackers. The attribute crunchy is generally a positive attribute for a cracker product. For wakame crackers with percentages of 15% or above, this attribute is rated lower than others. At 20% added wakame seaweed, the attribute hard also dominates with crunchy during the initial stages of oral processing, whilst the negative attribute of fishy becomes dominant at 10 seconds oral processing time and later. This is in contrast to crackers with wakame seaweed additions of 5%, where fishy does not reach significance at all.

TDS curves for additions of sea spaghetti to the cracker base recipe show similar patterns to the wakame seaweed crackers (Figure 3), although fishy is less dominant at higher concentrations of seaweed in the crackers (i.e., 15% and above). Interestingly, the attribute salty is shown in sea spaghetti crackers at 10, 15, and 20%, which is not shown in any of the other crackers.

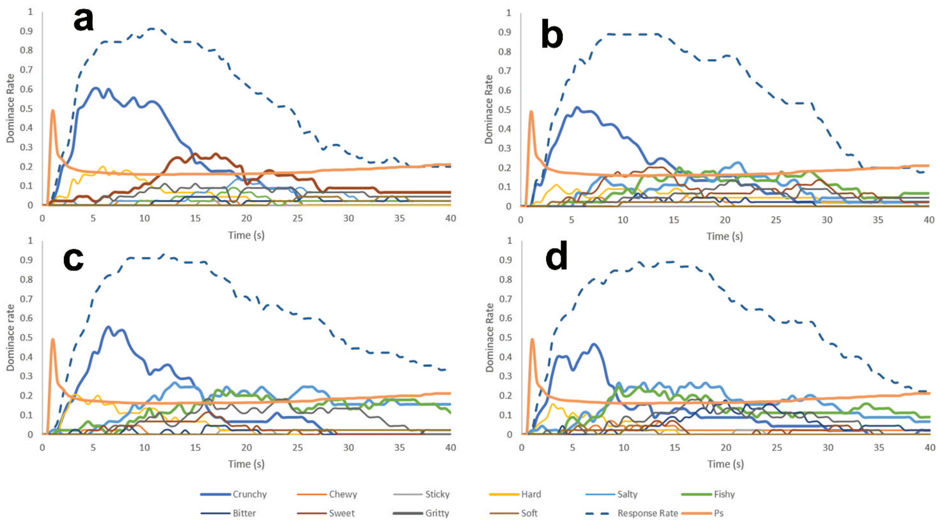

Figure 3. A representative figure for each seaweed cracker with differing percentage of Sea Spaghetti (a) 5%, (b) 10%, (c) 15% and (d) 20%.

Texture of seaweed crackers

When seaweed was added to the cracker mix at 15% or 20%, it showed textural force needed to break the cracker similar to having no seaweed within the product (Figure 4). However, the smaller concentration of seaweed within the cracker (5% and 10%) showed a harder texture and more force required to break the cracker.

A univariate analysis was conducted on the texture of the cracker products, where significant differences were observed between concentrations of seaweeds added to crackers (F[4.614]; p = .05). However, variety and concentration against variety were not significantly different (F[0.064]; p = .801; F[1.228]; p = .306, respectively). The samples were subjected to a post hoc analysis, where significant differences were observed between 5% and 20% (p = .040) and 10% and 20% (p = .042). No other significant differences were discovered.

Digestion of seaweed crackers

Figure 5(a) and 5(b) show the change in cumulative concentration of glucose liberated through starch digestion of crackers containing 0–20% wakame and sea spaghetti seaweeds, respectively. For all systems, there was no lag phase observed in the digestion kinetics; the release of glucose started immediately after the digestion was initiated. The digestion progressed in earnest, as indicated by the rapid increase in glucose concentration until the systems reached a plateau at about 2–3 minutes for the sea spaghetti systems and at 3–4 minutes for the wakame systems. The glucose concentration in the systems then remained at a similar level towards the end of the experiment, suggesting that the digestion had completed. It was noted that the initial rate of glucose release decreased with the increase of wakame seaweed in the crackers (Figure 5(a)), indicating that the presence of the seaweed slowed down starch digestion. However, it appeared that this was not the case for crackers with sea spaghetti (Figure 5(b)). There was no clear correlation between the initial rates of starch digestion with the seaweed concentration.

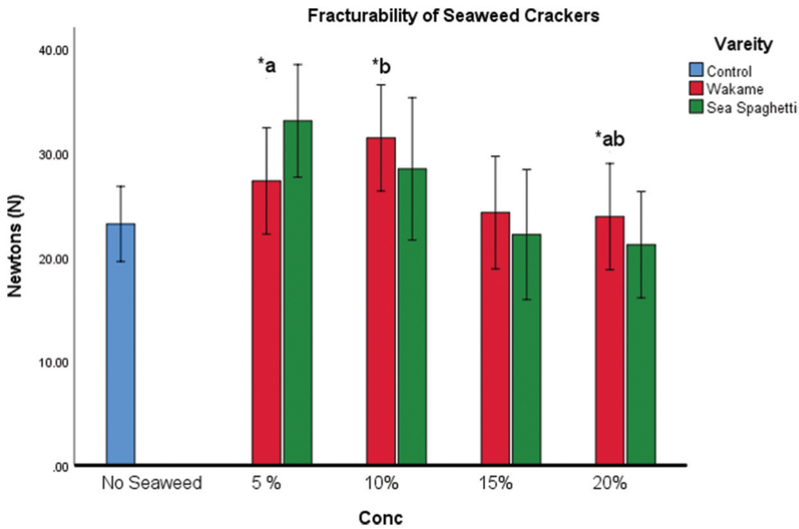

Figure 4. Fracturability of the seaweed crackers against concentration of seaweed and variety of seaweed.

Discussion

TDS discussion

Interestingly, the data collected regarding the seaweed concentration and its effect on the fracturability and TDS show some correlation. The higher the concentration of seaweeds into the cracker produced a less crunchy cracker, where fracturability showed a reduction in force required to break the cracker and whereas in the TDS data, the attribute crunchiness still appeared to dominate the first 10 seconds of oral processing in all crackers. This may be due to seaweed in larger quantities absorbing greater water and could 'dry out' the cracker making it crumbly or increase the bulk, therefore impeding the fracturability. Indeed, the ratio of gluten was reduced because of the addition of seaweed into the cracker, which could increase the crumbliness of the cracker and therefore reduce the texture of the product.

TDS is a useful measurement for better understanding the role of attributes in consumer preferences, although in this study some differences between texture analysis and TDS were found. Both methods observed a higher hardness/crunchiness at the beginning of oral processing for those crackers with lower concentration of added seaweed. It was interesting that 20% wakame required similar force to break the cracker as the 15% wakame cracker. However, when exploring the TDS data, the wakame cracker at 15% showed no hardness attribute, unlike 20% wakame crackers. Sea spaghetti crackers correlate well between the TDS and texture analysis. The crackers with 15 and 20% sea spaghetti had a crunchy attribute at the beginning of the oral process (less elevated in 15% sea spaghetti crackers) but also had a salty attribute appearing immediately after crunchiness. The texture profiles show that sea spaghetti had a higher fracturability at 5% than wakame and were below wakames with 10% or above.

When *Gracilaria gracilis* seaweed species was added to a rice cracker the gelatin content of the seaweed had an effect on crispness at 10% (Sumana et al. 2018). Other studies have shown similar effects of the addition of seaweeds, including *Ecklonia cava* (Yu and Han 2018), *Eucheuma spinosum* (Ardani and Buwono 2018), and *Garcililaria gracilis* (Sumana et al. 2018), where each study reported that increasing the concentration of seaweed decreased the lightness and crispness. The current study concurs with previous authors and shows that the addition of seaweed into the product decreased lightness and crispiness. Temporal dominance of sensations showed that fortification of seaweed at 20% interfered with the texture of the product for both seaweeds, changing a crunchy cracker into

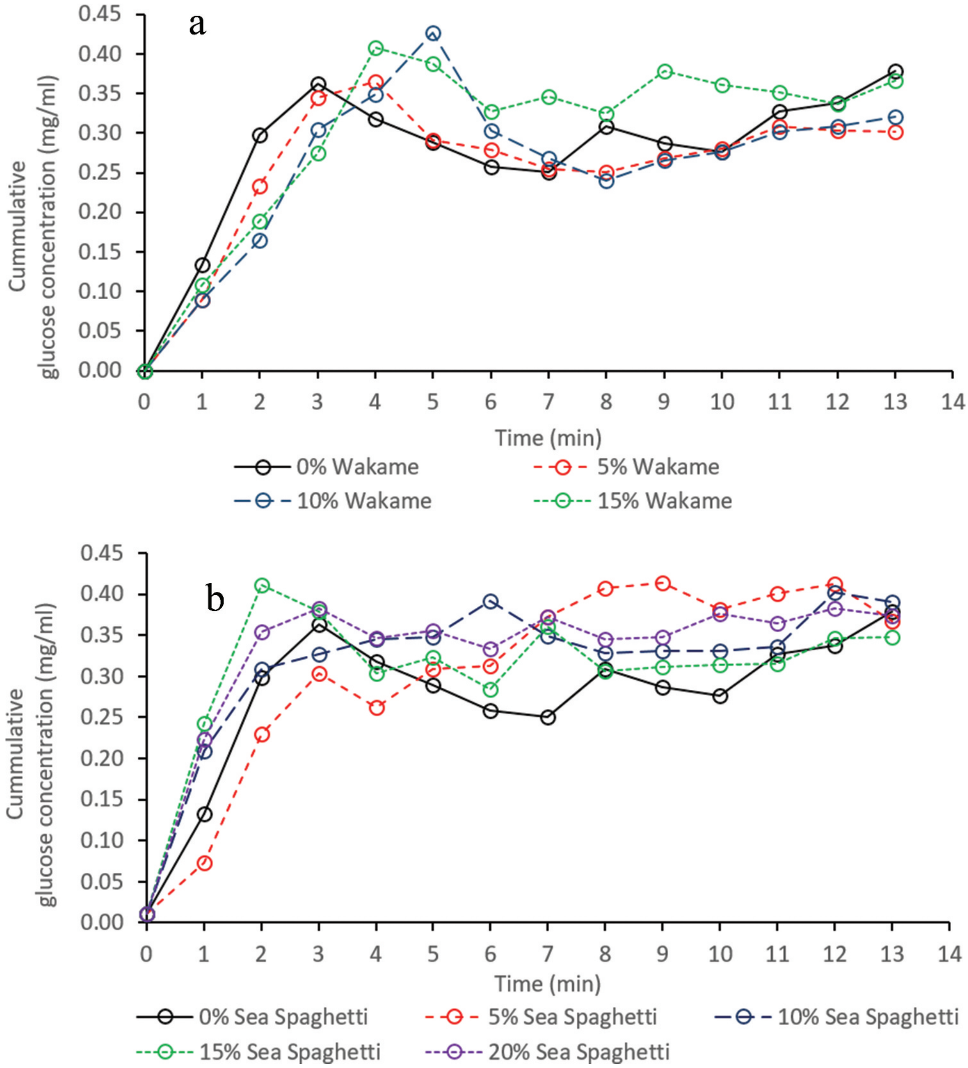

Figure 5. *In vitro* starch digestion of wheat crackers containing (a) Wakame and (b) Sea Spaghetti seaweeds at different concentrations. The kinetics of the digestion was followed by measuring the glucose released by porcine pancreatin during the course of digestion.

a hard texture. Excessive concentrations of seaweed were detrimental to the product, where increased concentration increased fishiness and salty attributes and reduced the sweetness attribute.

During the first stage of the oral processing of the control samples, crunchy was the initial dominant attribute followed by sweetness. Oral processing of foods is the first step of food consumption and affects the consumer acceptability of the food product prior to digestion and absorption (Wang and Chen 2017). Sweetness and crunchiness were the main attributes for the cracker with 5% wakame. In crackers with wakame seaweed concentration over and including 10%, there was an intensity of fishiness, and at higher concentration of seaweed, saltiness. Results for wakame seaweed in this study are similar to previous studies (Takeungwongtrakul and Benjakul 2017), where the authors describe the addition of 6% microencapsulated shrimp oil being added to a biscuit product was the highest concentration it was observed by the panelists. Other studies have shown that defatted mussel powder could be added to a biscuit product at 15% with the addition of a flavor masker, in this case mixed spices (Klunklin and Savage 2018).

Sea spaghetti crackers were predominately crunchy (5% and 10% sea spaghetti added crackers). The next attribute that followed, similar to the wakame 5% cracker, was sweetness. The 10% and 15% sea spaghetti crackers were perceived differently during the TDS experiment when compared with wakame 10% and 15% crackers. Sea spaghetti crackers had an overpowering crunchy attribute for 10%, and fishy was masked by the salty flavor. Previous studies have shown that bitter, acidic and salt flavors can significantly reduce the fish flavor of salmon in sauces (Paulsen et al. 2012). In a recent review, ambiguity was described in terms of dominance of attributes, which suggests that the lack of training on the description of terms, alongside the limitations of the methodology to overlook all attributes other than those that are dominant is a limitation of this type of sensory and consumer research (Oliver et al. 2018). Within this study, the panel of consumers were those who have undergone some basic sensory training, some are even members of trained panels; therefore, this could have mitigated some of the issues of understanding attributes. In addition, the warmup procedure is also a good mechanism to partially train consumers prior to taking part in TDS.

The starch digestion experiment showed that the presence of wakame seaweed, but not sea spaghetti seaweed, in the crackers reduced the rate of starch digestion. The reason for this observation is not immediately apparent. However, it is known that some dietary fibers such as gums and pectins can significantly change the viscosity of the intestinal content, which in turns influence the kinetics of food digestion (Yi et al. 2015). Alginate, fucans, and laminarans are the main soluble polysaccharides of both wakame and sea spaghetti seaweeds (Jiménez-Escrig and Sánchez-Muniz 2000; Lahaye 1991), and it was possible that wakame seaweed in the crackers became hydrated and its soluble polysaccharides with high water holding capacity thickened the digesta. This limited the flow and mixing of the digesta and the accessibility of the enzymes to starch, resulting in a slower release of glucose (Figure 3(a)). It was interesting that such enzyme inhibiting effect was not seen in the sea spaghetti systems; the addition of the seaweed did not appear to slow down the digestion. In fact, if anything, the sea spaghetti crackers had a tendency to speed up the starch digestion as indicated by an increase in the initial rate of the digestion (Figure 3(b)).

Understanding the role of seaweeds as an ingredient to fortify everyday products would be of great benefit to the food industry. Seaweed is a natural product that is under consumed and potentially has a great role to play in human health. The results from this study show that fortification into a cracker can be achieved at low levels of addition but could be a way of introducing seaweed to the consumer. The increase in consumption of seaweed would produce a valid industry that can grow.

Conclusion

At lower concentration of seaweeds, both species expressed similar attribute dominance, but as the concentration of seaweed increased, specific flavors associated with the species overpowered the cracker and produced less desirable consumer products. The starch digestibility of the seaweed crackers appeared to be affected by the type of seaweed. Incorporation of wakame seaweed into the crackers slowed down starch digestion, but the opposite was true for the crackers prepared with sea spaghetti seaweed. Seaweeds offer a different source of dietary fiber, and the application of seaweed in food products can help promote its consumption as well as increase its commercial value.

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Disclosure statement

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ORCID

Jonathan D. Wilkin http://orcid.org/0000-0001-8272-6865

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