International Food Research Journal 26(6): 1677-1687 (December 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Application of seaweed (*Kappaphycus alvarezii*) in Malaysian food products

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Article history

Received: 15 August, 2018
Received in revised form: 24 May, 2019
Accepted: 25 September, 2019

Keywords

Seaweed, Kappaphycus Alvarezii, Fish sausages, Noodle, Physicochemical properties, Consumer acceptance

Abstract

Kappaphycus alvarezii is a species of red algae, and one of the most important carrageenan sources for food, cosmetic, and pharmaceutical industries. It is commercially cultivated in the eastern part of Malaysia. Although *K. alvarezii* is rich in nutrients, it is limited in its integration into Malaysian food products. Therefore, the present work was conducted to investigate the quality characteristics, sensorial attributes, and antioxidant activity of *K. alvarezii* in Malaysian food products. Seaweed puree (SP) from *K. alvarezii* at 10%, 20% and 30% concentrations were prepared in the formulations of fish sausages, flat rice noodles and yellow alkaline noodles. Proximate analysis, physicochemical analysis, microbial count, total phenolic content (TPC), sensory evaluation, and consumer acceptance survey of the formulated food were conducted. The incorporation of *K. alvarezii* significantly increased the fibre, moisture, and ash content in formulated foods. In addition, the TPC content of *K. alvarezii* food also significantly increased up to 42 mg GAE/100 g. The presence of SP in food at higher concentration decreased the microbial counts. Sensory analysis confirmed that only fish sausages added with SP was overall acceptable as compared to control. Based on customer survey, functional foods that are "research proven" were the most preferred. In conclusion, *K. alvarezii* has the potential to be incorporated in Malaysian food products and developed as functional food.

Introduction

Nowadays, foods are consumed not just to satisfy hunger but also to fulfil nutritional requirements and reduce disease risks. To meet consumers demand, various food products have been introduced to offer health benefits beyond basic nutrition. Such foods are termed "functional foods". In 2012, the global market value for functional foods was US$162 billion (Euromonitor International, 2010). This prompted food manufacturers and entrepreneurs to be innovative in developing new functional food products. Foods with natural active ingredients are especially highly preferred by consumers due to safety concern. Still, there are numerous edible plants which are not well studied for commercial food production.

Kappaphycus alvarezii is red seaweed that can grow up to two meters long, and is one of the biggest tropical red macroalgae. *K. alvarezii* has the highest growth rate among other *Kappaphycus* seaweeds which is favourable for food and pharmaceutical applications (Patterson Edward and Bhatt, 2012; Kumar *et al*., 2015). Since 2008, Malaysia has produced more than 118,298 tons of seaweed with increased market value of 97.3% from 2007 to 2008 (Kaur and Ang, 2009). *K. alvarezii* is rich in fibre (29.4%) and carbohydrates (27.4%) (Fayaz *et al*., 2005). Intake of dietary fibre helps to improve blood glucose level, serum lipid concentrations, and immune function as well as lowering blood pressure and assisting in weight loss (Anderson *et al*., 2009).

K. alvarezii is a kappa carrageenan source, a phyllocoloid that is generally used as stabilising and thickening agent in food, cosmetic, and pharmaceutical industries. It is rich in phytochemical constituents such as phenolic compounds (Cox *et al*., 2012) and micro and macronutrients (Lorenzo *et al*., 2017). This compound offers a wide range of physiological functions such as antioxidant, anti-allergenic, anti-inflammatory, anti-atherogenic, anti-thrombic,

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antimicrobial, cardioprotective, and vasodilatory effects (Manach *et al*., 2004). The incorporation of seaweed in food matrix has been found to improve the physicochemical and nutritional properties, and technological aspects of foods (Roohinejad *et al*., 2017; Agregán *et al*., 2018; Parniakov *et al*., 2018; Žugčić *et al*., 2018).

Currently, seaweed is used as food ingredient in making ice-creams, yogurts, pastas, cheeses, breads, snacks, frankfurters, and beef patties (López-López *et al*., 2009; Prabhasankar *et al*., 2009; Shon *et al*., 2009; Haghighimanesh and Farahnaky, 2011; Cox and Abu-Ghannam, 2013). To promote seaweed incorporation in food products, *K. alvarezii* is added to local and frequently consumed products such as noodles and fish sausages which are very common in Southeast Asia, especially Malaysia. Every Asian nation produces at least one type of rice-based noodles and considers it as its own attraction because noodles and rice are the staple foods in Asia (Ismail *et al*., 2016). In Malaysia, flat rice noodles and yellow alkaline noodles are known by the locals as "*koay teow*" and "*mi kuning*", respectively. Wheat flour is the basic ingredient in noodle making but it is a poor source of fibre (Gunathilake and Abeyrathne, 2008). Fish sausages ("*keropok*" in Malay) are Malaysia's popular traditional fried fish snack made of sago starch, fish meat, salt, and water. It originated from the east coast of peninsular Malaysia. It is manufactured in small scale, but it has great market potential because more than 100 small-scale producers are identified in Malaysian east states alone.

Thus, it is of great interest to incorporate *K. alvarezii* in food products such as fish sausages, flat rice noodles, and yellow alkaline noodles. The aim of the present work was to investigate the effect of *K. alvarezii* puree on the physicochemical properties, nutritional value, textural, and sensorial properties of fish sausages, flat rice noodles, and yellow alkaline noodles.

Materials and method

Samples

Dried *K. alvarezii* seaweed was purchased from a traditional market in Kota Kinabalu, Sabah in January 2017. Sardine, sago starch and salt were purchased from local hypermarket.

Seaweed puree preparation

Seaweed puree was prepared following the method described by Dewi (2011). Dried seaweed (30 g) was soaked in water overnight, and then washed and drained. The dried seaweed was finely chopped and blended in water (3:1). The resulting mixture was added with more water (1:4) and boiled.

Fish sausage preparation

Sardines were clean thoroughly by removing the gills, intestines, and bones. The fish meat was minced with water, and the mixture was poured into a bowl. Then, sago starch and seaweed puree were added. The mixture was stirred until becoming a soft batter. The dough was rolled into a cylindrical shape, boiled until it floated, and then drained and cooled before packed into a sealed bag.

Flat rice noodle preparation

Wheat flour and rice flour were mixed in a bowl. Seaweed puree was later added and mixed until becoming smooth. Egg white, salt, and water were mixed in another bowl. Then, it was poured into the flour and seaweed mixture. The mixture was kneaded into dough. To obtain a noodle sheet, the dough was machine-rolled using cutting blade. The noodles were boiled for 2 min and drained. Next, 20 g of cooking oil was added into the noodles to prevent sticking.

Yellow alkaline noodle preparation

Yellow alkaline noodles were prepared following the method described by Norlaili *et al*. (2014) with few modifications. The flour was mixed thoroughly before addition of alkaline solutions (stepwise: sodium chloride, potassium carbonate, and potassium carbonate with water). Then, seaweed puree was added into the mixture. It was further mixed before it was kneaded into dough. The dough was taken out and rolled out using a roller. The dough was passed through a pair of rollers of pasta machine for five successive times and was allowed to rest for 15 min at room temperature (27 ± 2°C) before further sheeting. The dough sheet was placed on cutting roll after two subsequent sheeting. Then, the noodle strands were boiled, drained, cooled, and coated with vegetable oil before they were packed and stored at 4°C for further analysis.

Proximate analysis

Proximate analyses were performed based on American Association for Clinical Chemistry's method (AACC, 2000) for moisture (method 44-15), ash (method 08-01), fat (method 30-10), protein (method 46-10), and fibre (method 32-10). Total carbohydrate was calculated using Eq. 1:

$$\text{Total carbohydrate: } [100 - (\%\text{ moisture} + \%\text{ ash} + \%\text{ protein} + \%\text{ fat})] \quad \text{(Eq. 1)}$$

Cooking properties

Cooking yield and cooking loss were determined according to Zawawi *et al*. (2014). For cooking yield, 10 g of food sample was cooked in distilled water using a beaker covered in aluminium foil. Later, it was drained, cooled, and weighed. The cooking yield was measured using Eq. 2:

$$\text{Cooking yield (\%)} = (\text{Weight of food after cooking} / \text{Weight of food before cooking}) \times 100 \quad \text{(Eq. 2)}$$

For cooking loss, the cooking water from cooking yield was poured into a volumetric flask. Water was added to calibration mark before it was shaken. The solution (10 mL) was dried on a crucible in an oven (at 105°C) until dry constant was obtained. The cooking loss was measured using Eq. 3:

$$\text{Cooking loss (\%)} = [(A - B) / (\text{Noodle sample weight} - C)] \times 25 \quad \text{(Eq. 3)}$$

where, A = weight of crucible + dry cooked water sample, B = weight of crucible, and C = noodles moisture content.

Texture profile analysis

The texture of food was measured using a texture analyser (TA.XT2i, MA). Samples were penetrated using P/36R probe. Fish sausage was analysed according to Santana *et al*. (2015). Meanwhile, alkaline noodles and flat rice noodles were analysed according to Norlaili *et al*. (2014) and Zawawi *et al*. (2014), respectively. Food samples were placed horizontally on the platform and then compressed by a compression platen (P.75) at a constant 1 mm/s rate. The trigger force used was 10 g for 2 s with 3 mm/s of pre-test speed and post-test speed, and the return distance was 35 mm. The hardness, springiness, cohesiveness, and chewiness were then determined.

Colour analysis

Lightness (L^*), redness (a^*) and yellowness (b^*) were measured using Minolta CR-41Chroma meter (Konia Minolta, Inc. Japan) according to Santana *et al*. (2015). The data collected were then processed using Easy-Match QC Data System software.

Total phenolic content determination

Fish sausage, flat rice noodle, and yellow alkaline noodle extracts were prepared according to Norlaili *et al*. (2014) with modification. Food samples were mixed with methanol at a ratio of 1:10 before being homogenised. Whatman No. 1 filter paper was used to filter the mixture, and the supernatant was collected. The supernatant was freeze-dried for 2 d and stored in freezer before analysis.

TPC was measured according to Choo and Aziz (2010). Freeze-dried extract (5 mg) was dissolved in 5 mL of DMSO. The solution (0.5 mL) was pipetted into 1 mL of 50% Folin-Ciocalteu reagent, incubated for 3 min and added with 3 mL of 1% Na_2CO_3. The solution was vortexed briefly and incubated for 30 min. The absorbance was measured at 760 nm and the results were expressed as milligrams of Gallic acid equivalents per 100 g of sample (mg GAE/ 100 g).

Shelf life study

The shelf life study was performed on all food formulations according to Li *et al*. (2012). Food samples (25 g) were put into 225 mL of 0.1% peptone water (Oxoid, UK), and the mixture was shaken in a stomacher bag using a stomacher machine for 2 min. Serial dilutions (10^{-1} to 10^{-4}) were prepared using peptone water and 0.1 mL of diluent was spread onto plate count agar (Oxoid, UK) before incubated aerobically for 48 h at 37°C. The viable colonies were enumerated and expressed as log10 CFU/mL.

Sensorial evaluation

Four different formulations of fish sausages, flat rice noodles and alkaline noodles were prepared for sensorial evaluation. Fish sausages were sliced 1.5 cm while 10 g of noodles were served in chicken soup as carrier.

The sensory attributes of the cooked noodles were analysed by 50 panellists for each food type (fish sausages, flat rice noodles, and alkaline noodles) to a total of 150 panellists. The panellists consisted of students and staff of Faculty of Food Science and Technology, UPM. A 9-point Hedonic scale with 1 = disliked extremely to 9 = liked extremely was used. Noodles were analysed for aroma, colour, elasticity, firmness, surface, taste, texture, and overall acceptability. Meanwhile, fish sausages were analysed for appearance, colour, aroma, texture, flavour and overall acceptability. Then, a questionnaire form comprising six questions was distributed to determine the consumers' acceptance of the food products given. Panellists were asked if they were aware of functional foods, what were the factors affecting buying and buying of new products, and if they were interested to buy the formulated products.

Statistical analysis

The data were obtained in triplicate (n = 3) and results were expressed as means ± standard deviations (where applicable). Data were analysed using Minitab version 16 using ANOVA. If ANOVA test indicated significant result ($p < 0.05$), then the significant means were separated using Tukey's test.

Results and discussion

Proximate analysis

Table 1 shows the proximate composition of fish sausages, flat rice noodles, and yellow alkaline noodles prepared using different concentrations of seaweed puree (SP). The fibre and ash contents significantly increased ($p < 0.05$) with increasing SP. Fish sausages with 30% SP recorded the highest value of 1.29% and 2.36% of fibre content and ash content, respectively. Our findings are similar to those of Dewi (2011) where noodles mixed with seaweed *E. cottoniii* and *G. verucossa* significantly increased the fibre and ash content to 2.00% and 1.46%, respectively. As mentioned previously, K alvarezii is rich in fibre but it also contains high mineral from 10,997.62 to 29,939.61 mg/100 g dry weight which corresponds to the ash value obtained by Kumar *et al*. (2015).

Moreover, the moisture contents also increased (61.74 to 73.49%) with increasing SP concentration. Fibre and polysaccharide in *K. alvarezii* seaweed have great water holding capacity during dough/food formation. Therefore, it increases the water content in food (Dewi, 2011). However, in processing food like noodles, low moisture content is more desirable as the gluten level will be lowered and sheeting capacity can be enhanced (Zawawi *et al*., 2014).

Carbohydrate content was the lowest in 30% SP fish sausages (14.08%). The value significantly decreased as the SP concentrations increased across all food samples. Protein content also significantly decreased in flat rice noodles and yellow alkaline noodles while there was no significant difference in fish sausages. This can be due to the small carbohydrate and protein content contributed by seaweed as compared to sago starch and wheat flour. This agrees with a study by Kumar *et al*. (2015) where *K. alvarezii* (from Sabah) only had 25.87 g carbohydrate/100 g and 12.69 to 23.61 g protein/100 g which were lower than those in sago and wheat flours.

Cooking properties

The cooking properties of food samples are tabulated in Table 2. For fish sausages, the highest cooking yield and the lowest cooking loss were recorded with 30% SP. Cox and Abu-Ghannam (2013) also found that addition of brown seaweed (*Himanthalia elongata*) in beef patties improved the cooking yield and reduced the cooking loss. According to Huda *et al*. (2012), increase in protein content from meat could affect the water binding capacity in food. Besides protein from meat, this property could also be attributed to dietary fibre in seaweed. However, the results were different for noodles samples.

Table 1. Proximate analysis of fish sausages, flat rice noodles and yellow alkaline noodles incorporated with different percentages of seaweed puree.

SP (%)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fibre (%)	Carbohydrate (%)
Fish sausages						
0	59.68 ± 0.03^{a}	1.51 ± 0.08^{a}	11.64 ± 0.43^{a}	1.43 ± 0.49^{a}	0.23 ± 0.05^{a}	25.51 ± 0.26^{a}
10	62.42 ± 0.13^{b}	2.05 ± 0.04^{b}	12.28 ± 0.43^{a}	1.17 ± 0.16^{a}	0.41 ± 0.08^{b}	21.67 ± 0.58^{b}
20	64.53 ± 0.10^{c}	2.15 ± 0.05^{b}	12.47 ± 0.29^{a}	1.10 ± 0.02^{a}	0.60 ± 0.07^{c}	19.47 ± 0.80^{c}
30	68.80 ± 0.07^{d}	2.36 ± 0.03^{c}	12.51 ± 0.30^{a}	0.96 ± 0.18^{a}	1.29 ± 0.06^{d}	14.08 ± 0.10^{d}
Flat rice noodles						
0	63.90 ± 0.40^{a}	0.29 ± 0.10^{a}	3.28 ± 0.18^{a}	4.39 ± 0.48^{a}	0.18 ± 0.13^{a}	27.95 ± 0.88^{a}
10	72.36 ± 0.49^{b}	0.63 ± 0.09^{b}	3.20 ± 0.56^{a}	4.49 ± 0.33^{a}	0.39 ± 0.04^{a}	18.93 ± 0.17^{b}
20	72.90 ± 0.40^{bc}	0.66 ± 0.04^{b}	2.73 ± 0.44^{a}	4.47± 0.21^{a}	0.47 ± 0.21^{a}	18.77 ± 0.43^{b}
30	73.49 ± 0.35^{c}	0.67 ± 0.01^{b}	1.20 ± 0.03^{b}	4.67 ± 0.22^{a}	0.80 ± 0.03^{b}	19.17 ± 0.92^{b}
Yellow Alkaline noodles						
0	57.04 ± 0.24^{a}	0.38 ± 0.06^{a}	3.68 ± 0.11^{a}	2.25 ± 0.14^{a}	0.12 ± 0.02^{b}	36.76 ± 0.18^{a}
10	61.74 ± 0.13^{b}	0.38 ± 0.03^{a}	3.42 ± 0.03^{b}	1.82 ± 0.49^{ab}	0.37 ± 0.04^{ab}	32.01± 0.40^{b}
20	65.59 ± 0.53^{c}	0.46 ± 0.03^{a}	3.20 ± 0.04^{b}	1.31 ± 0.25^{ab}	0.27 ± 0.14^{ab}	29.03 ± 0.89^{c}
30	65.17 ± 0.36^{c}	0.63 ± 0.02^{b}	3.19 ± 0.02^{b}	2.02 ± 0.12^{b}	0.42 ± 0.14^{a}	28.74 ± 0.43^{c}

Data are means of three replicates (n = 3) ± standard deviation. Means in the same column with different letters are significantly different ($p < 0.05$). SP = seaweed puree.

Table 2. Cooking properties, colour parameter and texture analysis of fish sausages, flat rice noodles and yellow alkaline noodles incorporated with different percentages of seaweed puree.

SP (%)	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (Nm)	Adhesiveness (Ns)	Cooking Properties		Colour Parameter		
						Cooking yield (%)	Cooking loss (%)	*L**	*a**	*b**
Fish sausages										
0	5273.90 ± 388.90^{a}	0.81 ± 0.01^{ab}	0.57 ± 0.02^{a}	1723.60 ± 476.60^{a}	ND	100.68 ± 2.39^{a}	0.32 ± 0.13^{a}	62.84 ± 0.01^{a}	6.01 ± 0.04^{a}	8.40 ± 0.06^{a}
10	4844.60 ± 90.90^{a}	0.78 ± 0.02^{b}	0.54 ± 0.01^{a}	1921. 50 ± 540.80^{a}	ND	101.52 ± 1.21^{a}	0.28 ± 0.09^{a}	67.72 ± 0.24^{b}	4.86 ± 0.08^{b}	7.29 ± 0.02^{b}
20	4491.80 ± 410.40^{ab}	0.81 ± 0.01^{ab}	0.52 ± 0.12^{a}	2019.10 ± 40.70^{a}	ND	102.01 ± 0.46^{a}	0.25 ± 0.19^{a}	67.87 ± 0.03^{c}	5.53 ± 0.03^{c}	7.27 ± 0.00^{b}
30	3954.50 ± 277.80^{b}	0.83 ± 0.02^{a}	0.52 ± 0.11^{a}	2408.70 ± 202.30^{a}	ND	102.47 ± 0.20^{a}	0.18 ± 0.09^{a}	70.86 ± 0.03^{d}	7.76 ± 0.06^{d}	13.14 ± 0.20^{c}
Flat rice noodles										
0	3654.40 ± 373.50^{a}	1.05 ± 0.44^{a}	0.91 ± 0.01^{a}	2722.00 ± 757.30^{a}	-25.02 ± 3.86^{a}	429.42 ± 39.36^{a}	0.05 ± 0.03^{a}	79.88 ± 0.16^{a}	0.07 ± 0.01^{a}	15.26 ± 0.19^{a}
10	2893.80 ± 376.80^{ab}	0.84 ± 0.13^{a}	0.81 ± 0.01^{b}	1995.60 ± 551.50^{a}	-24.42 ± 3.80^{a}	326.30 ± 24.98^{b}	0.11 ± 0.01^{b}	79.23 ± 0.16^{ab}	0.17 ± 0.03^{b}	15.85 ± 0.59^{a}
20	2675.90 ± 457.30^{ab}	0.82 ± 0.02^{a}	0.82 ± 0.02^{b}	2081.10 ± 447.10^{a}	-21.64 ± 2.74^{a}	326.44 ± 27.09^{b}	0.09 ± 0.01^{ab}	78.33 ± 0.67^{ab}	0.36 ± 0.06^{c}	14.05 ± 0.17^{b}
30	1899.60 ± 1104.90^{b}	1.01 ± 0.32^{a}	0.80 ± 0.04^{b}	1643.20 ± 1406.90^{a}	-17.77 ± 8.97^{a}	314.54 ± 27.09^{b}	0.12 ± 0.01^{b}	78.00 ± 0.84^{b}	0.62 ± 0.04^{d}	13.80 ± 0.25^{b}
Yellow alkaline noodles										
0	3094.90 ± 215.50^{a}	0.83 ± 0.09^{ab}	0.75 ± 0.01^{a}	2151.20 ± 48.00^{a}	-10.66 ± 1.35^{a}	239.12 ± 14.44^{a}	0.24 ± 0.01^{a}	72.35 ± 0.12^{a}	0.82 ± 0.06^{ab}	21.61 ± 0.15^{a}
10	1535.50 ± 383.10^{b}	0.90 ± 0.02^{a}	0.78 ± 0.04^{a}	1419.70 ± 48.40^{b}	-2.63 ± 0.63^{b}	126.88 ± 2.13^{b}	0.74 ± 0.41^{ab}	72.11 ± 0.46^{a}	0.84 ± 0.05^{ab}	22.91 ± 0.30^{b}
20	1297.20 ± 153.50^{b}	0.73 ± 0.03^{b}	0.77 ± 0.04^{a}	689.50 ± 146.90^{c}	-0.50 ± 0.36^{c}	112.24 ± 4.82^{b}	1.36 ± 0.62^{b}	72.41 ± 0.47^{a}	0.89 ± 0.06^{a}	22.49 ± 0.45^{bc}
30	1468.70 ± 359.70^{b}	0.95 ± 0.05^{a}	0.80 ± 0.04^{a}	86.70 ± 154.50^{c}	-0.12 ± 0.01^{c}	112.83 ± 7.97^{b}	1.03 ± 0.01^{ab}	71.66 ± 0.38^{a}	0.77 ± 0.06^{b}	21.87 ± 0.31^{ac}

Data are means of three replicates (n = 3) ± standard deviation. Means in the same column with different letters are significantly different ($p < 0.05$). SP = seaweed puree, ND = not determined.

The cooking yield decreased, and the cooking loss increased with increasing percentages of SP in noodles. This contradicts the study by Chang and Wu (2008) where Chinese noodles with 6% seaweed had higher cooking yield (122.60%) than control. Unlike fish sausages, wheat flour contributes higher protein content than seaweed. Thus, the substitution of wheat flour with seaweed does not give enough gluten to create protein network to bind water molecules in the flat rice noodles. Furthermore, the water binding capacity of seaweed depends on the type and amount of polysaccharides involved in gelation (Sánchez-Alonso *et al*., 2006).

Colour analysis

Colour is one of the parameters that attract consumers and one the first criteria to be checked before taste and texture. The colour analysis is shown in Table 2. L^* value significantly increased ($p < 0.05$) (became lighter) with increasing concentration of SP in fish sausages, but significantly decreased in flat rice noodles. Higher moisture content in meat products leads to the highest lightness (Aleson-Carbonel *et al*., 2005; Cox and Abu-Ghannam, 2013). Noodle brightness is inversely related to protein content and to flour-grade colour as reported by Moss *et al*. (1986). Degradation of noodle colour might be explained on the basis of polyphenol oxidase activities as suggested by other researchers (Davies *et al*., 2003: Jukanti *et al*., 2003; Ma *et al*., 2013).

Textural analysis

The texture profile analysis is listed in Table 2 with different parameters for fish sausages and noodles. The presence of *K. alvarezii* significantly reduced ($p < 0.05$) the hardness of food samples. The lowest hardness (N) was recorded in 30% of SP in fish sausages, flat rice noodle, and yellow alkaline noodles with value of 3954.90 N, 1899.6 N and 1468.70 N, respectively. Chang and Wu (2008) also found lowest tensile strength in 6% M. nitidum seaweed noodles. They further suggested that noodles' textural properties are influenced by matrix structure of ingredients (starch, glutens, and proteins) which can either weaken or strengthen the hydrogen bonds within noodle structure. Furthermore, 80% gluten (composed of gliadins and glutenins) in wheat flour promotes dough strength and extensibility. Substitution of wheat flour with seaweed had probably reduced these properties. The chewiness and adhesiveness of yellow alkaline noodles with SP were significantly higher than control. Addition of 30% SP increased the chewiness and adhesiveness of yellow alkaline noodles to 886 Nm and -0.12 Ns respectively.

It was found that SP substitution rendered different effects on the food textural parameters based on food type formulated in the present work. Different techniques and processing methods are applied in food making, thus, fibre effect on the texture may vary, as suggested by Cofrades (2011).

Total phenolic content

Acting as a natural antioxidant, phenol can preserve food by retarding deterioration, rancidity or discoloration due to oxidation (Shahidi and Wanasundara, 1995) by donating hydrogen and chelating metal ions (Pereira *et al*., 2009). Folin-Ciocalteu reagents could determine the TPC and is chosen for its convenience, simplicity and efficiency (Singleton *et al*., 1999).

From Figure 1, the highest TPC was measured in

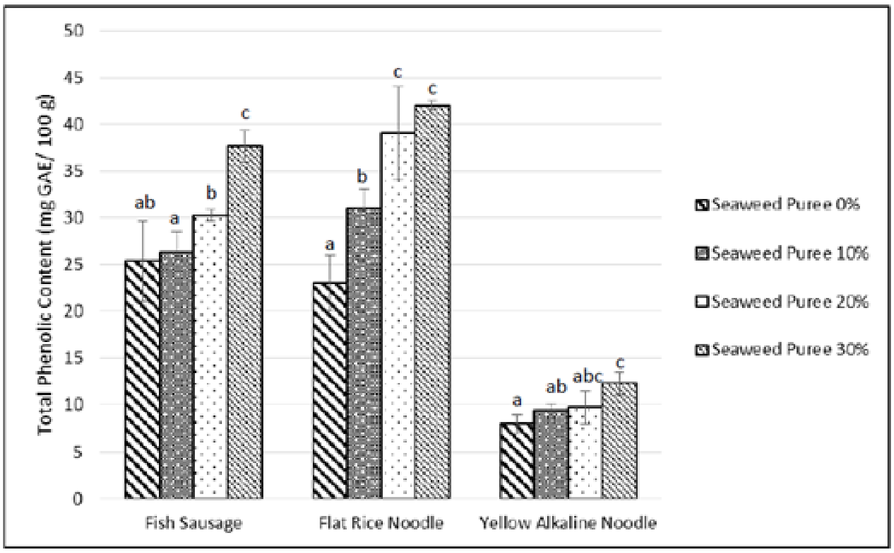

Figure 1. Total phenolic content (mg GAE/ 100 g) of fish sausages, flat rice noodles and yellow alkaline noodles incorporated with different percentages of seaweed puree. Different letters represent significant difference ($p < 0.05$).

30% SP in flat rice noodles, yellow alkaline noodles, and fish sausages. TPC significantly increased ($p < 0.05$) when SP substitution in food samples increased. Similarly, Prabhasankar *et al*. (2009) found edible Japanese seaweed, wakame (*Undaria pinnatifida*), increased TPC in pasta, while Cox and Abu-Ghannam (2013) blanched seaweed led to higher TPC in beef patties. Phenol is a secondary metabolite found in various plants and algae and is highly correlated with antioxidant activity (Yao *et al*., 2010). However, Chew *et al*. (2008) found that *K. alvarezii* yielded the lowest TPC content but highest antioxidant activity among other seaweed varieties studied. But the assay conducted was not specified to any antioxidant molecules in seaweed. Phenols present in seaweed include phenolic acid, flavonoids, and tannins, and they exhibit high antioxidant activity (Li *et al*., 2012).

Shelf life study

Figure 2 shows microbial count changes of food samples during 6 d storage. The total microbial count (log CFU/g) decreased when SP substitution in food samples increased except for flat rice noodles. The microbial count increased as the storage period increased. The plate count for entire food samples was in the range of log 0.57 - 6.00 CFU/g. The cut-off point between spoiled and unspoiled in fresh noodles and fish products can be considered at level below 10^6 CFU/g (≈log 6 CFU/g). Therefore, the microbial count in the present work was acceptable. Examples of antibacterial compounds found in seaweeds are fatty acids, lipophilic and phenolic compounds, lectins, acetogenins, terpenes, alkaloids, polyphenolics, isoprenoid metabolites, and hydrogen peroxide. Our findings however, differed from those of Cofrades *et al*. (2011) where the total viable count for reformulation of poultry steak with addition of H. elongata seaweed exceeded log 6 CFU/g. Meanwhile, a study by López-López (2010) found beef patties with Wakame seaweeds had total viable counts of log 6.0 - 6.4 CFU/g. The results were higher than that of the present work which could probably be due to the fact that both food samples were uncooked while the present work used cooked samples.

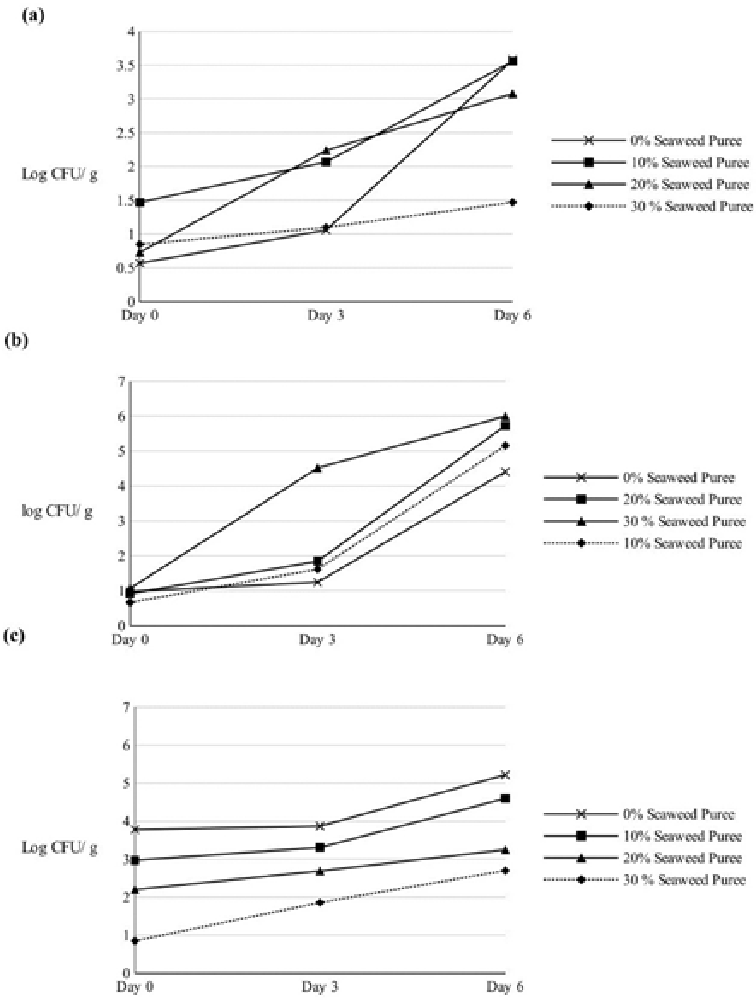

Figure 2. Microbial count for (a) fish sausages, (b) flat rice noodles and (c) yellow alkaline noodles incorporated with different percentages of seaweed puree.

Table 3. Sensory evaluation for fish sausages, flat rice noodles and yellow alkaline noodles incorporated with different percentages of seaweed puree.

SP (%)	Aroma	Colour	Elasticity	Firmness	Surface smoothness	Taste	Texture	Overall acceptance
Fish Sausages								
0	6.06 ±1.38^{a}	6.52 ± 1.40^{a}	ND	ND	ND	4.62 ± 1.64^{a}	4.62 ± 1.64^{a}	5.72 ± 1.54^{a}
10	6.08 ± 1.53^{a}	6.52 ± 1.22^{a}	ND	ND	ND	4.98 ± 1.84^{a}	4.98 ± 1.84^{ab}	5.72 ± 1.44^{a}
20	6.18 ± 1.47^{a}	6.52 ± 1.20^{a}	ND	ND	ND	5.80 ± 1.55^{a}	5.80 ± 1.55^{bc}	6.48 ± 1.20^{b}
30	6.12 ± 1.44^{a}	6.20 ± 1.55^{a}	ND	ND	ND	6.40 ± 1.54^{a}	6.40 ± 1.54^{c}	6.78 ± 1.61^{b}
Flat Rice Noodles								
0	6.54 ± 1.05^{a}	7.06 ± 1.27^{a}	6.04 ± 1.55^{a}	6.22 ± 1.48^{ab}	7.02 ± 1.35^{a}	6.60 ± 1.31^{a}	6.50 ± 1.42^{ab}	6.72 ± 1.14^{a}
10	6.54 ± 1.27^{a}	7.18 ± 1.30^{a}	6.16 ± 1.49^{a}	6.68 ± 1.17^{a}	6.84 ± 1.20^{ab}	6.70 ± 1.31^{a}	6.82 ± 1.21^{a}	6.88 ± 1.26^{a}
20	6.10 ± 1.43^{a}	6.92 ± 1.48^{a}	5.78 ± 1.57^{ab}	5.84 ± 1.75^{bc}	6.46 ±1.33^{ab}	6.18 ± 1.49^{a}	6.00 ± 1.71^{bc}	6.30 ± 1.42^{ab}
30	6.40 ± 1.43^{a}	6.68 ± 1.43^{a}	5.00 ± 1.84^{b}	5.40 ± 1.78^{c}	6.16 ± 1.41^{b}	6.12 ± 1.61^{a}	5.64 ± 1.87^{c}	5.92 ± 1.44^{b}
Yellow Alkaline Noodles								
0	6.92 ± 1.19^{a}	6.94 ±1.35^{a}	6.28 ± 1.57^{a}	6.56 ± 1.45^{a}	6.68 ± 1.39^{a}	6.16 ± 1.68^{a}	ND	6.70 ± 1.34^{a}
10	6.76 ± 1.10^{ab}	6.40± 1.36^{ab}	6.08 ± 1.54^{a}	6.32 ± 1.60^{a}	6.56 ± 1.30^{a}	6.48 ± 1.47^{ab}	ND	6.54 ± 1.31^{ab}
20	6.28 ± 1.29^{b}	5.92 ± 1.40^{b}	5.26 ± 1.70^{b}	5.74 ± 1.56^{b}	5.72 ± 1.57^{b}	5.58 ± 1.88^{b}	ND	5.64 ± 1.56^{c}
30	6.56 ± 1.21^{ab}	6.28 ± 1.38^{b}	5.80 ± 1.46^{ab}	6.06 ± 1.33^{ab}	6.18 ± 1.51^{ab}	5.84 ± 1.54^{ab}	ND	5.94 ± 1.35^{bc}

Data are means of three replicates ($n = 3$) ± standard deviation. Means in the same column with different letters are significantly different ($p < 0.05$). SP = seaweed puree, ND = not determined .

Sensorial evaluation

Table 3 summarises the sensory score for fish sausages, flat rice noodles, and yellow alkaline noodles. There was no significant difference ($p < 0.05$) in sensorial attributes among fish sausages formulation with seaweeds except for texture. The sensory score for texture and overall acceptability of fish sausages increased at higher SP substitution. Fish sausages (30% SP) yielded the highest score for overall acceptability at 6.78. However, substitution of SP in yellow alkaline noodles affected all the sensory scores while SP in flat rice noodles only affected the elasticity, firmness, surface smoothness, and texture scores. The overall acceptability significantly decreased for both noodles as SP substitution increased.

Customers' acceptance of *K. alvarezii* in the food products formulated in the present work varied. As reported by Kim *et al*. (2010), the most important factor influencing sensorial attributes is the food type in which seaweed is applied. This is proven by the present work as application of *K. alvarezii* in different food products (fish sausages and noodles) yielded diverse responses among the consumers. Thus, different formulation for each food product needs to be further investigated and developed to suit customer preference.

Consumer acceptance survey

Figure 3(a) shows the panellists' awareness on functional fish sausages, flat rice noodles, and yellow alkaline noodles available in the market. Most panellists were unaware of functional fish sausages and yellow alkaline noodles. But they are aware of functional yellow alkaline noodles. This suggested that most panellists were not aware and did not practice healthy lifestyle. One of the possible reasons is that they are students, thus, consuming food was based on budget rather than healthy choice. Figure 3(b) displays the factors that affected customers' preference in buying functional foods. According to the survey, the most chosen factor was taste while the lowest was aroma. This is as expected because food taste is the most important attribute in fulfilling satiety. However, in promoting functional food, health value / functionality should be superior. Verbeke (2006) studies on Belgium consumers' compromise on taste for nutritional value showed that females and the elderlies tend to compromise taste in 2001, but later, the difference faded in 2004.

Factors that influence customers to buy new products are displayed in Figure 3(c). The highest influence factor was "research proven" and the lowest is "commentary from internet/websites".

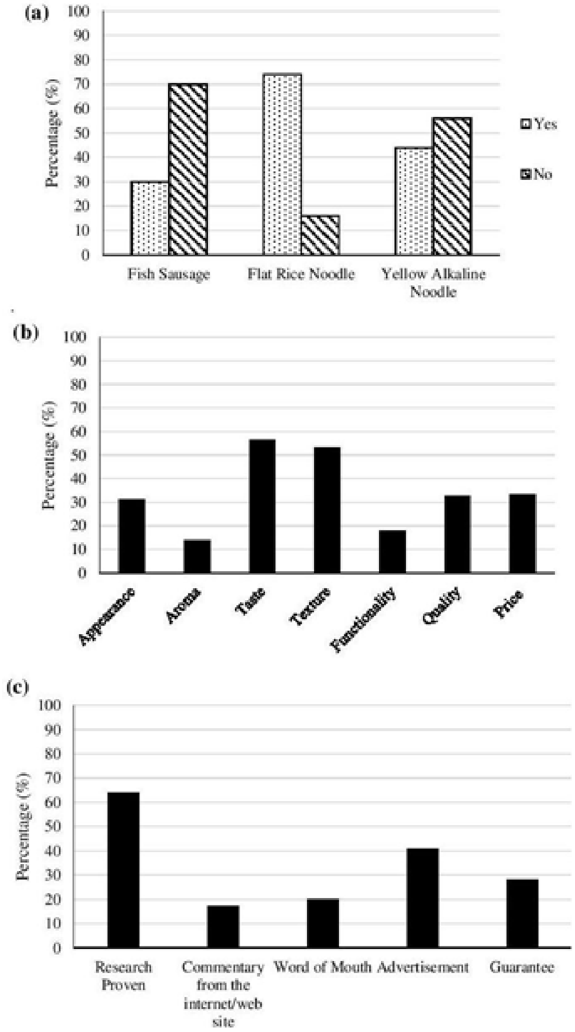

Figure 3. (a) customers' awareness on functional fish sausages, flat rice noodles and yellow alkaline noodle available in market, (b) factors that might affect the customers' preferences to buy the products, and (c) factors that might influence the customers when deciding to buy new product.

Therefore, when products have scientific evidence to support their health claims, the consumers will be more confident to buy it. Thus, research on functional foods is becoming more relevant and highly required. However, functional food development and marketing requires robust research efforts from laboratory work to clinical trials with collaboration between various parties, not just among researchers (Siró *et al*., 2008).

Based on the survey, 58.5% and 61.3% of panellists were "somewhat interested" to buy these products and "interested" if available in the market, respectively. Most customers (53%) were "very interested" in buying these food products if they provide health benefits. This proves that functional food has the potential to be further researched, invested, and marketed.

Conclusion

The incorporation of seaweed *K. alvarezii* in food systems could offer great potential for marketing in the food industry. SP substitution in fish sausages, flat rice noodles, and yellow alkaline noodles increased the fibre, ash, and moisture content as compared to control. Total phenolic content increased while microbial count decreased with higher SP substitution in food systems. *K. alvarezii* was overall accepted by consumers when incorporated in fish sausages but not in noodles. The sensory attributes were influenced by type of food in which the seaweed is applied. Further studies should focus on reformulating the ingredients and methods of preparation for incorporation of *K. alvarezii* in noodles or other food products in order to better appeal to consumers in terms of taste. Overall, seaweed *K. alvarezii* has the potential to be incorporated in food products to provide functional food high in fibre, antimicrobial properties and high total phenolic content.

Acknowledgement

The authors acknowledge the contribution from the laboratory staff and assistants of Faculty of Food Science and Technology, UPM for assisting in the laboratory works and in providing all the equipment needed for making the food products.

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