

Seaweed Tea: Fucoidan-Rich Functional Food Product Development from Malaysian Brown Seaweed, *Sargassum binderi*

(Teh Rumpai Laut: Pembangunan Produk Makanan Berfungsi Berasaskan Fukoidan
daripada Rumpai Laut Perang Malaysia, *Sargassum binderi*)

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ABSTRACT

Our previous studies on fucoidan from Malaysian brown seaweed, Sargassum binderi, found that it exhibited significant secondary anti-oxidative activity and showed non-toxicity. In order to exploit its health benefits, fucoidan-rich seaweed tea was developed in this study. A total of 4 different brewing time treatments were performed on Sargassum binderi at 5, 10, 15 and 20 mins (F_{105} , F_{110} , F_{115} and F_{120} , respectively). It was found that F_{120} showed significantly ($p < 0.05$) highest fucoidan content (27.22 ± 0.07 mg/200 mL), superoxide anion scavenging activity ($16.46 \pm 2.83\%$) and hydroxyl radical scavenging activity ($89.83 \pm 4.11\%$) compared to that of F_{105} , F_{110} and F_{115} . Both the secondary antioxidant activities were significantly positive correlated to the fucoidan content tests (superoxide anion scavenging activity at $r = 0.97$, $p = 0.0052$; and hydroxyl radical scavenging activity at $r = 0.99$, $p = 0.0011$). Masking of the seaweed odour was performed using lemon essence and discriminative test found that masking was most effective using lemon essence at a concentration of 0.3% (v/v). Therefore, there is potential for this seaweed tea to be commercialised, thus, consumers may acquire the health benefit of fucoidan.

Keywords: Fucoidan; functional food; Sargassum binderi; seaweeds; tea

ABSTRAK

Kajian terdahulu kami terhadap fukoidan yang diekstrak daripada rumpai laut perang Malaysia, Sargassum binderi, menunjukkan bahawa fukoidan ini mengandungi aktiviti antipengoksidaan sekunder yang bererti dan merupakan produk tidak toksik. Dalam usaha mengeksploitasi manfaat kesihatan fukoidan, teh rumpai laut telah dibangunkan dalam kajian ini. Sebanyak 4 perlakuan masa pendidihan pada Sargassum binderi pada sela masa 5, 10, 15 dan 20 min (masing-masing F_{105} , F_{110} , F_{115} dan F_{120}) digunakan. Didapati bahawa F_{120} menunjukkan kandungan fukoidan (27.22 ± 0.07 mg/200 mL), aktiviti pemerangkapan superoksida anion ($16.46 \pm 2.83\%$) dan aktiviti pemerangkapan radikal hidroksil ($89.83 \pm 4.11\%$) tertinggi secara bererti ($p < 0.05$) berbanding F_{105} , F_{110} dan F_{115} . Kedua-dua aktiviti antipengoksidaan sekunder menunjukkan korelasi yang bererti dengan kandungan fukoidan (aktiviti pemerangkapan superoksida anion pada $r = 0.97$, $p = 0.0052$; dan aktiviti pemerangkapan radikal hidroksil pada $r = 0.99$, $p = 0.0011$). Penopengan bau rumpai laut telah dijalankan menggunakan pati lemon dan ujian diskriminatif telah menunjukkan bahawa penopengan yang paling berkesan adalah penggunaan pati lemon pada kepekatan 0.3% (v/v). Oleh itu, produk teh rumpai laut ini mempunyai potensi untuk dikomersialkan, supaya pengguna dapat menikmati manfaat kesihatan fukoidan.

Kata kunci: Fukoidan; makanan berfungsi; rumpai laut; Sargassum binderi; teh

INTRODUCTION

Malaysia has been identified as a main aquatic plant producer worldwide. According to the Food and Agricultural Organization of the United Nations (FAO 2016), Malaysia produced 245,332 tonnes of aquatic plants worth USD 63.75 million in 2014, making Malaysia the 7th largest producer of aquatic plants worldwide behind China, Indonesia, Philippines, South Korea, North Korea and Japan. However, the value of Malaysian aquatic plants are relatively low compared to those from the other major producer countries. For example, Japan produced 363,400 tonnes of aquatic plants worth USD 746.51 million in 2016 (USD 2054/tonne), which overshadows Malaysia's USD 260/tonne of aquatic plant production.

Brown seaweed has been known to be a natural source for bioactive compounds compared to red and green seaweeds (Prabhasankar et al. 2009). In general, brown seaweed contains fucoxanthin as its natural pigment, which gives a distinct greenish-brown colour, from which it gets its name (Wu et al. 2014). It has been reported that brown seaweed produces various active components, including unique secondary metabolites, such as phlorotannins, which exhibit specific biological activities. Other components found in brown seaweeds such as polyphenols and flavonoids were also reported to have strong anti-oxidative activities. The push for the use of natural antioxidants has been greater as synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT)

and tert-butyl hydroxyquinone (TBHQ) were reported to be toxic and exert carcinogenic effects (Chen et al. 1992). Aside from that, polysaccharides produced by brown seaweed have been used as pharmaceuticals, nutraceuticals, cosmeceuticals and functional food (Gupta & Abu-Ghannam 2011; Wijesinghe & Jeon 2012). Since the past decade, there has been a lot of focus on bioactive sulphated polysaccharides extracted from brown seaweeds. These functional polysaccharides, including fucoidan, alginates and laminarans, exhibited diverse biological characteristics, including anti-coagulant, anti-inflammatory, antiviral, anti-tumour and anti-oxidative activities (Li et al. 2008). It was reported by Wang et al. (2010) that the presence of sulphate groups in fucoidan promotes its anti-oxidative activity. The sulphate group function as electron withdrawing groups, thus increasing its anti-oxidative activity. Therefore, this study was conducted to promote the utilisation of high-value bioactive compounds from Malaysian seaweeds and thus, increasing the value of Malaysian seaweeds.

In our previous work, fucoidan has been isolated, characterised (Lim et al. 2016a) and has its anti-oxidation capacity determined (Lim et al. 2014), in addition to the toxicology studies performed (Lim et al. 2016b). It was found to exhibit significant secondary anti-oxidative activity as well as it being safe for consumption (non-toxic). Therefore, it was prudent to have these results translated into a functional product, i.e. seaweed tea. Seaweed tea came into consideration as this product is a minimally processed product, where there will be less effect towards the quality and bioactivity of fucoidan. At the same time, the hot water used to brew the seaweed tea is similar to the extraction procedure of fucoidan performed in the laboratory. Therefore, consumers will be able to enjoy the health benefits of fucoidan from seaweed tea. In this study, several brewing time treatments were performed on the seaweed tea and the tea was then analysed for fucoidan content, secondary anti-oxidative activities, masking of seaweed odour, and sensory evaluation to investigate the effectiveness of masking.

MATERIALS AND METHODS

MATERIALS

Sargassum binderi from Semporna, Sabah, was harvested in November 2010. Commercial food grade fucoidan was supplied by Yaizu Suisankagaku Industry Co., Ltd., Yaizu, Japan (F_{ysk}). Empty tea bags were purchased from AEON Mid Valley, Malaysia (Top Valu Brand, Tokyo, Japan). Lemon essence (FE-6744) was purchased from Peresscol, Damah Trading Sdn Bhd (Kuala Lumpur, Malaysia). All other chemicals were of analytical grade and purchased from Sigma (UK), unless otherwise stated.

PREPARATION OF SEAWEED SAMPLE

Fresh *Sargassum binderi* samples were washed to remove salt and impurities and dried at 40°C in an oven (Protech,

Texas, USA) for 72 h. They were then cut into small pieces with a knife mill before being ground into powder using a high-speed grinder (Kuoao Fung Electronic and Machine, Taiwan). The samples were kept at 4°C until use.

DEVELOPMENT OF SEAWEED TEA FROM *SARGASSUM BINDERI*

Powdered form of *Sargassum binderi* was weighed into empty tea bags, at 5 g per tea bag, and the tea bags folded to close them. These tea bags were then boiled (brewed) in 200 mL distilled water for 5, 10, 15 and 20 min (F_{05} , F_{10} , F_{15} and F_{20}). Distilled water was added from time to time to ensure the boiling water was at the 200 mL mark. The tea bags were then removed from the brews after the allocated time, and the brews were filled up to 200 mL. Figure 1 shows the seaweed tea preparation process.

DETERMINATION OF FUCOIDAN CONTENT IN SEAWEED TEA

As discussed in our former work, Lim et al. (2014), *Sargassum binderi* contains fucoidan and the fucoidan isolated from *Sargassum binderi* (F_{sar}) demonstrates anti-oxidative activities, especially in terms of secondary anti-oxidative activities. Therefore, the fucoidan content in the seaweed tea with four different time treatments (F_{05} , F_{10} , F_{15} and F_{20}), were tested for its fucoidan content using a colorimetric method. A fucoidan standard curve was prepared using 0.1-0.5 mg/mL of commercial food grade fucoidan (F_{ysk}). The samples and standards were pipetted at 1 mL each into test tubes submerged in an ice-water bath. The samples and standards were added with 4.5 mL concentrated sulphuric acid (1:6, $H_2O:H_2SO_4$), stirred and allowed to stand for 1 min, before transferring the tubes into a boiling water bath for exactly 10 min. The samples and standards were then allowed to cool and added with 0.1 mL 3% L-cysteine solution, stirred and left at room temperature for 30 min. Absorbance at 396 nm and 427 nm were determined using *BioTek Epoch* microplate spectrophotometer (Vermont, USA) and the difference in both absorbance ($\Delta\lambda_{396-427}$) were due to that of fucose (Hellebust & Craigie 1978; Lim et al. 2014). The fucoidan content was calculated using the following equation:

$$\text{Fucoidan content (mg/200 mL)} = R \times 200, \quad (1)$$

where R is the reading obtained from the standard curve and 200 represents 200 mL of the sample serving size. A replication of ($n=3$) was performed.

ANALYSIS OF ANTI-OXIDATIVE ACTIVITIES IN SEAWEED TEA

The significant secondary anti-oxidative activities of F_{sar} from our previous work (Lim et al. 2014) initiated this functional food product development, i.e. the seaweed tea. In order to demonstrate that the seaweed tea still exhibits secondary anti-oxidative activities, two secondary antioxidant assays were performed on seaweed tea with

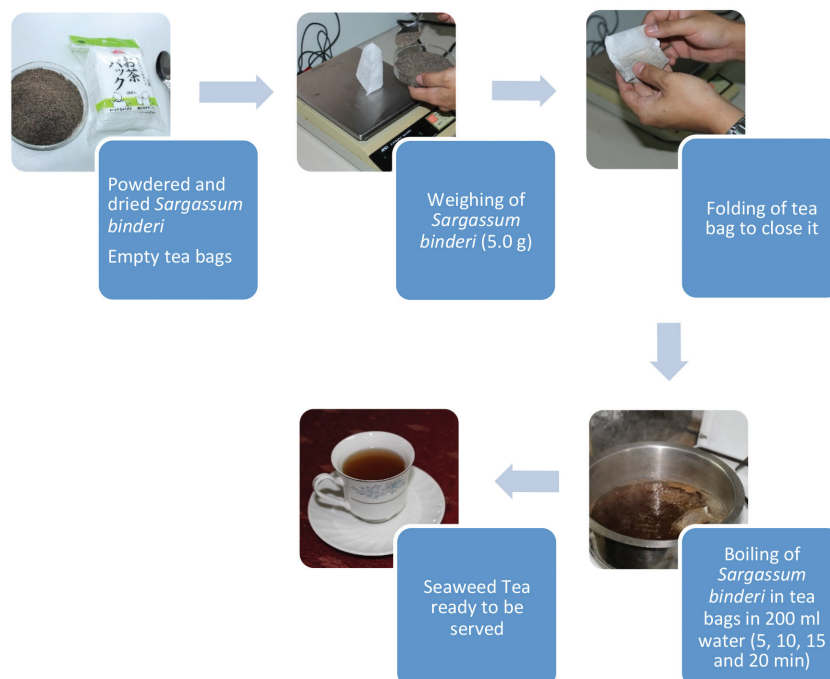


FIGURE 1. Preparation of seaweed tea

four different time treatments (F_{105} , F_{110} , F_{115} and F_{120}), namely superoxide anion scavenging activity and hydroxyl radical scavenging activity.

Superoxide anion scavenging activity The superoxide anion scavenging activity was determined by measuring the inhibition of pyrogallol auto-oxidation (Lim et al. 2014; Marklund & Marklund 1974). All samples (F_{105} , F_{110} , F_{115} and F_{120}) were pipetted at 0.3 mL each into 2.6 mL phosphate buffer (50 mM, pH 8.24) and 90 μ L of 3 mM pyrogallol solution (dissolved in 10 mM HCl). A blank was prepared using distilled water in place of sample. The absorbance, which reflects the inhibition rate of pyrogallol auto-oxidation, was measured at 325 nm in a 96-well plate (200 μ L) using a *BioTek Epoch* microplate spectrophotometer in which the absorbance was recorded every 1 min interval for 10 min. A replication of $n=3$ was performed in the superoxide anion scavenging activity test. The percentages of superoxide anion scavenging activities of the samples were calculated using the following equation:

$$\text{Scavenging rate (\%)} = [1 - (A_2 - A_1) / A_0] \times 100, \quad (2)$$

where A_1 is the absorbance of the sample at zero min; A_2 is the absorbance of the sample at the tenth min; and A_0 is the autoxidation rate of pyrogallol for the blank (the change of absorbance in the blank from zero min to ten min).

Hydroxyl radical (\cdot OH) scavenging activity The hydroxyl radical scavenging activity (\cdot OH) was determined according to the method reported by Lim et al. (2014). The hydroxyl radical was generated using the Fenton reaction,

where 0.5 mL of 9 mM ferrous sulphate (FeSO_4) solution was added to 1.0 mL of 8.8 mM hydrogen peroxide (H_2O_2) (35% purity, HmbG Chemicals) solution. This mixture was then added to 1 mL of F_{105} , F_{110} , F_{115} and F_{120} samples, before adding 0.2 mL of 9 mM salicylic acid solution. Another set of reaction solutions was prepared as above without the addition of salicylic acid. A blank with salicylic acid was also prepared. The mixtures were allowed to stand at 37°C for 1 h before the absorbance was measured at 510 nm in a 96-well plate (200 μ L) using the *BioTek Epoch* microplate spectrophotometer. The hydroxyl radical scavenging activity was determined in triplicate ($n=3$) and the activity was calculated as follows:

$$\text{Hydroxyl radical scavenging rate (\%)} = \left[1 - \frac{(A_1 - A_2)}{A_0} \right] \times 100, \quad (3)$$

where A_0 is the absorbance of the blank (without sample); A_1 is the absorbance of the reaction containing the sample and salicylic acid; and A_2 is the absorbance of the reaction containing the sample but without salicylic acid.

Correlation analysis of fucoidan content with anti-oxidative activities Correlation analyses between fucoidan content and anti-oxidative activities were carried out using Microsoft Excel 2013. Pearson correlation analysis was performed where the p-value determines the significance of the correlation.

SENSORY EVALUATION OF SEAWEED TEA

The seaweed tea produced has a rather unpleasant seaweed smell. Therefore, a smell masking technique was employed

in this research. Since the antioxidant analysis showed that F_{120} has the highest activity, the masking procedure was employed to this sample. The masking of the unpleasant seaweed smell was performed using lemon essence (FE-6744). Lemon essence was added into the F_{120} sample at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5% (v/v), at room temperature. A discriminative model was used to determine the minimum lemon essence concentration necessary to mask the seaweed smell in the seaweed tea. The samples with lemon essence were evaluated by a panel consisting of 74 undergraduate and postgraduate students from Universiti Kebangsaan Malaysia. The sensory evaluation was conducted in a sensory laboratory with proper panels separating the tables. Samples were served in 20 mL universal bottles with screw caps at room temperature. Each universal bottle was filled with 10 mL of samples. The panel were asked to identify the seaweed odour from the reference sample (untreated F_{120}) and then detecting the samples (with lemon essence) that do not contain the smell of the seaweed odour present in the reference sample. Panel were allowed to choose more than one sample that does not contain the smell in the reference sample. Samples were arranged randomly and labelled with random numbers to minimise bias (Aminah 2004). The results were presented as a score graph, where each sample chosen was given a score of 1, while samples which were not chosen given a score of 0.

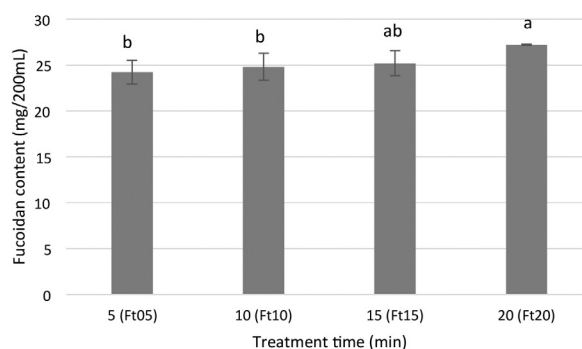
STATISTICAL ANALYSIS

Analysis was performed in triplicate ($n=3$), except for the sensory evaluation, where 74 subjects were involved. Data were obtained as the mean and standard deviation and analysed using one-way ANOVA followed by Duncan's multiple range test (DMRT) using SAS version 6.12 for Windows. The difference in mean values was considered significant when $p<0.05$.

RESULTS AND DISCUSSION

FUCOIDAN CONTENT IN SEAWEED TEA

The fucoidan content in F_{05} , F_{10} , F_{15} and F_{20} are shown in Figure 2. The fucoidan content is presented in mg/200 mL, as the samples were extracted in a serving size of 200 mL. It was found that the fucoidan content in F_{120} (27.22 ± 0.07 mg/200 mL) was the highest and it was significantly ($p<0.05$) different to that of F_{05} and F_{10} . The F_{15} sample did not show any significant ($p>0.05$) difference with other samples. This indicates that the extraction time of 20 min successfully extracted significantly higher fucoidan compared to the other extraction times tested. A higher fucoidan content in the seaweed tea increases its value, as consumers will be able to enjoy the benefits of fucoidan, whereby it was reported to possess functional properties (Ale et al. 2011; Li et al. 2008).



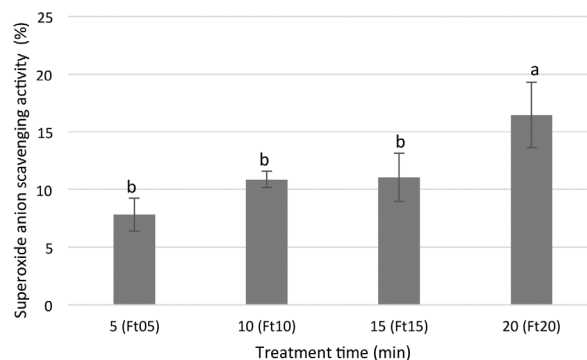
^{a,b} Different letters indicate statistical difference ($p<0.05$)

FIGURE 2. Fucoidan content in seaweed tea with four different time treatment ($n=3$)

ANTI-OXIDATIVE ACTIVITIES OF SEAWEED TEA

Superoxide anion scavenging activity The superoxide anion scavenging activity of seaweed tea with four different time treatment (F_{05} , F_{10} , F_{15} and F_{20}) are shown in Figure 3. It was found that F_{20} ($16.46 \pm 2.83\%$) had the significantly ($p<0.05$) highest superoxide anion scavenging activity compared to that of the other samples. The other samples, F_{05} , F_{10} and F_{15} , did not show any significant difference ($p>0.05$) among them. Superoxide anion scavenging is of great importance to the human body, as superoxide anions are constantly being formed in the human body through cellular oxidation reactions. Although it is a relatively weak oxidant, it decomposes to produce stronger oxidative species, such as hydrogen peroxide and hydroxyl radicals, through dismutation and other types of reactions (Sarikurcu et al. 2010; Yangthong et al. 2009). The significant superoxide anion scavenging activity in seaweed tea shows prominent health benefit, which is important to consumers.

Hydroxyl radical ($\cdot OH$) scavenging activity Figure 4 shows the hydroxyl radical scavenging activity of seaweed

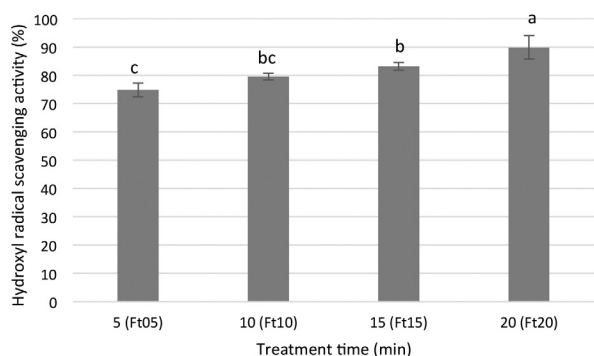


^{a,b} Different letters indicate statistical difference ($p<0.05$)

FIGURE 3. Superoxide anion scavenging activity of seaweed tea with four different time treatment ($n=3$)

tea with four different time treatments (F_{105} , F_{110} , F_{115} and F_{120}). It was found that F_{120} ($89.83 \pm 4.11\%$) had the significantly highest ($p < 0.05$) hydroxyl radical scavenging activity compared to that of the other samples. The F_{115} and F_{105} samples differed significantly ($p < 0.05$), while F_{110} did not show significant difference ($p > 0.05$) compared to that of F_{115} and F_{105} in terms of hydroxyl radical scavenging activity. According to Aruoma (1998), the hydroxyl radical is the most reactive free radical which can be formed from superoxide anions and hydrogen peroxides. It causes damage to nearly all types of biomolecules, which in turn accelerates the aging process, leading to cancer and several diseases (Aruoma 1998; Zhu et al. 2006). Therefore, the ability of this seaweed tea to scavenge hydroxyl radical brings essential health benefits to consumers by preventing the abovementioned diseases.

Since both secondary antioxidant data (superoxide anion and hydroxyl radical scavenging activities) showed that seaweed tea had the significantly highest ($p < 0.05$) activity when heated with time treatment of 20 min (F_{120}), the masking procedure as well as the sensory evaluation were carried out using the F_{120} sample.



^{a-c} Different letters indicate statistical difference ($p < 0.05$)

FIGURE 4. Hydroxyl radical scavenging activity of seaweed tea with four different time treatment ($n=3$)

Correlation between fucoidan content and anti-oxidative activities Correlation analyses were performed on fucoidan content with anti-oxidative activities conducted to relate how fucoidan content affect the anti-oxidative activities in seaweed tea, as illustrated in Figure 5. The results showed positive correlations between fucoidan content and superoxide anion scavenging activity ($r=0.97$, $p=0.0052$) and hydroxyl radical scavenging activity ($r=0.99$, $p=0.0011$). Both the correlations were significant ($p < 0.05$) and were highly correlated (very high r-values). This indicates that the increase of fucoidan content in the seaweed tea does actually increase the anti-oxidative activities of the tea.

SENSORY CHARACTERISTICS OF SEAWEED TEA

The sensory evaluation on the masking capabilities of the lemon essence are as shown in Figure 6. In this

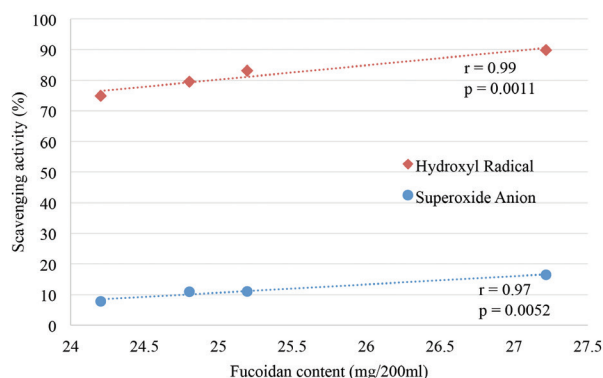


FIGURE 5. Correlation between fucoidan content and antioxidant activities in seaweed tea

discriminative test, the sample with the highest frequency at lowest concentration was deemed as the most effective concentration of lemon essence in masking the seaweed smell. It was evident that lemon essence in seaweed tea (F_{120}) at the concentration of 0.3% (v/v) had the most efficient masking effect, where it achieved a score of 52 frequencies. Both F_{120} samples with 0.1 and 0.2% (v/v) lemon essence had the lowest score of 31 frequencies. Increasing the lemon essence concentration to 0.4 and 0.5% (v/v) in the seaweed tea (F_{120}) did not increase the efficiency of masking effect, where the scores were 46 and 51 frequencies, respectively. Therefore, the minimum concentration of lemon essence in seaweed tea (F_{120}) that effectively masked the seaweed smell is at 0.3% (v/v).

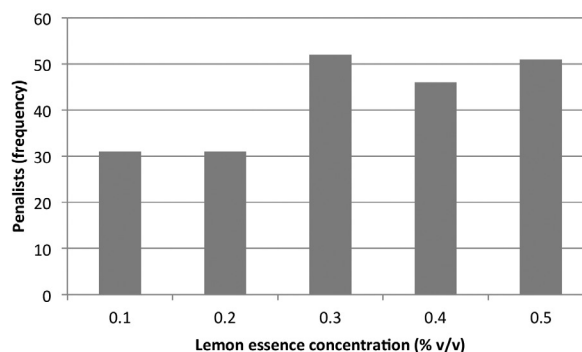


FIGURE 6. Masking efficiency frequency of seaweed smell in seaweed tea (F_{120}) using different concentrations of lemon essence ($n=74$)

A previous study by Haas (1935) showed that the unpleasant smell from seaweeds is from decomposing seaweeds, which is the putrefactive reduction of the organically combined sulphates. Haas (1935) identified the smell from *Polysiphonia fastigiata* seaweed, as coming from methyl sulphide volatile compounds. Another study by Izzreen and Ratnam (2011) identified a few compounds, namely, z-8-tetradecen-1-yl acetate, isobutyl nonyl ester and cis-7-dodecen-1-yl acetate as the volatile compounds which are responsible for the unpleasant smell

from *Sargassum polycystum*, *Kappaphycus alvarezii* and *Caulerpa lentillifera*, respectively. It was discussed in this report that these volatile compounds were products of seaweed decomposition.

Seo et al. (2012) reported that the brown seaweed *Laminaria japonica* extract has high expected odour intensity (EOI) from a few compounds, namely, isovaleric acid (41.2%), allyl isothiocyanate (22.6%), octanal (11.1%) and acetaldehyde (10.6%). All these compounds were responsible for 85.5% of the total odour strength in the extract, and this suggests that the odour from *Laminaria japonica* extract is mainly attributed to these compounds. Therefore, in the present study, lemon essence was used to mask the smell from volatile compounds which are responsible to the unpleasant smell from the seaweed tea. According to a review by Sohi et al. (2004), numerous products, including oral pharmaceuticals, food and beverage products and bulking agents have unpleasant, bitter-tasting components. Therefore, taste masking is generally applied on these products to mask the unpleasant flavour, which increases the acceptance of consumers. Various synthetic flavours are used to mask the bitterness, among them, chlorpheniramine maleate and phenylpropanolamine HCl (orange flavour and cream flavour), famotidine (lemon flavour) and acetaminophen (cherry flavour). Since taste masking is an important part of pharmaceutical and food industry, the masking agent should not affect the bioactivity of the product. In this study, the lemon essence used was minimal 0.3% (v/v), and thus will not affect fucoidan content and antioxidative properties of the seaweed tea.

CONCLUSION

The functional food product in this research, seaweed tea, was successfully developed using *Sargassum binderi* as its raw material. In this research, several parameters need to be prioritised in order to increase the anti-oxidative effects and also reduce the unpleasant seaweed smell. This was to ensure that consumers can enjoy the seaweed tea health benefits while consumer acceptance to this product could be improved. Therefore, it was found in this research that the most suitable parameters for the preparation of seaweed tea from the *Sargassum binderi* powder in tea bags (5 g) was brewing it in 200 mL boiling water for 20 min to ensure higher fucoidan extraction and thus significantly ($p < 0.05$) higher secondary anti-oxidative activities. The fucoidan content in this seaweed tea showed significant ($p < 0.05$) positive correlation with superoxide anion scavenging activity ($r = 0.97$) and hydroxyl radical scavenging activity ($r = 0.99$). The seaweed tea need to be supplemented with 0.3% (v/v) lemon essence, that is 0.6 mL lemon essence in 200 mL seaweed tea, to mask the unpleasant seaweed flavour, and thus increasing consumer acceptance towards the product.

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