

Physicochemical and antioxidant properties of chilli sauce incorporated with fermented *Kappaphycus* spp. extracts

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Abstract

In this study, three distinct *Kappaphycus* spp. var. Green Flower (GF), var. White Giant (WG), and var. Purple Giant (PG) were subjected to solid-state fermentation (SSF) for four days at 30°C using *Aspergillus oryzae* (10% inoculum). The fermented seaweed extracts were incorporated into chilli sauce to increase its nutritional quality and were characterised in terms of proximate composition, antioxidant activity, viscosity and serum separation. The results obtained indicated that samples incorporated with fermented seaweed extracts contained significantly lower carbohydrates and higher ash content in comparison to control. The addition of fermented seaweed extracts significantly ($p < 0.05$) increased the antioxidant activity. Serum separation was observed only in the control sample after eight weeks of storage. Therefore, adding fermented seaweeds can be incorporated into chilli sauce to increase the antioxidant properties and storage stability.

Introduction

The benefits of seaweeds include their abundant nutrients, such as dietary fibres, minerals, vitamins, polyphenols, proteins/peptides, and omega-3 fatty acids (Cardoso *et al.*, 2015). Several studies have shown that incorporation of seaweeds or isolates into foods can enhance shelf life, nutritional, textural, organoleptic, and nutritional properties of food products (Roohinejad *et al.*, 2017). Hence seaweed can be used as a functional ingredient in foods. Seaweeds can be used as texturing agents and stabilisers, and the food industry remains the primary industry that utilises seaweed hydrocolloids (Bixler and Porse, 2010). Soluble and insoluble dietary fibres in seaweed polysaccharides demonstrate better water-holding capacity than cellulosic fibres. The capacity to increase viscosity, create gels and/or serve as emulsifiers makes soluble dietary fibres useful when added to food (Elleuch *et al.*, 2011).

Capsicum annum (or chilli) is a herbaceous-vegetable commonly used worldwide in cuisine (Dubey

et al., 2015). Chilli sauce is the most prevalent common item derived from chilli, also referred to as hot sauce. Hot sauces are primarily consumed in Asian nations (particularly Korea and China), Mexico and the United States (Kim *et al.*, 2018). However, the use of hot sauce is less widespread in most European countries. Common raw ingredients for chilli sauce include chilli, water, garlic, preservatives, sugar, vinegar, salt, and hydrocolloids (Gamonpilas *et al.*, 2011). Chilli sauces are complicated multiphase suspensions comprising chilli particles and occasionally flexible fluid particles such as oil droplets. The working fluid is often water or a macromolecule solution in water. This solution may also include salt, hydrocolloids, organic acid, or other ingredients to create acidity and, as a result, play a positive role in extending the hot sauce shelf life (Sikora *et al.*, 2003; Sikora *et al.*, 2008). Colour, viscosity, and consistency are three qualities that, in general, influence the overall acceptability of sauce by consumers (Ali *et al.*, 2019).

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The main problem in chilli sauce is associated with the tendency to separate solids and liquid phases during storage (Rengsutthi and Charoenrein, 2011). Previously, in chilli sauce, corn starch is usually used as a thickener (Noraziah and Theng, 2013). However, because corn starch is a highly processed carbohydrate without protein, fat, vitamins, minerals, or fibre, it can add substantial calories when added to any processed food (Nur Atika *et al.*, 2014). Since seaweed contains a significant amount of soluble polysaccharides, it can act as a thickening agent with the added value of having higher phenolic content, related to antioxidant properties (Choudhary *et al.*, 2021). Thus, the possibility of using a more nutritious fermented seaweed extract to replace corn starch in chili sauce was investigated. The quality and properties of chilli sauce incorporated with fermented seaweed extracts were determined in terms of physicochemical and antioxidant properties. Findings in this study would lead to the development of food-based applications of fermented seaweed extracts to promote consumption among non-seaweed eaters and diversify the use of seaweed products in food products.

2. Materials and methods

2.1 Sample preparation

Three varieties of dried seaweed were purchased from Semporna, Sabah (*Kappaphycus* spp. var. Green Flower (GF), var. White Giant (WG), and var. Purple Giant (PG)). The samples collected were washed with tap water to remove dirt and contaminants. Figure 1 shows the three seaweed samples used in this study. It was then desalinated by soaking for 1 h in twice as much water. Desalinated samples were sliced 1-5 mm and then air-dried for five days at room temperature. The dry samples were pulverised using a Waring blender and kept at 4°C in tight cap bottles prior to the fermentation process.

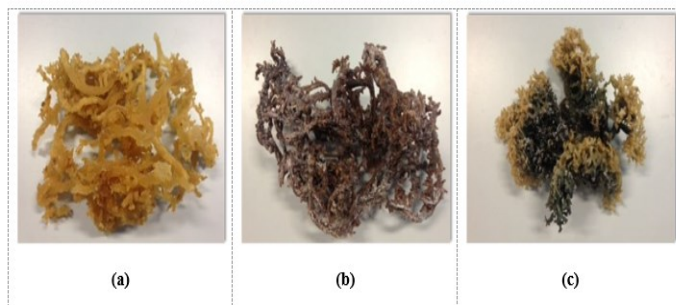


Figure 1. Seaweeds used in this study: (a) white seaweed-WG; (b) purple seaweed - PG; (c) green flower (GF).

2.2 Fermentation of seaweed

Solid state fermentation of seaweed was conducted according to Norakma *et al.*, 2022 with 10g working volume. Sucrose (0.03 g) and yeast extract (0.03 g) were added to the fermentation medium as supplementary carbon and nitrogen sources respectively. The inoculum

(1 mL) was added to a sterile medium that had an initial moisture content of 70%. This medium was then incubated at 30°C for four days. At the end of fermentation, the medium was added with distilled water (100 mL) and then vigorously mixed on a rotary shaker at a speed of 180 rpm for an hour. After 10 minutes of centrifugation at 8000 rpm, the supernatant that was collected was subjected to further filtration using a no 1. Whatman filter paper. The crude fermented seaweed was the clear supernatant. It was stored at -20°C and freeze-dried before being used as an ingredient in chilli sauce.

2.3 Preparation of chilli sauce

The method for chilli sauce preparation was carried out according to Rengsutthi and Charoenrein (2011). The chilli seed was removed, and chilli skins were rinsed with tap water. Then chilli (28%) and garlic (12%) were steamed for 20 minutes in boiling water. Steamed chilli and garlic were then blended with vinegar to get a uniform consistency. Sugar (17%), salt (2.5%) and fermented seaweed extract (1%) were added at 50°C, and then pasteurisation at boiling point was carried out for 10 minutes. The process began with hot filling of bottles equipped with screw caps, followed by cooling to room temperature. The sauce was divided into two batches prior to further analysis. The first batch was not subjected to further storage, whilst the second batch was kept at 37°C for a total of 8 weeks. The ingredients used to make the chilli sauce are summarised in Table 1. The remaining ingredient was water that made up 23.5% of the sauce.

Table 1. Composition of each ingredient (per 100 g) in chilli sauce incorporated with fermented seaweed extracts of white seaweed (WG), green flower (GF) and purple seaweed (PG).

Ingredients (%)	Samples			
	Control	WG	GF	PG
Chilli	28	28	28	28
Sugar	17	17	17	17
Vinegar	16	16	16	16
Garlic	12	12	12	12
Salt	2.5	2.5	2.5	2.5
Corn starch	1	0	0	0
Seaweed extract	0	1	1	1
Total	76.5	76.5	76.5	76.5

2.4 Chilli sauce extracts

For determination of total phenolic content (TPC) and antioxidant analysis, an extract of the chilli sauce was prepared (Pereira *et al.*, 2016). The combination was agitated using a magnetic stirrer for 1 hour and spun at

7,000 rpm for 10 minutes after adding 10 g of chilli sauce to distilled water (100 mL). The recovered supernatant was the chilli sauce extract.

2.5 Proximate evaluation

The protein, moisture content, fat, ash, and fibre content of all samples were analysed (AOAC, 2013). Fat content was determined using the Soxhlet technique, moisture content was determined using the oven method, and protein content was determined using the Kjeldahl method. A muffle furnace was used to evaluate the ash level of the chilli sauce by drying it at 550°C for 24 hours until constant weight was achieved. The protein content was determined by multiplying g/100 g of nitrogen by 6.25, whereas the carbohydrate amount was estimated using the following formula:

$$\% \text{carbohydrates} = 100 - \% \text{moisture} - \% \text{protein} - \% \text{lipid} - \% \text{mineral}$$

2.6 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was performed as previously described by Bhuiyan and Hoque (2010). Chilli sauce extract (1 mL) was added to 0.1 mM DPPH solution (1 mL). The solution was refluxed well and incubated at room temperature for 30 minutes under dark conditions. The absorbance was recorded at 517 nm using a spectrophotometer. At various concentrations ranging from 0 - 0.7 mg/mL, ascorbic acid was used as a positive reference. Mixing 1 mL of methanol and 0.1 mM DPPH solution (1 mL) yielded the control. Using the following formula, the radical scavenging activity was reported as the radical scavenging percentage:

$$\% \text{ Scavenging activity} = \left[\frac{Ac - As}{Ac} \right] \times 100\%$$

Where Ac = absorbance of the control, As = absorbance of the sample solution

2.7 Serum separation

Serum separation of chilli sauce was determined according to the method of Rengsutthi and Charoenrein (2011) with slight modifications for samples used in the storage study. The samples (50 mL) were placed in measuring cylinders of the same volume, covered with parafilm, and kept at 37°C for 8 weeks. Serum separation was determined on a weekly basis (in cm). All measurements were done in duplicates.

2.8 Viscosity measurement

The viscosity (Pa.s) and shear rate of all sauce samples were determined using a rheometer (Physica, model: MCR 300) according to Gamonpilas et al. (2011).

3. Results and discussion

3.1 Proximate composition

The proximate composition of chilli sauce was comparable to commercial chilli sauce in the market. Based on the results obtained in Table 2, the control chilli sauce showed the highest carbohydrate composition compared to the rest of the chilli sauce incorporated with fermented seaweed extracts. This finding shows that the use of fermented seaweed extracts can reduce the calorie obtained from corn starch that was used as a thickener agent in the control chilli sauce. The control chilli sauce also showed the highest dietary fibre (DF) composition compared to chilli sauce from fermented seaweed extracts. DF is the edible portion of carbohydrate polymer, which is resistant to digestion and requires complete or partial bacterial fermentation located in the large intestine (Lattimer and Haub, 2010). DF refers to oligosaccharides, polysaccharides, and related plant components (Dhingra et al., 2011). Chilli sauce incorporated with fermented seaweed extract contained higher ash content than control chilli sauce, suggesting that it contained a higher total mineral content (Uchida et al., 2017).

Table 2. Proximate composition of chilli sauce produced from fermented seaweed extracts of white seaweed (WG), green flower (GF) and purple seaweed (PG).

Composition	Sample			
	Control	WG	GF	PG
Moisture	59.00 ^a ±0.00	59.00 ^a ±0.01	59.94 ^a ±0.00	59.16 ^a ±0.00
Ash	11.23 ^c ±0.09	14.82 ^a ±0.01	14.05 ^a ±0.04	13.41 ^b ±0.11
Dietary fiber	2.03 ^a ±0.06	1.70 ^b ±0.01	1.62 ^c ±0.01	1.65 ^c ±0.01
Lipids	0.11 ^b ±0.00	0.16 ^a ±0.01	0.10 ^b ±0.00	0.13 ^b ±0.00
Protein	1.52 ^a ±0.05	1.05 ±0.00	1.57 ^a ±0.00	1.14 ^b ±0.00
Carbohydrate	26.10 ^a ±0.15	23.26 ±0.01	22.71 ^c ±0.03	24.51 ^b ±0.11

^{a,b} show significant different at p<0.05

^c shows insignificant different at p<0.05

3.2 DPPH radical scavenging activity

The results obtained for the DPPH radical scavenging activity of chilli sauce incorporated with fermented seaweed concentrates are shown in Figure 2. It was found that the DPPH radical scavenging activity of chilli sauce incorporated with fermented seaweed extracts was significantly higher compared to raw fermented seaweed extracts. The highest DPPH radical scavenging activity was found in GF (89.11%), followed by PG (87.15%), WG (75.12%) and control (72.19%). These results suggest that fermented seaweed extracts in chilli sauce formulation can provide better antioxidant properties than conventional chilli sauce (control). The heat treatment during chilli sauce preparation also did not reduce the antioxidant capacity of fermented seaweed extracts used in this study. The antioxidant activity of processed food is strongly affected by cooking method, time, temperature and portion size (Hwang *et al.*, 2012).

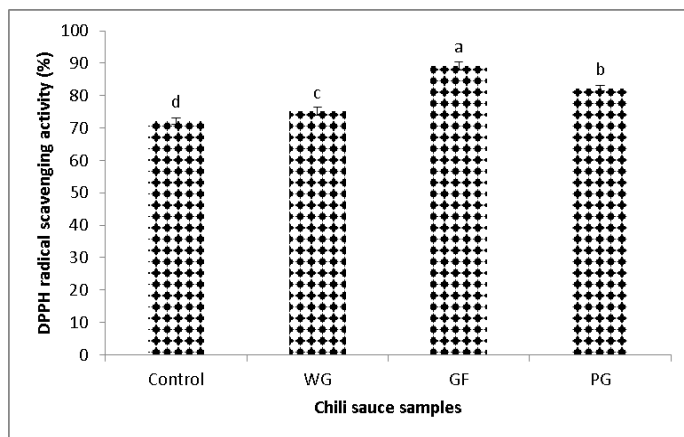


Figure 2. DPPH radical scavenging of chilli sauce incorporated with fermented seaweed extracts from white seaweed (WG), purple seaweed (PG) and green flower (GF). a, b, c and d denote significant differences between samples at $p < 0.05$.

In this present study, the antioxidant activity of chilli sauce incorporated with fermented seaweed extracts was significantly higher than the antioxidant activity of raw fermented seaweed extracts. The DPPH free-radical scavenging activity was increased by 15.27% in chilli sauce produced from WG sample, 29.62% in PG sample and 22.96% in GF sample, respectively. Previous studies reported that thermal treatment caused a deterioration in antioxidant activity (Maghsoudlou *et al.*, 2019; Zarei *et al.*, 2019), while some reports demonstrated an increase or no change in antioxidant properties (Ioannou *et al.*, 2020) and oxidative stability (Cai *et al.*, 2021). The increment in antioxidant ability suggests that formation of new antioxidant compounds after cooking was more than the heat degradation of antioxidant compounds. The breakdown of cell walls and subcellular structures can release antioxidant compounds. The production of new antioxidants by thermal reaction and deactivation of certain oxidative enzymes by heat may explain the

increase in antioxidant capacities after heat treatment (Yamaguchi *et al.*, 2001).

Likewise, thermal-oxidative degradation of certain antioxidants can be prevented by other antioxidants present in the foods, for example, anthocyanins (Oancea, 2021). The synthesis of new antioxidant molecules during thermal processing or heat treatment might also attribute to the high antioxidant properties due to the formation of Maillard reaction products. Maillard products are potent antioxidant compounds that are formed at elevated temperatures due to the reaction of carbonyl groups in sugar and amino acids in protein (Ioannou *et al.*, 2020). Some Maillard reaction products, such as melanoidins, have advantageous impacts on health, including anti-oxidative and antimicrobials (Tamanna and Mahmood, 2015). It is further reported that Maillard reaction products may compensate for the loss of Vitamin C or even boost the hydrophilic antioxidant capability (Toyoizumi *et al.*, 2021).

3.3 Serum separation

This study showed no serum separation for all sauce samples in the first four weeks of storage at 37°C. Only at week 8, a small degree of serum separation was observed in control (1 cm). Serum separation occurs when the solids particles start to settle out of the solution, leaving a layer of transparent, straw-coloured serum on the upper side of the product. The results suggest that chilli sauce incorporated with fermented seaweed extract was potentially a better stabiliser than corn starch in high acid foods.

Water separation acts as a significant sensory attribute and may be a major issue in liquid products. Serum separation is reduced by a more homogenised and stable solution. To prevent serum separation, insoluble particles must stay in a stable suspension throughout the serum (Noraziah and Theng, 2013). Generally, less serum separation happens at higher viscosity. The results obtained indicated that chilli sauce incorporated with fermented seaweed extracts has the ability to reduce serum separation because no serum separation was observed during 8 weeks of storage. The separation of water has been reported to decrease with increase in hydrocolloid addition in chilli sauce, and increased with the increase in storage duration (Noraziah and Theng, 2013). It was reported that addition of jackfruit seed starch also showed lower levels of serum separation compared to chilli sauce with corn starch (Rengsutthi and Charoenrein, 2011).

3.4 The viscosity of chilli sauce

Figure 3 shows the relationship between viscosity (Pa.s) and the shear rate of all sauce samples. It is apparent that the viscosity of chilli sauce decreased with

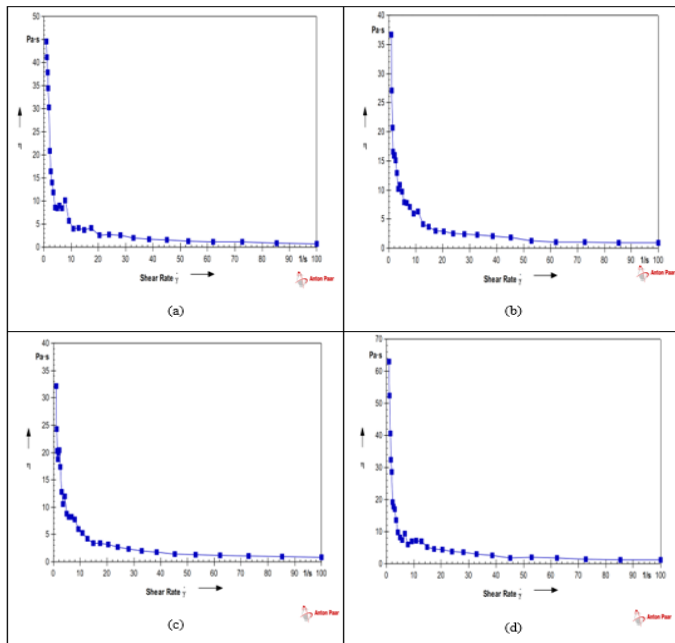


Figure 3. The relationship between viscosity (Pa.s) and shear rate (s⁻¹) of (a) Control (b) white seaweed-WG (c) green flower (GF) and (d) purple seaweed (PG) chilli sauce samples.

the increase in shear rate. Hence all chilli sauce samples incorporated with fermented seaweed extract had a noticeable apparent viscosity, which could be characterised within the Non-newtonian pseudoplastic flow behaviour. The curve showed a pseudoplastic pattern where the apparent viscosity was inversely related to the rate of shear. Even though the viscosity of the chilli sauce decreased with increasing storage time, the chilli sauce gel structure was maintained by corn starch (Control) and fermented seaweed extract (samples), which give a stickiness effect to chilli sauce. Based on the results obtained, PG showed the significantly highest viscosity, followed by control, WG and GF samples.

4. Conclusion

This study demonstrated that the antioxidant activity of chilli sauce incorporated with fermented seaweed extracts was higher compared to control chilli sauce. Proximate composition showed that control chilli sauce contained the highest carbohydrate content compared to the samples that were incorporated with seaweed extract. The colour of chilli sauce incorporated with fermented seaweed extracts had a similar redness index to the control chilli sauce sample. In addition, the chilli sauce produced using fermented seaweed extracts exhibited good serum separation stability. Incorporation of fermented seaweed extracts can be a useful substitute for corn starch because of its enhanced antioxidant activity and lower carbohydrate content. However, sensory analysis of the products should be evaluated to further determine consumer acceptability.

Conflict of interest

The authors no conflicts of interest to disclose.

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