

## Production of Fucoxanthin-Rich Powder from *Cladosiphon okamuranus*

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**Abstract:** Fucoxanthin, a major carotenoid of *Cladosiphon okamuranus* and other brown seaweed, exhibits anti-carcinogenic, anti-obesity, and anti-inflammatory activities. Despite these desirable activities, the utilization of fucoxanthin of a high purity in functional food preparations has been restricted because the instability of fucoxanthin makes its production difficult. In order to expand the industrial-scale use of *C. okamuranus*, the possibility of producing a fucoxanthin-rich product of a high purity was explored by using *C. okamuranus* as the source material. Several drying methods (hot-wind, vacuum, and freezing), drying temperatures (40-80°C), and pulverization methods (particle size: 50, 200, and 1000 µm) were employed and the outcomes were compared; the conditions were optimized to minimize the decomposition of fucoxanthin. The 50-µm freeze-dried powder was found to be the best. In addition, the algal extract showed a strong DPPH radical scavenging activity.

**Key words:** Brown seaweed, carotenoid, DPPH assay, functional food

### INTRODUCTION

Fucoxanthin is a major marine carotenoid found in brown seaweed (Chandini *et al.*, 2008). It has a unique structure, which contains an allenic bond and a 5,6-epoxide in its molecule. Some examples of familiar brown seaweed include *Sargassum*, *Undaria*, *Hizikia*, *Laminaria* and *Cladosiphon*. Kanazawa *et al.* (2008) reported that *Laminaria japonica* that grows in the northern regions is a good source for commercial-scale preparation of fucoxanthin in Japan (Kanazawa *et al.*, 2008). *C. okamuranus* that grows in the southern regions is the major fucoxanthin-containing alga cultured in Okinawa, with an annual harvest of around 20,000 tons (wet weight). Fucoxanthin exhibits anti-carcinogenic, anti-obesity, anti-inflammatory, anti-angiogenic, and anti-oxidative activities (Das *et al.*, 2005; Heo *et al.*, 2008; Kotake-Nara *et al.*, 2001; Maeda *et al.*, 2006; Okuzumi *et al.*, 1990; Shiratori *et al.*, 2005; Sugawara *et al.*, 2006). Orally administered fucoxanthin is safe in terms of mutagenic potential (Beppu *et al.*, 2009). Despite these desirable features, the utilization of fucoxanthin of a high purity in functional food preparations has been restricted because of the instability of fucoxanthin against oxidation and heat during processing. Nonetheless, although pure fucoxanthin is unstable, the fucoxanthin extracted from the alga is rendered stable by the coexisting antioxidants such as polyphenols. Therefore, the optimal conditions for the production of *C. okamuranus* powders enriched with stable fucoxanthin were tested and determined. The obtained powders would also have additional health

benefits derived from the coexisting fucoidan, fucosterol, fibers, minerals, and antioxidants. In this paper, the optimal conditions for the production of powdered form of *C. okamuranus* rich in fucoxanthin were investigated. Further, to expand our knowledge of the health benefits of *C. okamuranus*, the DPPH radical scavenging activity of a crude extract of the alga and of fucoxanthin and deacetyl fucoxanthin (fucoxanthinol) was investigated.

First, the efficiency of various solvents (acetone, ethanol, and methanol) and pulverized particle sizes (50, 200, and 1000 µm) were investigated. Second, various combinations of drying methods (hot-wind, vacuum, and freezing) and dehydration temperatures were compared, and the combination that afforded the best product were determined. The data thus obtained enabled us to set up conditions for the production of a fucoxanthin-rich powder of *C. okamuranus* that possessed good nutritional value. Third, the radical scavenging activity of an algal extract, fucoxanthin, and fucoxanthinol was assessed by using the DPPH assay. The extract showed a stronger DPPH radical scavenging activity than fucoxanthin and fucoxanthinol, probably because of the coexistent antioxidants such as polyphenols. We concluded that *C. okamuranus* has a great potential as a commercial-scale food material having beneficial effects on health.

### MATERIALS AND METHODS

**Determination of the optimum method for extraction:** Commercially available dry alga, *C. okamuranus*, was used to test the extraction efficiency of different solvents (acetone, ethanol, and methanol) and suitability of pulverized particle sizes (50, 200, and 1000 µm) for the

extraction of fucoxanthin. This experiment was conducted in June 2009. The 50 and 200  $\mu\text{m}$  powders were prepared using Supermasscolloider MKCA10-15J (MASUKO SANGYO), and the 1000  $\mu\text{m}$  powder was obtained using a cutter mill MKCM-5 (MASUKO SANGYO).

The algal powder (180 mg) was extracted with 30 mL of a solvent (acetone, ethanol, or methanol) at room temperature for 1 h on a shaker. After centrifugation (3500 rpm, 5 min, room temperature), the supernatant (1 mL) was transferred onto a small column (Wakogel 50C18, Wako; 300 mg), and fucoxanthin was eluted with MeOH (4 mL). The MeOH eluate was filtered through a 0.45  $\mu\text{m}$  membrane filter and used for HPLC analysis (Agilent HPLC, 1100 series). HPLC separations were performed on an ODS column (COSMOSIL 5C<sub>18</sub>-AR-II; 4.6 $\times$ 150 mm) under the following conditions: gradient elution was started with MeOH:H<sub>2</sub>O (9:1, 0 min) to MeOH (10 min); the flow rate was maintained at 0.4 mL/min; column oven temperature, 25°C; and injection volume, 10  $\mu\text{L}$ . Fucoxanthin was detected at 440 nm and quantified from the peak area by comparing with the standard curve obtained with pure fucoxanthin (Wako). All the experiments were conducted in triplicate.

**Determination of the optimum drying method:** Frozen *C. okamuranus* purchased from a fishery cooperative shop was defrosted, washed with tap water, and left on a sieve to drain the excess water. This experiment was conducted in July 2009. The following drying methods: hot-wind drying, vacuum drying, and freeze drying were compared. The temperature employed ranged from 40°C to 80°C. The duration of hot-wind drying was 24 h at 40°C, 16 h at 60°C, and 8 h at 80°C. The duration of vacuum drying was 46 h at 40°C, 25 h at 60°C, and 22 h at 80°C. Freeze drying was carried out for 112 h. The 50  $\mu\text{m}$  algal powder (180 mg) was extracted with 30 mL methanol at room temperature for 1 h on a shaker. Fucoxanthin contents were determined using an Agilent HPLC (1100 series), as described above.

**DPPH assay:** The DPPH radical scavenging activity was measured using a previously described method with slight modification (Duan *et al.*, 2006). This experiment was conducted in March 2010. In brief, 2 mL of the sample in methanol was mixed with 2 mL of 0.16 mM DPPH in methanol. The mixture was incubated at room temperature for 30 min in the dark. After incubation, the absorbance was measured at 517 nm (JASCO V-570). And the scavenging activity was showed using the follow equation as the percentage of remaining DPPH.

$$\text{Remaining DPPH (\%)} = [(A_{\text{sample}} - A_{\text{sample blank}})/A_{\text{control}}] \times 100$$

$A_{\text{control}}$  is the absorbance of the control (DPPH solution without the sample).  $A_{\text{sample}}$  is the absorbance of the sample (DPPH solution with the sample).  $A_{\text{sample blank}}$  is the absorbance of the sample solution without DPPH.

Fucoxanthin was isolated from *Undaria pinnatifida*, known as Wakame in Japan, as previously reported (Kadokaru *et al.*, 2008). Fucoxanthinol was prepared from fucoxanthin by the hydrolysis of fucoxanthin with the lipase derived from *Candida rugosa* (Sigma). First of all, 4 mL of an acetone solution containing 100 mg of fucoxanthin was mixed with 45 mL of a phosphate buffer solution (0.1 M, pH 7.0) containing 2 g lipase, and incubated at 37°C for 20 h. The solution was filtered (ADVANEK, filter paper No. 3), and the residue on the filter was eluted with 50 mL acetone. The acetone solution was allowed to evaporate, and the residue was dissolved in 4 mL acetone. The unhydrolyzed fucoxanthin in the eluate was again treated with the enzyme, filtered, eluted, evaporated, and dissolved with MeOH. Fucoxanthinol was further purified by HPLC. The purity of fucoxanthin and fucoxanthinol was determined by HPLC analysis and monitored at 440 nm.

## RESULTS AND DISCUSSION

**The optimum method for extraction:** To promote the commercial use of the fucoxanthin-rich powder of *C. okamuranus*, it is crucial to establish a rapid, simple, and inexpensive production method. First, the optimum method for the extraction of fucoxanthin from the powder of *C. Okamuranus* were investigated. The use of acetone, ethanol, and methanol for their efficiency to extract fucoxanthin from the dried powder of *C. Okamuranus* were compared. As shown in Table 1, methanol gave the best result, i.e., 270  $\mu\text{g}$  of fucoxanthin was extracted from 1 g powder. Acetone and ethanol were less efficient than methanol (226  $\mu\text{g}$ ). However, because ethanol is the best solvent for processing food from the viewpoint of safety, ethanol as an alternative to methanol would be recommended, although it is slightly more expensive. The fucoxanthin extractability was markedly enhanced as the powder size decreased. The best result was obtained with

Table 1: Comparative study of solvents (acetone, ethanol, and methanol) and pulverization methods (particle size: 50, 200, and 1000  $\mu\text{m}$ ).

Solvent	Size ( $\mu\text{m}$ )	Fucoxanthin ( $\mu\text{g/g}$ ) $\pm$ SD
EtOH (100%)	50	225.9 $\pm$ 6.4
	200	140.0 $\pm$ 7.0
	1000	39.6 $\pm$ 2.4
MeOH (100%)	50	270.0 $\pm$ 8.3
	200	185.5 $\pm$ 13.4
	1000	66.1 $\pm$ 8.1
Acetone (100%)	50	226.1 $\pm$ 16.4
	200	148.8 $\pm$ 3.8
	1000	46.5 $\pm$ 4.4

Each value is presented as mean  $\pm$  SD (n = 3)



Fig. 1: (a) *Cladosiphon okamuranus*; (b) Freeze-dried *C. okamuranus*; (c) The 50-µm powder of freeze dried *C. okamuranus*. All scale bars show 5 mm

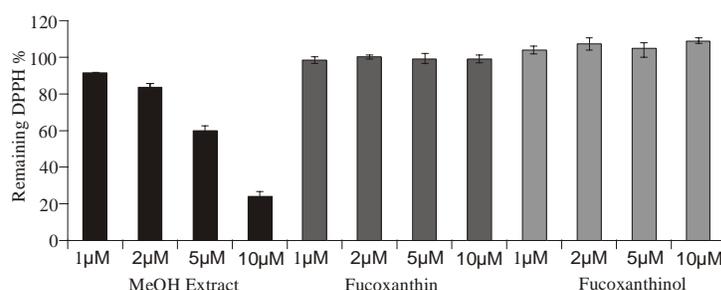


Fig. 2: DPPH radical scavenging activity of the *Cladosiphon okamuranus* extract, fucoxanthin (1-10 µM), and fucoxanthinol (1-10 µM) were showed as the percentage of remaining DPPH. Each extract was adjusted to contain 1-10 µM fucoxanthin

50 µm powder. However, finer powder is not preferable because of the longer time required for sieving and filtration of the extract.

**The optimum method for drying:** Second, the various combinations of drying methods (hot-wind drying, vacuum drying, and freeze drying) and temperatures were compared. Among the methods tested, freeze drying gave the highest fucoxanthin content (483.4 µg/g) (Fig. 1, Table 2). Air drying at an elevated temperature (80°C) offered the very low fucoxanthin content (132.9 µg/g) but produced a moderately good product at 40°C (322.6 µg/g). Comparable results were also obtained by vacuum drying at 40 and 60°C (330.9 and 329.3 µg/g, respectively). Although freeze drying was the best in preventing the decomposition of fucoxanthin, other methods, if used at an appropriate temperature, could be regarded as practical because of the lower cost and shorter time for production. The powdered product facilitates extraction of fucoxanthin at a low cost and by utilizing a simple process. Furthermore, the powder form is an appropriate ingredient to enrich nutritional value of other food, because the obtained powders would also have additional health benefits derived from the coexisting fucoidan, fucoxanthin, fibers, minerals, and antioxidants. The powdered product is expected to contribute to expand the utilization of *C. okamuranus* and thereby benefit the local fishery industry.

Table 2: Comparative study of drying methods (hot-wind drying, vacuum drying, and freeze drying) and dehydration temperatures (40-80°C) by using *Cladosiphon okamuranus*

Drying		Fucoxanthin (µg/g)±SD
Hot-wind drying	40°C	322.6±35.6
	60°C	234.6±34.1
	80°C	132.9±34.1
Vacuum drying	40°C	330.9±28.5
	60°C	329.3±27.2
	80°C	237.2±30.1
Freeze drying		483.4±8.6

Each value is presented as mean ± SD (n = 3)

**DPPH radical scavenging activity:** Many carotenoids have attracted attention because of their potential health benefits, and fucoxanthin is one of the latest topics of interest (Maeda *et al.*, 2008). Nevertheless, studies targeting fucoxanthin have been stalled because of the difficulties encountered in obtaining fucoxanthin of a high purity. Since fucoxanthin-rich freeze-dried powder and pure fucoxanthin were available, their DPPH radical scavenging activity was investigated (Fig. 2). The algal extract showed a strong DPPH radical scavenging activity, supposedly because of the presence of bioactive substances other than fucoxanthin (1-10 µM). In a previous study, the extract of *Hijikia fusiformis* showed a strong DPPH radical scavenging activity, and its major active compound was identified as fucoxanthin (Yan *et al.*, 1999). These results indicate the benefit of consuming the algal powder prepared in this study. Since

fishermen tend to overproduce *C. okamuranus*, utilization of this alga to produce powdered food material or for fucoxanthin extraction may contribute to the growth of the fishery industry.

### CONCLUSION

The 50 µm freeze-dried powder was found to be the best to produce powders enriched with stable fucoxanthin. In addition, the algal extract showed a strong DPPH radical scavenging activity. Furthermore, the powder form is an appropriate ingredient to enrich nutritional value of other food, because the obtained powders would also have additional health benefits derived from the coexisting fucoidan, fucosterol, fibers, minerals, and antioxidants. We concluded that *C. okamuranus* has a great potential as a commercial-scale food material having beneficial effects on health.

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