

# Research of Ultraviolet Radiation on Photosynthesis Vitamin D<sub>3</sub> Synthesis about Dry Juvenile Fish Fillet

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## Abstract

Growing evidence suggests that adequate levels of vitamin D are associated with superior health outcomes. Although vitamin D can be synthesized by exposure to ultraviolet radiation (UVR), UVR also has a detrimental effect on human health. Fish is the major natural source of vitamin D in the diet, but despite being a good source of vitamin D, considerable differences in vitamin D contents are observed among fish species. Dry juvenile fish fillet is a popular food, especially among children. However, environmental conditions, such as fish species, season, and habitat depth, affect the vitamin D content in dry juvenile fish. The present study shows that two light-filter solutions, Na<sub>2</sub>WO<sub>4</sub> and CoSO<sub>4</sub>/H<sub>2</sub>SO<sub>4</sub>, applied in one-step photosynthesis to change the UV wavelength from 280 nm to 320 nm can decrease harmful products and increase the vitamin D<sub>3</sub> content. Furthermore, the effects of different UV wavelengths, irradiation distances, irradiation times, fish species, fish maturities (adult and juvenile), and seasons on the vitamin D<sub>3</sub> content are investigated. The best conditions for vitamin D production were UV irradiation at 305 nm at a distance of 5 cm for 30 min using the two filter solutions. Photosynthesized vitamin D<sub>3</sub> levels were higher in spring than in autumn.

**Keywords:** Vitamin D<sub>3</sub>; Dry Juvenile Fish Fillet; Ultraviolet Radiation; Wavelength; Salangidae

## Introduction

Adequate vitamin D contents are associated with superior health outcomes [1,2]. Vitamin D deficiency is associated with increased risks of autoimmune disease, cardiovascular disease, cancer, osteoporosis, and type 1 diabetes [3-11]. Although vitamin D is synthesized in the skin by exposure to ultraviolet radiation (UVR), UVR has adverse effects on human health [12,13]. The World Health Organization (WHO) estimates that UVR exposure causes 12.8 million melanoma skin cancers, 30% of 8 million total cataracts, 60,000 premature deaths, and the loss of 1.5 million disability-adjusted life years worldwide annually [14,15].

Cheese, egg yolk, mushrooms, and fish are good sources of natural vitamin D [16]. Among them, fish is the major natural dietary source of vitamin D in many populations and generally possesses the higher levels of vitamin D [17-19], such as Lu, *et al.* [20] evaluated the vitamin D content in several species of fish. Surprisingly, farmed salmon had approximately 25% of the vitamin D content as wild salmon had. The vitamin D content in fish varied widely even within species. Lamberg-Allardt [16], reported good sources of vitamin D<sub>3</sub> are fish (not only fatty fish), salmon possess vitamin D content of 12.4 mg/100g. Although fish is a good source of vitamin D, there are considerable differences in vitamin D contents among fish species [13]. Other important factors are environmental conditions, such as fat content and season, especially in deep-sea fish, which receive little or no daylight. Therefore, more research is needed in this area.

Dry juvenile fish fillet is a popular food, especially among children. UVR light irradiation of dry juvenile fish fillet increases the vitamin D content in the fish body. However, environmental conditions, such as fish species, season, and habitat depth, affect the vitamin D content in dry juvenile fish. Little is known about the effect of UVR light irradiation conditions on the vitamin D content in fish. This study aimed to investigate changes in vitamin D levels in different dry juvenile fish fillet through UVR light irradiation. However, vitamin D has many photoisomers and harmful products can be produced during vitamin D synthesis, such as toxisterol, suprasterol, pyro vitamin D<sub>3</sub> and isopyro vitamin D<sub>3</sub> [21,22], depending on the UV radiation wavelength. Furthermore, the photo isomerization of vitamin D was performed using a dual wavelength filter to change the wavelength of a high-pressure mercury lamp from 280 to 320 nm for step photo isomerization with a special photochemical device (Figure 1 and 2), which decreased the contents of some harmful products and increased that of vitamin D<sub>3</sub> in dry juvenile fish fillet.



Salangidae

Half Mouth Sardine

Hemiculter Clupeoide

Figure 1: Photographs of salangidae, half mouth sardine, and hemiculter clupeoides fish species

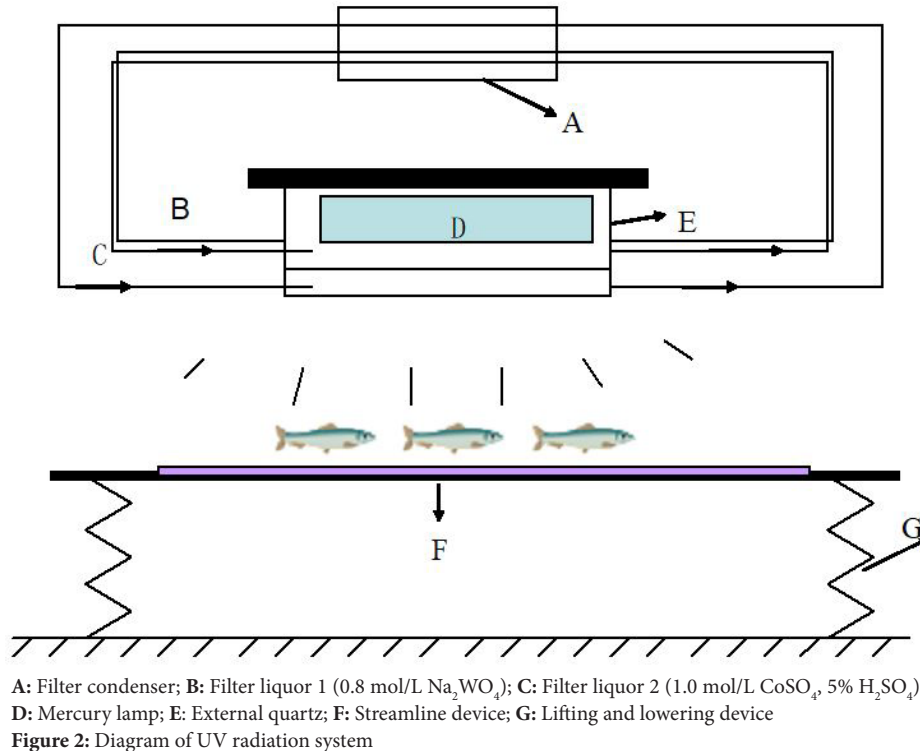


Figure 2: Diagram of UV radiation system

## Materials and Methods

### Materials

**Dry juvenile's fish fillet:** Salangidae, half mouth sardine, and hemiculter clupeoides were collected in Zhoushan market, Zhejiang Province (China) in spring and autumn. Pure vitamin  $\text{D}_3$  was purchased Sigma (Shanghai China). Major chemicals were purchased from Aldrich Chemical Corporation (Shanghai China) and were of the analytical grade. UV-lamp is slightly improved (ZF-4, made in Shanghai): wavelength, 254-365 nm; filter size, 200×80 mm; ultraviolet lamp tube, 6W×4 branches; ultraviolet Intensity, ultraviolet intensity of 20 MW/cm from sample.

### Isolation and purification of vitamin D in dry juvenile fish fillet

Vitamin D was extracted from dry juvenile fish fillets and isolated according to procedures described in a previous study [23]. Briefly, dry juvenile fish fillet (200 g) was irradiated with UVR for 10, 30, 60, or 90 min, using the dual wavelength filter (Filter liquor: 0.8 mol/L  $\text{Na}_2\text{WO}_4$  and 1.0 mol/L  $\text{CoSO}_4$ , 5%  $\text{H}_2\text{SO}_4$ ) to change the wavelength of the high pressure mercury lamp from 280 to 320 nm through step photo isomerization with a special photochemical device. The fillets were then crushed and extracted by refluxing with ethyl acetate for 2 h repeated three times. All ethyl acetate extracts were then combined, filtered, and concentrated under vacuum to afford oil. This oil was stored in a refrigerator at 4 °C.

### Saponification

Cod liver oil (2.0g), ascorbic acid (0.2g), anhydrous alcohol (50ml), and aqueous KOH (5ml; prepared by dissolving KOH (800 mg) in freshly boiled water (1000 ml) and cooling) were mixed in a round-bottomed flask and refluxed over a steam bath at 85 °C for 40 min. Aqueous NaCl solution (50ml, 5mg/ml) was then added, and the reaction mixture was cooled rapidly under running water. The saponified mixture was transferred to a 250 ml separatory funnel and the reaction flask was rinsed further with aqueous

NaCl solution (38 ml, 5 mg/ml) and ether/hexane (75ml, 1:1). The rinsing solutions were combined with the saponified mixture and shaken vigorously for 30 s. The mixture was allowed to stand until both layers were clear and separated, and the lower layer was discarded. The remaining ether/hexane extract layer was washed with alcoholic KOH solution (25 ml; prepared by dissolving KOH (1.5g) in freshly boiled water (25ml), adding ethanol (5ml), and diluting to 50 ml with more freshly boiled water) and three times with aqueous NaCl solution (25ml, 5mg/l). The upper layer was collected, filtered through anhydrous sodium sulfate (2.5g) using fast filter paper, washing the filter with further ether/hexane (1:1), into a 100ml flask suitable for a rotary evaporator. The solvent was evaporated under reduced pressure at a temperature not exceeding 30 °C and the flask was filled with nitrogen once evaporation was complete. Alternatively, the solvent was also evaporated under a gentle stream of nitrogen at a temperature not exceeding 30 °C. The resultant residue was dissolved in butylated hydroxytoluene (BHT) solution (1.5ml, 1 mg/ml in methanol).

### Preparation of Vitamin D<sub>3</sub> RP-HPLC and NP-HPLC

The saponified material was added to chromatography-grade methanol (3ml) and dissolved by ultra-sonication and centrifugation at 1200rpm for 5min. The vitamin D<sub>3</sub> content was analyzed by reverse phase (RP) and normal phase (NP) high-pressure liquid chromatography (HPLC) (LC-20A HPLC: Shimadzu, Japan). First, the saponified material was analyzed by RP-HPLC using a Venusil MP-C18 column (4.6µm, 250 × 4.6mm) and monitored at 280-320nm. The injection volume was 500µl. The mobile phase for RP-HPLC was methanol/acetonitrile/water (50:50:2) at a flow rate of 1.8ml/min at 25 °C. Fractions containing the purified material were combined and concentrated under vacuum to afford an oil. This oil was added to chromatography-grade hexane (3ml) and dissolved by ultra-sonication and centrifugation at 1,200 rpm for 5 min. NP-HPLC was then performed on 100-µl samples using a PrepHT XDB C18 column (250 mm × 21.2 mm). The mobile phase NP-HPLC for n-hexane/n-pentyl alcohol (997:3) had a flow rate of 15ml/min, with other conditions the same as those described for RP-HPLC.

### Results and Discussion

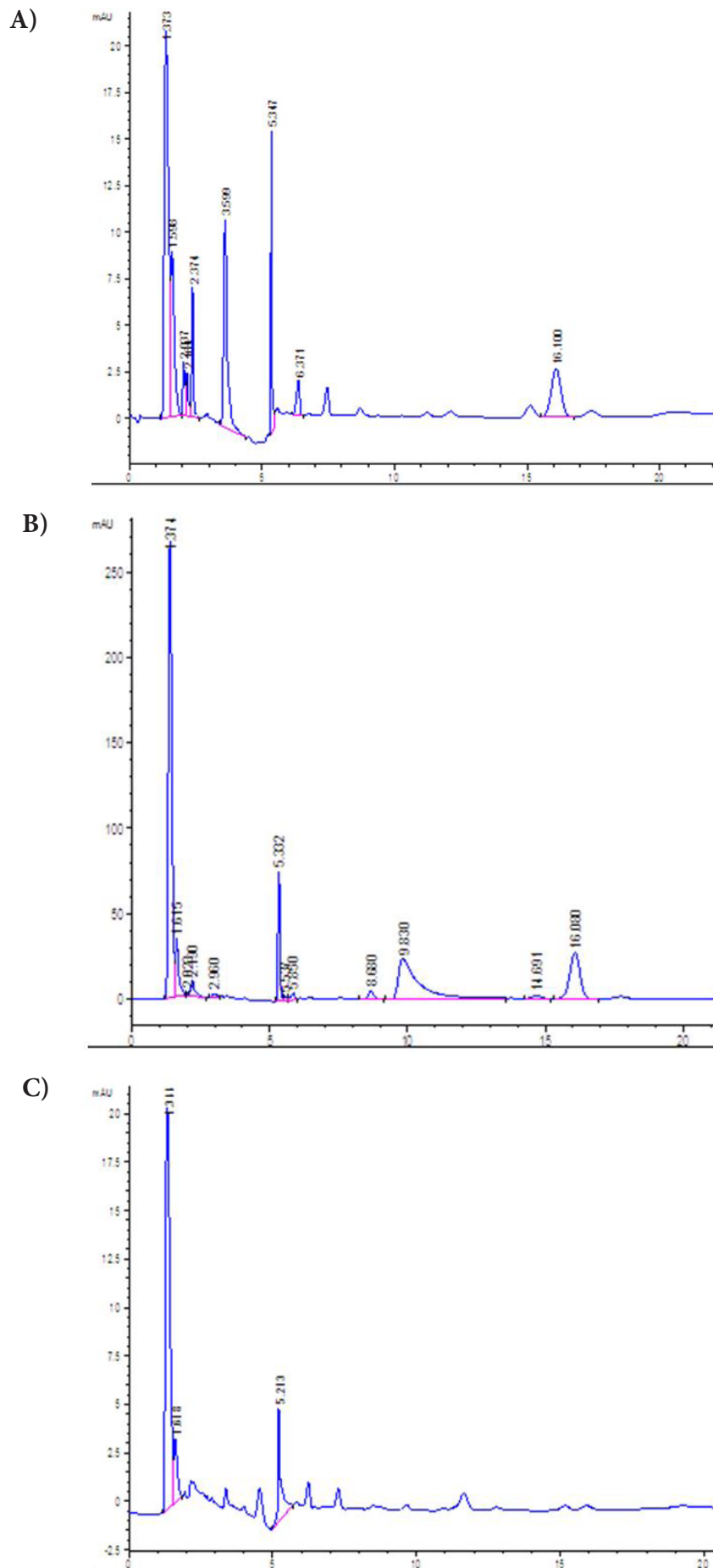
In recent work, vitamin D has been shown to be crucial in regulating the immune system [24]. Growing evidence suggests that adequate levels of vitamin D are associated with superior health outcomes. These include improved cardiovascular and bone health, lower incidence or activity of autoimmune diseases, reduced cancer incidence or mortality, and reduced all-cause mortality [25-28]. Vitamin D can be synthesized through UVR exposure, ingested in the diet, or provided through oral supplementation. Medical literature frequently states that humans obtain most of their vitamin D from sunshine and that UVR exposure is essential for maintaining vitamin D levels [29-31]. While some patterns of sunlight exposure have benefits (notably, reduced melanoma risk with intermediate lifetime sun exposure), there is strong evidence that overexposure to sunlight significantly increases the risk of cutaneous carcinogenesis and cataracts. The optimal dietary vitamin D intake remains undetermined and the acceptable level of exposure to UV-B radiation that would permit vitamin D synthesis without increasing the risk of UVR-related diseases is unconfirmed. Although a consensus statement from various societies suggests that suberythemal doses of midday UVR can help generate vitamin D, they are also known to have various effects on skin, including DNA damage and p<sup>53</sup> up regulation [32,33].

Vitamin D<sub>3</sub> is unstable and many photoisomers are produced during the synthesis of Vitamin D<sub>3</sub>, such as toxisterol, suprasterol, pyro vitamin D<sub>3</sub>, and isopyro vitamin D<sub>3</sub>. The production of these photoisomers depends on the UV wavelength, with the best UV wavelengths decreasing the harmful products and increasing the vitamin D<sub>3</sub> content. This study on the photo isomerization of vitamin D<sub>3</sub> was performed using a double filter to change the wavelength of a high-pressure mercury lamp from 280 to 320 nm in a special photochemical device for step photo isomerization (Figure 2). Two kinds of light filter solution, Na<sub>2</sub>WO<sub>4</sub> and CoSO<sub>4</sub> in 5% H<sub>2</sub>SO<sub>4</sub>, were adopted in the one-step photosynthesis to change the UV wavelength of the mercury lamp from 280 nm to 320 nm for the photosynthesis of vitamin D<sub>3</sub> [34]. The results showed that the vitamin D<sub>3</sub> content increased, while those of some harmful products decreased, when using the double filter (Na<sub>2</sub>WO<sub>4</sub> and CoSO<sub>4</sub> in 5% H<sub>2</sub>SO<sub>4</sub>) to change the UV wavelength (Table 1). Na<sub>2</sub>WO<sub>4</sub> and CoSO<sub>4</sub> are safe substances that can absorb ultraviolet light of certain wavelengths [35].

Material	Vitamin D <sub>3</sub>	Pre-vitamin D <sub>3</sub>	Tachysterol	Sterol
IU/10g	862	25	93	30

Table 1: Composition after photoperiod through the two filter solutions

Dry juvenile fish fillet is a popular food, especially among children. In this study, we investigated whether the vitamin D content of dry juvenile fish fillet was increased by UV light irradiation of the fish body. Notably, preliminary data on the fish species and environmental conditions indicated that different living conditions affected the vitamin D content. This method showed great potential for improving the vitamin D content, which is more pronounced after UV light irradiation. Vitamin D<sub>3</sub> levels in salangidae after UV light irradiation were analyzed by HPLC (Figure 3). Standard substances of Vitamin D<sub>3</sub> were ethylated according to the sample preparation procedure described above prior to injection into the HPLC system. One main peak was observed in the HPLC spectrum at 16.100 min (Figure 3A), which was also observed at 16.080 min in the saponified oil (Figure 3B). However, little vitamin D<sub>3</sub> was observed in salangidae without UV light irradiation (Figure 3C).



**Figure 3:** HPLC of vitamin D<sub>3</sub> levels of salangidae through UVR light irradiation. (A) vitamin D<sub>3</sub> standard substance; (B) vitamin D<sub>3</sub> levels through UVR light irradiation; (C) vitamin D<sub>3</sub> levels without through UVR light irradiation



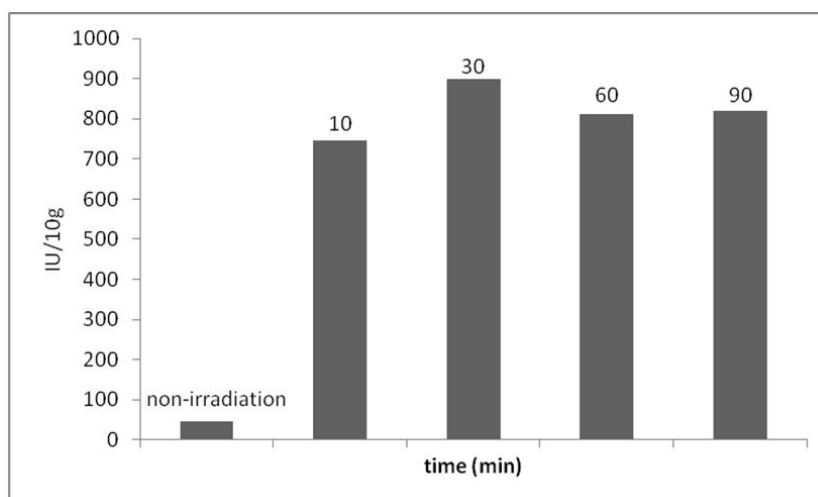
Recommendations exist for supplementing vitamin D in pregnant women, infants, and children up to 5 years old [36]. Furthermore, in population groups not exposed to sunlight during winter months, vitamin D levels can become very low during winter and early spring, and vitamin D supplements may be taken. The consumption of fish has long been known to be healthy. Although fish is generally a good source of vitamin D, and the major natural dietary source of vitamin D in many populations, there are considerable differences in vitamin D contents among fish species. Lean fish, mostly cod, does not increase vitamin D levels significantly, although it should be noted that the differentiation between lean and fatty may be arbitrary in some species, which could also be classified as medium-fatty fish. Other important factors could be environmental conditions, season, and habitat depth, with deep-sea fish receiving little or no light, resulting in very low vitamin D concentrations. Previous studies have shown that there is a huge potential for improving the vitamin D content, which is more pronounced after postmortem irradiation than after feeding. However, both technologies should be developed further, because the absolute amounts of vitamin D in the treated fish were still relatively low [37].

The synthesis of vitamin D<sub>3</sub> depends on incident UV-B radiation, which decreases the harmful products and increases the vitamin D<sub>3</sub> content in dry juvenile fish fillet. The impact of different UV wavelengths was investigated (Table 2), with vitamin D<sub>3</sub> contents of 834 IU/10g, 926 IU/10g, 845 IU/10g, and 828 IU/10g obtained using wavelengths of 285 nm, 305 nm, 315 nm, and 320 nm, respectively. Therefore, a wavelength of 305 nm produced the highest photosynthesized vitamin D<sub>3</sub> content of 67.4%. It may be when using the double filter (Na<sub>2</sub>WO<sub>4</sub> and CoSO<sub>4</sub> in 5% H<sub>2</sub>SO<sub>4</sub>) to change the UV wavelength. Na<sub>2</sub>WO<sub>4</sub> and CoSO<sub>4</sub> are safe substances that can absorb ultraviolet light of certain wavelengths.

Wavelength(nm)	IU/10g			
	Vitamin D <sub>3</sub>	Pre-vitamin D <sub>3</sub>	Tachysterol	Sterol
285	834	34	84	48
305	926	10	47	17
315	845	29	83	43
320	828	34	91	47

**Table 2:** The different UV-wavelength on two kinds of filter liquid

Next, we tested the effect vitamin D<sub>3</sub> contents in the different time. The dry juvenile salangidae were irradiated with UV light at 305 nm for 10, 30, 60, and 90 min using the two filter solutions. Different UV-B irradiation times influenced the photosynthesized vitamin D<sub>3</sub> contents, affording 746 IU/10g, 889 IU/10g, 811 IU/10g, and 819 IU/10g contents, respectively. The present study clearly showed that the vitamin D content increased with increasing UVR treatment time, with the best vitamin D content obtained after irradiation for 30 min (Figure 4). Little further change in the vitamin D content was observed with extended irradiation time. Murphy, *et al.* [38] studied children playing on open sunny ground at a school for 30 min (11.15–11.45 am) every day for six days in a week, with a total of 160 children invited to participate in the study, of which parents of 85 children (41 boys, 44 girls) gave their written consent and 71 children (boys 36, girls 35) completed the study. The results showed that sun exposure caused a significant increase in 25-Hydroxyvitamin D levels. However, there is strong evidence that individuals overexposed to sunlight carry a significantly increased risk of cutaneous carcinogenesis and cataracts.



**Figure 4:** Impact of the photosynthesis vitamin D<sub>3</sub> for different radiation times

Dry juvenile salangidae were irradiated at 305 nm with the mercury lamp at different distances from the fish of 5, 8, 10, and 15 cm for 30 min using the two filter solutions. The resultant photosynthesized vitamin D<sub>3</sub> contents were 934 IU/10g, 831 IU/10g, 763 IU/10g, and 700 IU/10g, respectively. Therefore, the best irradiation distance was 5 cm. The irradiation distance also affected vitamin D photosynthesis. Dry juvenile salangidae was irradiated with a mercury lamp at 305 nm from distances of 5, 8, 10, and 15 cm for 30 min using the two filter solutions. The results showed that the highest photosynthesized vitamin D<sub>3</sub> content was obtained with a distance of 5cm (Figure 5).

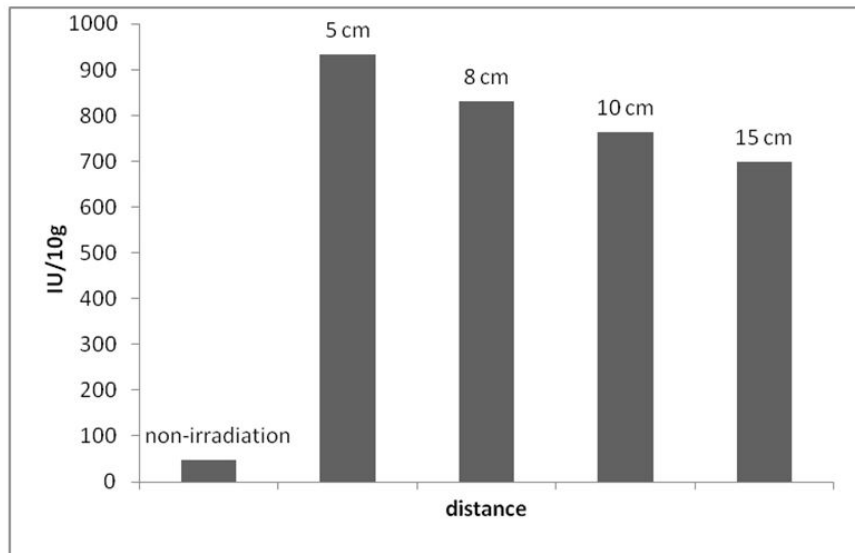


Figure 5: Impact of the photosynthesis vitamin D<sub>3</sub> for different radiation distances

The vitamin D content of dry juvenile salangidae after UV light irradiation was different depending on the growing season, with photosynthesized vitamin D<sub>3</sub> contents higher in spring (901 IU/10g) than in autumn (851 IU/10g). Dry juvenile salangidae were irradiated at 305 nm in spring and autumn purchased at a distance of 5 cm for 30 min using the two filter solutions. The results showed that vitamin D<sub>3</sub> levels in the fish were higher in spring than in autumn, with photosynthesized vitamin D<sub>3</sub> contents of were 901 IU/10g and 851 IU/10g, respectively (Figure 6).

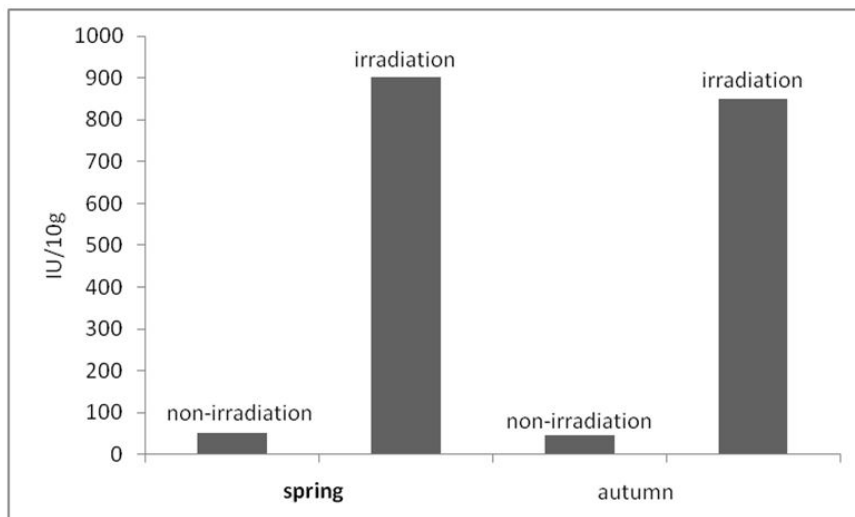


Figure 6: Different seasonal variation in photosynthesis vitamin D<sub>3</sub>

Knowledge of the variation in vitamin D contents in dry juvenile fish fillet is limited. It may be assumed that, even within a given fish species, the vitamin D content varies widely depending on growth, feed, and other factors, such as habitat depth and season. Figure 7 shows that the photosynthesized vitamin D<sub>3</sub> contents were higher in salangidae than in half mouth sardine and hemiculter clupeoides species analyzed in this study, with vitamin D<sub>3</sub> contents of 903 IU/10g, 312 IU/10g, and 286 IU/10g, respectively. This may be because salangidae is a white-skinned fish. This suggested that skin pigmentation negatively influenced vitamin D synthesis. Libon *et al.* [39], investigation into whether dark skin produces less vitamin D under UV-B irradiation than fair skin remains controversial. The results showed that all volunteers were severely vitamin D deficient. On day 2, the 25-Hydroxyvitamin D levels of fair-skinned volunteers increased significantly (median: 11.9–13.3 ng/mL,  $p < 0.0001$ ), but not in dark-skinned people (median: 8.60–8.55 ng/mL,  $p = 0.843$ ). On day 6, the 25-(OH)-D levels of fair-skinned volunteers had again increased significantly (median: 11.9–14.3 ng/ml,  $p < 0.0001$ ), but had not in dark-skinned people (median: 8.60–9.57 ng/mL,  $p = 0.375$ ). This study suggested that skin pigmentation negatively influenced vitamin D synthesis. Murphy, *et al.* [38] also studied predictors of serum vitamin D levels in African American and European American men in Chicago, with the results showing that black skin, blood sampling during a cold season, elevated body mass index, and lack of vitamin D supplementation increased the risk of vitamin D deficiency. Supplementation is a high-impact, modifiable risk factor. Skin color and sunlight exposure should be taken into account in recommended daily allowances for vitamin D intake.

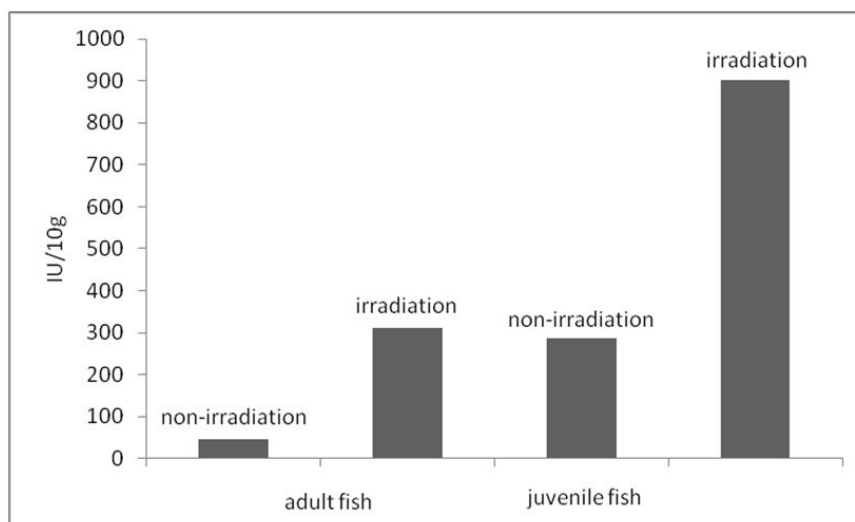


Figure 7: Vitamin D<sub>3</sub> concentration on adult fish and juvenile fish

In an eight-part study, vitamin D contents in different types of juvenile fish have been investigated after exposure to UV-B radiation. The study showed the huge potential of UV irradiation treatment for increasing vitamin D contents, which was more pronounced in salangidae than in half mouth sardine and hemiculter clupeoides species. However, both technologies should be developed further because the absolute amounts of vitamin D in the treated fish were still relatively low. In this respect, it is interesting to note that preliminary data on freshwater fish species have also indicated the effect of different living conditions on vitamin D content [40]. Dry juvenile salangidae, half mouth sardine, and hemiculter clupeoides were irradiated at 305 nm at a distance of 5 cm for 30 min using the two filter solutions. The results showed that vitamin D<sub>3</sub> levels were higher in salangidae than those in half mouth sardine and hemiculter clupeoides, with photosynthesized vitamin D<sub>3</sub> contents of 910 IU/10g, 851 IU/10g, and 632 IU/10g, respectively (Figure 8).

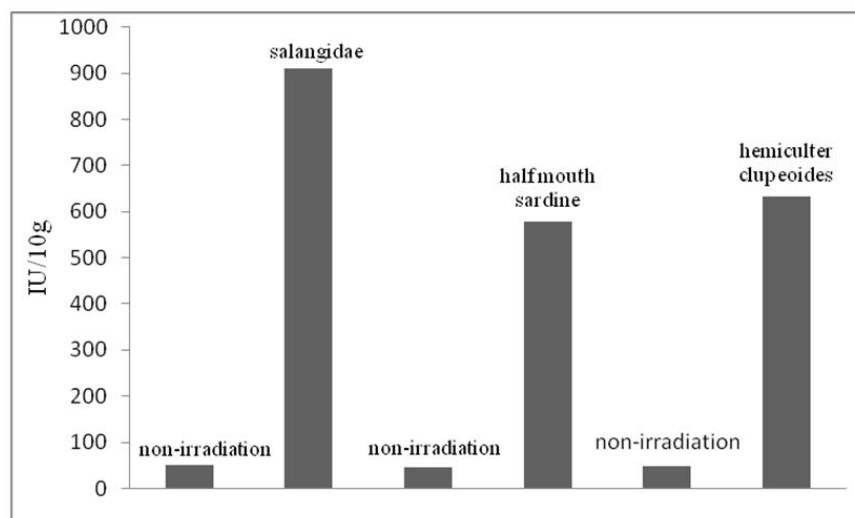


Figure 8: Vitamin D<sub>3</sub> concentration

## Conclusion

The current study has demonstrated that two light-filter solutions, Na<sub>2</sub>WO<sub>4</sub> and CoSO<sub>4</sub>/H<sub>2</sub>SO<sub>4</sub>, can be adopted in one-step photosynthesis to change the UV wavelength from 280 nm to 320 nm, which can decrease harmful products and increase the vitamin D<sub>3</sub> content. Furthermore, the effects of different UV wavelengths, irradiation distances, irradiation times, fish species, fish maturities (adult and juvenile), and seasons were discussed in this paper. The best conditions for the production of vitamin D were irradiation at 305 nm at a distance of 5 cm for 30 min using the two filter solutions. Photosynthesized vitamin D<sub>3</sub> contents were higher in spring than in autumn.

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