Nguyen Phuoc Minh et al /J. Pharm. Sci. & Res. Vol. 11(3), 2019, 925-929

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Green Pigment Extraction from Pandan (*Pandanus Amaryllifolius)* and its Application in Food Industry

Nguyen Phuoc Minh[1,*], Tan Thanh Vo[2], Le Van Man[3], Ta Dinh Phong[4], Nguyen Van Toan[5], Vo Hoang Nam[6]

[1]*Faculty of Chemical Engineering and Food Technology, Nguyen Tat Thanh University, Ho Chi Minh, Vietnam*
[2]*NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam*
[3]*Can Tho University, Can Tho City, Vietnam*
[4]*Soc Trang Community College, Soc Trang Province, Vietnam*
[5]*Can Tho University, Can Tho City, Vietnam*
[6]*Dong Thap University, Dong Thap Province, Vietnam*

Abstract.

Leaves of *Pandanus amaryllifolius* are used as natural colourant due to presence of high chlorophyll content. Chlorophyll can replace synthetic dyes which may affect health. Chlorophyll can be extracted from pandan leaves. Different aspects affecting to chlorophyll extraction were examined including blanching, solvent, solvent: solid, extraction time and temperature under ultrasound, carrier and temperature for drying. Optimal results were clearly shown that the panda leaves should be blanched under 95^{o}C in 10 seconds; extraction should be conducted by ethanol 90%, ethanol: solid (2.0:1.0), 60^{o}C, 8 minutes; vaccum drying should be executed at 60^{o}C with the support of carrier (0.5 maltodextrin: 0.5 gelatin: 1.0 pandan fluid). Ultrasonic had positive effect on chlorophyll extraction and this method could be utilized to optain the most chlorophyll yield.

Keywords: *Pandan, chlorophyll, ultrasonic, blanching, solvent, extraction, carrier, drying*

I. INTRODUCTION

The leaf of *Pandanus amaryllifolius* Roxb., commonly known as pandan, is often used to give a refreshing, fragrant flavor (Ampa Jimtaisong and Panvipa Krisdaphong, 2012). Besides its culinary value, pandan leaves are used in the perfume industry and also medicinally as a diuretic, cardio-tonic and anti-diabetic (Wakte KV et al., 2010). The diuretic effect of the leaves has been reported by Ysrael et al. (1995). The leaves are used to refresh the body, reduce fever, and relieve indigestion. Pandanus contains phytochemicals like steroids, carbohydrates, phenols, isoflavones, coumestrol, lignans, alkaloids, glycosides, amino acids and vitamins, terpenoids, flavonoids, saponins. Chief characters of transverse section of leaves include sclerenchymatous patches, parenchymatous mesophyll cells, bundle sheath cells (Bindu Gopalkrishnan et al., 2015). Significant *p*harmacological activities reported by scientists are anti-microbial, anti-diabetic, anti-viral, anti neoplastic, anti-oxidant, anti-diuretic, analgesic and neuroprotective (Singh Gurmeet, Parle Amrita, 2015). Faras A. F et al., (2014) proved the effect of leaf extract of *Pandanus amaryllifolius* (Roxb.) on growth of *Escherichia coli* and *Micrococcus* (*Staphylococcus*) *aureus.* The leaves are to be used preferably fresh but can also be used in dried form (Wongpornchai et al., 2003). Pandanus leaves is rich in terpenes and sesquiterpene hydrocarbons (Yoshihashi, 2002). Beside the aromatic compound, the leaves also have been reported to contain maltodextrin (Chaiseri and Cheetangdee, 2006). Mahmoud A. M. Al-Alwani et al. (2017) examined natural dye extracted from *Pandannus amaryllifolius* leaves as sensitizer in fabrication of dye-sensitized solar cells.

Natural colorants from plant-based materials have gained increasing popularity due to health consciousness of consumers. Among the many steps involved in the production of natural colorants, pigment extraction is one of the most important. Soxhlet extraction, maceration, and hydrodistillation are conventional methods that have been widely used in industry and laboratory for such a purpose. Recently, various non-conventional methods, such as supercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction, ultrasound-assisted extraction, pulsed-electric field extraction, and enzyme-assisted extraction have emerged as alternatives to conventional methods due to the advantages of the former in terms of smaller solvent consumption, shorter extraction time, and more environment-friendliness. Prior to the extraction step, pretreatment of plant materials to enhance the stability of natural pigments is another important step that must be carefully taken care of (Luxsika Ngamwonglumlert et al., 2017).

Chlorophylls are the major light-harvesting green pigments in photosynthetic organisms, including green plants, algae and bacteria. Stability of chlorophylls in foods has also been studied, widely. Heat and acidity are two major process conditions that enhance chlorophyll degradation (Schanderl et al., 1962; Schwartz et al., 1981; LaBorde & von Elbe, 1994). In the present study, we examined different parameters influencing to chlorophyll extraction such as blanching, solvent, solvent: solid, extraction time and temperature under ultrasound, carrier and temperature for drying.

II. MATERIALS AND METHOD

2.1 Material

We collected Pandan leaves in Mekong river delta, Vietnam. They must be cultivated following VietGAP to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. They were washed thoroughly under turbulent washing to remove dirt, dust and adhered unwanted material. Besides

Pandan leaves we also used other materials during the research such as ethanol, acetone, ethyl acetate, maltodextrin, gelatin, DMSO. Lab utensils and equipments included oven, ultrasonicator, vaccum dryer, centrifugator, water bath.

Figure 1. Pandan (*Pandanus amaryllifolius)*

2.2 Researching procedure

2.2.1 Effect of blanching on chlorophyll recovery (%)

Different blanching conditions were performed (100^{o}C, 5 sconds; 95^{o}C, 10 seconds; 90^{o}C, 15 seconds; 85^{o}C, 20 seconds) to verify the effectiveness (% recovery) of blanching to chlorophyl extraction.

2.2.2 Effect of different solvents on chlorophyll recovery (%)

Different solvents (water, ethanol, acetone, ethyl acetate) were used to examine the effectiveness (% recovery) of chlorophyl extraction.

2.2.3 Effect of solvent ratio: material on chlorophyll recovery (%)

Different ratios of solvent: solid (1.0:1.0, 1.5:1.0; 2.0:1.0; 2.5:1.0; 3.0:1.0) were verified to demonstrate the effect of ratio between solvent and material on the effectiveness (% recovery) of chlorophyl extraction.

2.2.4 Effect of extraction temperature by ultrasonic combination with solvent on chlorophyll recovery (%)

Different temperature conditions (30^{o}C, 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C) under ultrasonic (37 kHz) combined solvent were examined to prove the effectiveness (% recovery) of chlorophyl extraction.

2.2.5 Effect of extraction interval by ultrasonic combination with solvent on chlorophyll recovery (%)

Different time intervals of ultrasonic treatment (2 minutes, 4 minutes, 6 minutes, 8 minutes, 10 minutes) were investigated to verify the effectiveness (% recovery) of chlorophyl extraction.

2.2.6 Effect of ratio of carrier for drying

Different ratios of carrier: pandan (1 maltodextrin: 1 pandan; 1 gelatin: 1 pandan; 0.5 maltodextrin: 0.5 gelatin: 1 pandan) were demonstrated to show the effectiveness of drying to chlorophyl recovery (%).

2.2.7 Effect of drying temperature

Vaccum drying was applied at different temperature (50^{o}C, 55^{o}C, 60^{o}C, 65^{o}C, 70^{o}C) to the final moisture content of dried powder 6 ± 1%.

2.3 Physico-chemical and biological analysis

Chlorophyl (µg/g) was performed by Nianwei Qiu et al., (2018). The ground panda samples in water were collected by centrifugation at 5000 *g* at 4 °C for 15 min. The precipitated panda were suspended with 2 mL DMSO. Then the sample was transferred to a 15 mL centrifuge tube, and the tube was incubated at 65 °C for 1–2 h in the dark until the sample turned white. After cooling, the chlorophyll extract was diluted with 8 mL 90% acetone, before centrifugation at 5000 *g* for 5 min. The absorbance values of the supernatants at 750, 664, 647 and 630 nm were used for the calculation of chlorophyll concentrations by the trichromatic equations.

2.4 Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT). Statistical analysis was performed by the Statgraphics Centurion XVI.

III. RESULT & DISCUSSION

3.1 Phytochemical composition in Pandan (*Pandanus amaryllifolius)*

Phytochemical composition (chlorophyll) in fresh Pandan leaves was analyzed. Result was clearly depicted in table 1 representing that Pandan leaves were suitable for utilization to collect this healthy pigment.

Table 1. Phytochemical composition in Pandan (*Pandanus amaryllifolius)*

Material	Pandan (*Pandanus amaryllifolius)*
Major composition	Chlorophyl (7,493.2 mg/kg)

3.2

3.3 Effect of blanching chlorophyll extraction recovery (%)

Different blanching conditions were performed (100^{o}C, 5 seconds; 95^{o}C, 10 seconds; 90^{o}C, 15 seconds; 85^{o}C, 20 seconds) to verify the effectiveness (% recovery) of blanching to chlorophyll extraction. Results were depicted in table 2.

Table 2. Effect of blanching to chlorophyll extraction recovery (%)

Blanching	100^{o}C, 5 seconds	95^{o}C, 10 seconds	90^{o}C, 15 seconds	85^{o}C, 20 seconds
Chlorophyll extraction recovery (%)	72.16÷0.03^b	74.29÷0.01^a	68.18÷0.04^c	65.31÷0.01^d

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 3. Effect of different solvents on chlorophyll extraction recovery (%)

Solvent	Water	Ethanol 90%	Acetone	Ethyl acetate
Chlorophyll extraction recovery (%)	43.01÷0.04^d	78.39÷0.01^a	74.04÷0.02^c	75.92÷0.01^b

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 4. Effect of solvent ratio: material on chlorophyll extraction recovery (%)

Solvent: solid	1.0: 1.0	1.5: 1.0	2.0: 1.0	2.5: 1.0	3.0: 1.0
Chlorophyll extraction recovery (%)	65.04÷0.01^{e}	67.40÷0.03^{d}	80.19÷0.02^{a}	78.23÷0.02^{b}	70.48÷0.01^{c}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Haecheon Ahn, Eunok Choe (2015) examined the effects of blanching and drying on pigments and antioxidants of daraesoon. Blanching caused a significant (p<0.05) increase in the total chlorophyll content to 4,629.8 mg/kg from 2,932.5 mg/kg. Nartnampong, A. et al., (2016) demonstrated that 30 s blanching better preserved and also promoted more nutrients and antioxidant properties in green leafy vegetables than steaming. C. Severini et al., (2016) studied the influence of different blanching methods on colour, ascorbic acid and phenolics content of broccoli.

3.4 Effect of different solvents on chlorophyll extraction recovery (%)

Different solvents (water, ethanol 90%, acetone, ethyl acetate) were used to examine the effectiveness (% recovery) of chlorophyll extraction. Results were elaborated in table 3.

Norbert Wasmund et al., (2006) demonstrated that the extraction efficiency was much better in 96% ethanol than in 90% acetone – extraction. Meanwhile, Nadia R.A. El-Mouhty, Ashraf Yehia El-Naggar (2014) found that the suitable solvent of extraction was 70% ethanol.

3.4 Effect of solvent ratio: material on chlorophyll extraction recovery (%)

Different ratios of solvent: solid (1.0:1.0, 1.5:1.0; 2.0:1.0; 2.5:1.0; 3.0:1.0) were verified to demonstrate the effect of ratio between solvent and material on the effectiveness (% recovery) of chlorophyl extraction. Results were elaborated in table 4.

Zurina Zainal Abidin et al., (2016) found that at 80 °C in 90 minutes with 2:5 solid to solvent ratio was the most favorable condition to extract chlorophyll a and b.

3.5 Effect of extraction temperature by ultrasonic combination with solvent on chlorophyll recovery (%)

Different temperature conditions (30^{o}C, 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C) under ultrasonic (37 kHz) combined solvent were examined to prove the effectiveness (% recovery) of chlorophyl extraction. Results were elaborated in table 5.

Meilana Dharma Putra et a., (2017) examined the effects of temperature, 30–70 °C and agitation speed, 100–400 rpm on chlorophyll extraction from pandan leaves, using ethanol. The optimal conditions of extraction was obtained at 60 °C and 300 rpm; the chlorophyll concentration was 107.1 mg L^{-1}

Table 5. Effect of extraction temperature by ultrasonic combination with solvent on chlorophyll recovery (%)

Extraction temperature (^{o}C)	30^{o}C	40^{o}C	50^{o}C	60^{o}C	70^{o}C
Chlorophyll extraction recovery (%)	68.36÷0.02^{e}	73.19÷0.01^{d}	77.36÷0.02^{c}	82.39÷0.01^{a}	80.08÷0.03^{b}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 6. Effect of extraction interval by ultrasonic combination with solvent on chlorophyll recovery (%)

Extraction time (minutes)	2	4	6	8	10
Chlorophyll extraction recovery (%)	73.08÷0.01^{d}	77.29÷0.03^{c}	80.24÷0.04^{b}	85.48÷0.01^{a}	85.79÷0.01^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 7. Effect of ratio of carrier for drying

Carrier: Pandan	1 maltodextrin: 1 pandan	1 gelatin: 1 pandan	0.5 maltodextrin: 0.5 gelatin: 1 pandan
Chlorophyll drying recovery (%)	80.29÷0.02^{c}	83.17÷0.03^{b}	88.73÷0.01^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 8. Effect of drying temperature on chlorophyll recovery (%)

Drying temperature (^{o}C)	50	55	60	65	70
Chlorophyll drying recovery (%)	84.22÷0.01^{d}	87.45÷0.01^{b}	90.01÷0.02^{a}	86.29÷0.01^{c}	83.12÷0.03^{e}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

3.6 Effect of extraction interval by ultrasonic combination with solvent on chlorophyll recovery (%)

Different time intervals of ultrasonic treatment (2 minutes, 4 minutes, 6 minutes, 8 minutes, 10 minutes) were investigated to verify the effectiveness (% recovery) of chlorophyl extraction. Results were elaborated in table 6.

Woon Yong Choi and Hyeon Yong Lee (2018) obtained the optimal ultrasonification extraction conditions of 20.52 kHz for the frequency, 32.59 °C for the temperature, and 4.91 h for the process time, 17.98 mg/g of chlorophyll. It was much higher than 13.81 mg/g from conventional 70% ethanol extraction and even higher than other data from *Spirulina.*

3.7 Effect of ratio of carrier for drying on chlorophyll recovery (%)

Different ratios of carrier: Pandan (1 maltodextrin: 1 pandan; 1 gelatin: 1 pandan; 0.5 maltodextrin: 0.5 gelatin: 1 pandan) were demonstrated to show the effectiveness of drying to chlorophyl recovery (%). Results were elaborated in table 7.

Porrarud, S. and Pranee, A. (2010) produced microencapsulated compound of Zn-chlorophyll derivatives extracted from pandan leaf by using spray dried method. Three different wall material types, gum arabic (GA), maltodextrin (MD) and osa-modifed starch (MS), were studied based on their physicochemical properties and stabilities of the encapsulated powder. Results showed that MS powder was spherical and smooth, whereas GA and MD powders exhibited shrinkage on the surface.

3.8 Effect of drying temperature on chlorophyll recovery (%)

Vaccum drying was applied at different temperature (50^{o}C, 55^{o}C, 60^{o}C, 65^{o}C, 70^{o}C) to the final moisture content of dried powder 6 ± 1%. Results were elaborated in table 8.

Haecheon Ahn, Eunok Choe (2015) examined the effects of blanching and drying on pigments and antioxidants of daraesoon. Drying of blanched *daraesoon* caused a significant (p<0.05) loss of chlorophylls and carotenoids, and carotenoids were more affected by light than chlorophylls.

IV. CONCLUSION

Pandanus plants revealed its huge biological potential. The plant had multifaceted medicinal properties like anti-convulsant, anti-oxidant, anti-viral, anti-diabetic, antiinflammatory, neuroprotective, analgesic, anti-neoplastic, anti-microbial, anti-diuretic. It is used to treat nocturnal enuresis. The oil yielded from the pandanus plant is used to treat headaches, earache and rheumatic pains. The plant contains lignans, is of lavones, phenolic compounds, steroids, saponins, terpenoids, glycosides, tannins, flavonoids in the extract. We have successfully optimized different aspects affecting to chlorophyll extraction such as blanching, solvent, solvent: solid, extraction time and temperature under ultrasound, carrier and temperature for drying. Natural chlorophyll colorant has the potential to be used as acceptable additive in foods as natural and potential health benefits.

REFERENCES

1. Ampa Jimtaisong and Panvipa Krisdaphong (2012). Antioxidant activity of *pandanus amaryllifolius* leaf and root extract and its application in topical emulsion**.** *Tropical Journal of Pharmaceutical Research June* 12 (3): 425-431.
2. Bindu Gopalkrishnan, Seema Agashe, Urmila Kumavat (2015). Pharmacognostical screening of flavouring leaves *Pandanus amaryllifolius* Rox. *International Journal of Pharmacognosy and Phytochemical Research* 7(4): 745-749.
3. Chaiseri, S. and Cheetangdee, V. (2006). Free amino acid and reducing sugar composition of pandan (*Pandanus amaryllifolius*) leaves. *Kasetsart Journal: Natural Science* 40(2): 67-74.
4. Faras A. F., Wadkar S. S. and Ghosh J.S (2014). Effect of leaf extract of *Pandanus amaryllifolius* (Roxb.) on growth of *Escherichia coli* and *Micrococcus* (*Staphylococcus*) *aureus. International Food Research Journal* 21(1): 421-423.
5. Haecheon Ahn, Eunok Choe (2015). Effects of blanching and drying on pigments and antioxidants of daraesoon (shoot of the Siberian gooseberry tree, *Actinidia arguta* Planchon). *Food Science and Biotechnology* 24(4): 1265-1270.
6. LaBorde, L.F. & von Elbe, J.H. (1994). Chlorophyll degradation and zinc complex formation with chlorophyll derivatives in heated green vegetables. *Journal of Agricultural and Food Chemistry* 42(5): 1100–1103.
7. Luxsika Ngamwonglumlert, Sakamon Devahastin & Naphaporn Chiewchan (2017). Natural colorants: Pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods. *Food Science and Nutrition* 57(15): 1-8.
8. Mahmoud A. M. Al-Alwani, Abu Bakar Mohamad, Abd Amir H. Kadhum, Norasikin A. Ludin , N. E. Safie, M. Z. Razali, M. Ismail, Kamaruzzaman Sopian (2017). Natural dye extracted from *Pandannus amaryllifolius* leaves as sensitizer in fabrication of dye-sensitized solar cells. *Int. J. Electrochem. Sci.,* 12: 747 – 761.
9. Meilana Dharma Putra, Agus Darmawan, Ilham Wahdini, Ahmed E. Abasaeed (2017). Extraction of chlorophyll from pandan leaves using ethanol and mass transfer study. *Journal of the Serbian Chemical Society* 82(7): 921-931.
10. Nadia R.A. El-Mouhty, Ashraf Yehia El-Naggar (2014). Extraction of chlorophyll and carotene from irradiated parsley plant. *International Journal of Innovative Research in Science, Engineering and Technology* 3(1): 8522-8527.
11. Nartnampong, A., Kittiwongsunthon, W. and Porasuphatana, S. (2016). Blanching process increases health promoting phytochemicals in green leafy Thai vegetables. *International Food Research Journal* 23(6): 2426-2435.
12. Nianwei Qiu, Xiushun Wang, Feng Zhou (2018). A new method for fast extraction and determination of chlorophylls in natural water. A new method for fast extraction and determination of chlorophylls in natural water. *Z Naturforsch C.* 73(1-2): 77-86.
13. Norbert Wasmund, Ina Topp, Dirk Schories (2006). Optimising the storage and extraction of chlorophyll samples. *Oceanologia* 48(1): 125–144.
14. Porrarud, S. and Pranee, A. (2010). Microencapsulation of Zn-chlorophyll pigment from Pandan leaf by spray drying and its characteristic. *International Food Research Journal* 17: 1031-1042.
15. Schanderl, S.H., Chichester, C.O. & Marsh, G.L. (1962) Degradation of chlorophyll and several derivatives in acid solution. *Journal of Organic Chemistry* 27: 3865–3868.
16. Schwartz, S.J., Woo, S.L. & Von Elbe, J.H. (1981). High-performance liquid chromatography of chlorophylls and their derivatives in fresh and processed spinach. *Journal of Agricultural and Food Chemistry* 29(3): 533– 535.
17. C. Severini, R. Giuliani, A. De Filippis, A. Derossi, and T. De Pilli (2016). Influence of different blanching methods on colour, ascorbic acid and phenolics content of broccoli. *J Food Sci Technol.* 53(1): 501–510.
18. Singh Gurmeet, Parle Amrita (2015). Unique pandanus - Flavour, food and medicine. *Journal of Pharmacognosy and Phytochemistry* 5(3): 08-14.
19. Wakte KV, Thengane RJ, Jawali N, Nadaf AB (2010). Optimization of HS-SPME conditions for quantification of *2-acetyl-1-pyrroline* and study of other volatiles in *Pandanus amaryllifolius* Roxb. *Food Chem.* 121: 595–600.

20. Wongpornchai, S., Sriseadka, T. and Choonvisase, S. (2003). Identification and quantitation of the rice aroma compound, 2-acetyl-1-pyrroline, in bread flowers (Vallaris glabra Ktze). *Journal of Agricultural and Food Chemistry* 51: 457-462.
21. Zurina Zainal Abidin, Wan Noraznorin Mohamad, Mahtab Samadi (2016). Optimization of chlorophyll extraction from *Gynura procumbens. Malaysian Journal of Analytical Sciences* 20(6): 1421 – 1428.
22. Yoshihashi, T. (2002). Quantitative analysis on 2-acetyl-1- pyrroline of an aromatic rice by stable isotope dilution method and model studies on its formation during cooking. *Journal of Food Science* 67: 619–622.
23. Ysrael, M.C, Santiago, M. and Nonato, M.G. (1995). Diuretic studies of five Pandanus species. *Acta Manilana* 43: 25-30.
24. Woon Yong Choi and Hyeon Yong Lee (2018). Enhancement of Chlorophyll a Production from Marine *Spirulina maxima* by an Optimized Ultrasonic Extraction Process. *Appl. Sci.* *8*(1), 26-36.