J.Natn.Sci.Foundation Sri Lanka 2016 44 (1): 11 - 21
DOI: http://dx.doi.org/10.4038/jnsfsr.v44i1.7977

RESEARCH ARTICLE

Extraction and degradation of chlorophyll *a* and *b* from *Alternanthera sessilis*

M.A.M. Jinasena[1*], A.D.U.S. Amarasinghe[2], B.M.W.P.K. Amarasinghe[2] and M.A.B. Prashantha[3]
[1]*Department of Chemical and Process Engineering, Faculty of Engineering, University of Peradeniya, Peradeniya.*
[2]*Department of Chemical and Process Engineering, Faculty of Engineering, University of Moratuwa, Katubedda, Moratuwa.*
[3]*Department of Chemistry, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda.*

Revised: 20 March 2015; Accepted: 26 August 2015

Abstract: The use of *Alternanthera sessilis,* which is commonly known as *Mukunuwenna* in Sri Lanka as a source of chlorophyll was examined. The extraction of chlorophyll was carried out using buffered 80 % (v/v) aqueous acetone. The optimum operating conditions such as solvent to *A. sessilis* ratio, extraction temperature and extraction time were found to be 5 mL/g, 50 °C and 45 minutes, respectively. The yield of chlorophyll *a* and chlorophyll *b* under these optimum operating conditions were 659 and 261 μg/g of *A. sessilis,* respectively. Mechanical grinding of *A. sessilis* gave a higher yield as compared to blanching and drying. Refrigeration at 15 °C was found to be ideal for storing of fresh *A. sessilis* up to 3 days without a considerable loss of chlorophyll content. Chlorophyll extraction could be modelled successfully using basic mass transfer equations up to 30 °C. It failed above this temperature due to the degradation effect. Kinetic study on the degradation of chlorophyll extracted from *A. sessilis* confirmed first order reaction model and the effect of temperature on the rate constant was also adequately modelled by the Arrhenius equation.

Keywords: *Alternanthera sessilis*, chlorophyll degradation, chlorophyll extraction, kinetics, mass transfer, operating condition.

INTRODUCTION

Chlorophyll is the green pigment found in plants, and is formed in the chloroplasts of the plant cells influenced by sunlight. This pigment is necessary for the process of photosynthesis. Chlorophyll exists mainly as chlorophyll *a* and chlorophyll *b*.

Chlorophyll and chlorophyll derivatives, which are known as chlorophyllin, are widely used in industry as a stable, non-toxic, physiologically harmless colourant for dairy products, edible oils, soups, chewing gums, sugar confections, drinks, cosmetics, toiletries and medicines (Madrid & Madrid, 1990; Francis, 2000; Marquez & Borrmann, 2009). Various therapeutic properties of chlorophyll such as anti-inflammatory activity, acceleration of wound healing, immune modulator properties and body deodorisation in geriatric and ileostomy patients have been reported (Chernomorsky & Segelman, 1988). Furthermore, the anti-mutagenic and anti-carcinogenic activities of chlorophyll and its derivatives have been proposed (Matney, 1980; Dashwood *et al*., 1991; Sarkar *et al*., 1994; Ferruzi *et al*., 2002; Ferruzzi & Blakeslee, 2007). Due to the anti-oxidant, anti-atherogenic, anti-inflammatory and detoxification properties of chlorophyll and its derivatives, it is used in medicines and food supplements (Chernomorsky *et al*., 1999; Kamat *et al*., 2000; Fernandes *et al*., 2007; Ferruzzi & Blakeslee, 2007). Chlorophyll can also be used to produce fuel additives to enhance the combustion characteristics of carbonaceous fuels (Jordan, 1998). The potential of the photo-conversion efficiency of a chlorophyll derivative in dye-sensitised solar cells have also been studied (Wang *et al*., 2005).

The production of chlorophyll from algae and higher plants is a key topic of scientific and commercial interest. Chlorophyll is widely extracted for industrial applications from stinging nettle, spinach, algae (Macias-Sanchez *et al*., 2007), silkworm excreta, alfalfa, pine needles, other pasture grasses and plant harvest by-products (Marquez & Borrmann, 2009).

* Corresponding author (mamjinasena@yahoo.com)

Alternanthera sessilis (L.) R. Br. ex D.C., which is commonly known as sessile joy weed or dwarf copperleaf around the world, while it is known as *Mukunuwenna* (Sinhala) or *Ponnannkannjkkirai* (Tamil) in Sri Lanka, was selected because of the low cost, ready availability and medicinal and nutritional value. *A. sessilis* is a perennial plant belonging to the class Magnoliopsida of the family Amaranthaceae. It is widespread throughout the tropics and subtropics. This plant is commonly used as a leafy vegetable and a medicine in Sri Lanka. *A. sessilis* is used in Ayurveda for the treatment of biliousness, chronic congestion of liver, acute and chronic pyelitis, cystitis, gonorrhea, and snake bite in Sri Lanka (Gayathri *et al*., 2006). Further, its antimicrobial properties, wound healing abilities (Jalalpure *et al*., 2008) and the ability to increase the production of milk in nursing mothers (Jayaweera, 1981) have also been reported. The objective of the present study was to evaluate *A. sessilis* as a potential source of extractable chlorophyll and to examine the ideal extraction, pre-processing and storage conditions. The applicability of general mass transfer equations in modelling the chlorophyll extraction was also examined.

There are a few different forms of chlorophyll that occur naturally. Since the most widely distributed forms in the terrestrial plants are chlorophyll *a* and *b*, the study was focused on those two forms. Various solvents such as aqueous acetone, methanol, ethanol and dimethylsulfoxide (DMSO) are used for the extraction of chlorophyll (Shoaf & Lium, 1976; Sartory & Grobbelaar, 1984; Simon & Helliwell, 1998; Porra *et al*., 1999). Although methanol, ethanol and DMSO proved to be superior to acetone (Sartory & Grobbelaar, 1984; Barnes *et al*., 1992), 80 % acetone was selected as the solvent in the present study since it is the most suitable solvent for the selected quantification method (Lichtenthaler, 1987; Wellburn, 1994), produces a more stable product, is cheaper and less toxic (Thompson *et al*., 1999).

Food that have added commercial chlorophyll are processed thermally for microbiological safety; nevertheless, the addition of heat to food causes losses in texture, flavour, colour, and nutrients. The stability of chlorophyll is mainly affected by temperature (Ryan-Stoneham & Tong, 2000). The kinetics of chlorophyll degradation have been studied on a variety of vegetables, including spinach (Canjura *et al*., 1991), snapbeans, okra and turnip greens (Jones *et al*., 1963), asparagus, green beans and green peas (Hayakawa & Timbers, 1977), broccoli (Sweeney & Martin, 1958) as well as on chlorophyll derivatives (Koca *et al*., 2007). Generally, chlorophyll degradation has been shown to follow a first-order model (Hayakawa & Timbers, 1977; Lichtenthaler, 1987; Canjura *et al*., 1991; Steet & Tong, 1996; Ryan-Stoneham & Tong, 2000; Koca *et al*., 2007; Erge *et al*., 2008; Rudra *et al*., 2008; Seema & Keshav, 2010). The effect of temperature on the rate constant was also adequately modelled by the Arrhenius equation (Lichtenthaler, 1987; Ryan-Stoneham & Tong, 2000; Koca *et al*., 2007). The degradation kinetics of the chlorophylls extracted from *A. sessilis* was examined in the present work to ensure the prediction of quality loss during thermal processing and storage.

METHODS AND MATERIALS

Materials

Mature and healthy *A. sessilis* samples were collected from a farm in Kesbewa, Sri Lanka. All parts of the plant except the roots were taken to prepare the samples. Double distilled acetone (analytical grade) was used as the solvent. A buffer solution of pH 7.8 was prepared with sodium dihydrogen phosphate and disodium hydrogen phosphate.

Analytical technique

A dual beam recording UV-visible spectrophotometer (Shimadzu: model 1800) with 1 cm square glass cuvettes was used to determine the chlorophyll concentration. A Panasonic MX-AC-300 mixer-grinder was used for mechanical grinding of *A. sessilis* and REMI R-4C laboratory centrifuge was used to centrifuge the extract.

Aqueous acetone with a concentration of 80 % (v/v) was used as the solvent and 2.5 mM phosphate buffer of pH 7.8 was added to the solvent. Fresh *A. sessilis* (5 g) were cut into pieces and ground using the mixer-grinder. The ground *A. sessilis* was added to the solvent and placed in a water bath. The extraction was carried out in a dark environment with frequent agitation. After the extraction, samples were centrifuged for 10 min at 2,000 rpm and the supernatant was separated for analysis of chlorophyll.

Chlorophyll concentrations were determined using the spectrophotometer according to the procedure used by Wellburn (1994) and Lichtenthaler (1987). Absorption spectrum was recorded in the UV-visible spectrophotometer. The solutions were diluted using the solvent (80 % acetone) to obtain the absorbance values in the range of 0.1 to 0.7. The concentrations of chlorophyll *a*, *b* and *a* plus *b* were calculated using the following formulae (Lichtenthaler, 1987; Wellburn, 1994).

$$C_a = 12.25\, A^{663.2} - 2.79\, A^{646.8} \quad ...(1)$$

$$C_b = 21.5\ A^{646.8} - 5.1\ A^{663.2} \quad ...(2)$$

$$C_{a+b} = 7.15\ A^{663.28} + 18.71\ A^{646.8} \quad ...(3)$$

Where $A^{646.8}$ and $A^{663.2}$ are the absorbance at wave lengths 646.8 and 663.2 nm, respectively. C_a and C_b are the concentrations of chlorophyll *a* and *b* in plant extract in micrograms per millilitre, respectively.

Concentrations of the extracts were calculated by multiplying the concentrations of diluted samples with the respective dilution factor. The weights of chlorophyll *a*, *b* and *a* plus *b* were calculated by multiplying the concentrations of chlorophyll *a*, *b* and *a* plus *b* with the volume of the sample. Experiments were triplicated and the average value was taken. The weights have been expressed on wet basis unless stated otherwise.

Optimum solvent volume to *A. sessilis* ratio

Chlorophyll was extracted by following the analytical techniques described previously. Solvent volume to *A. sessilis* weight ratio was changed from 3 to 10 mL/g. Temperature of the water bath was kept at 30 °C and the extraction time was selected as 300 min in order to provide sufficient time for the extraction of chlorophyll.

Optimum temperature of extraction

Solvent volume to *A. sessilis* ratio was taken as 5 mL/g based on the results of the previous experiment. Five water baths were maintained at temperatures 20, 30, 40, 50 and 60 °C. The extraction time varied from 60 to 300 min with 60 min interval. Five samples were tested for each temperature and extraction time.

Optimum time for extraction

A solvent volume to *A. sessilis* ratio of 5 mL/g and an extraction temperature of 50 °C was selected based on the results of the previous experiments. The extraction time varied from 15 min to 60 min with 15 min intervals, and then up to 300 min with 60 min intervals.

Effective storage conditions for *A. sessilis*

In order to determine the effective method of storage for harvested *A. sessilis*, six commonly used storage conditions were tested (Table 1).

Effective method of pre processing of *A. sessilis*

Five different commonly used pre-processing methods were used to disintegrate the structure of the plant cells as described in Table 2.

Kinetics of chlorophyll degradation

Four samples of fresh *A. sessilis* were prepared for extraction by the method Pre 1 (Table 2). Solvent to *A. sessilis* ratio of 5 mL/g, temperature of 50 °C and time of 45 min were selected as the extraction conditions based on the results of previous experiments. The extracted chlorophyll samples as solutions were used to examine the degradation kinetics at four different temperatures; 15, 30, 40 and 50 °C. This temperature range was selected based on the normal storage and transportation conditions and the possible processing temperatures. Each sample was tested for chlorophyll *a* and *b* for 0 – 6 hours at 1 hour

Table 1: Tested storage conditions for the determination of effective storage conditions for harvested *A. sessilis*

Storage condition	Description
Env 1	Fresh *A. sessilis* was stored in a closed container in a freezer section of a refrigerator, where the average temperature was 5 ± 2 °C and relative humidity (RH) was 50 ± 2 %.
Env 2	Fresh *A. sessilis* was stored in a closed container in a cooler section of a refrigerator, where the average temperature was 15 ± 2 °C and RH was 60 ± 2 %
Env 3	Fresh *A. sessilis* was stored in open air, where the average temperature was 32 ± 2 °C and RH was 76 ± 2 %
Env 4	Fresh *A. sessilis* was stored in a closed dark container, where the average temperature was 32 ± 2 °C and RH was 76 ± 2 %
Env 5	Fresh *A. sessilis* was dried in an oven for six hours (until weight becomes a constant) at a temperature of 40 ± 2 °C and then stored in a closed transparent container, where RH was 76 ± 2 %
Env 6	Fresh *A. sessilis* was dried in an oven at a temperature of 70 ± 2 °C until weight becomes a constant (about four hours) and then stored in a closed transparent container, where RH was 76 ± 2 %

Table 2: Tested pre processing methods for the determination of the effective pre processing method

Method	Description
Pre 1	Fresh *A. sessilis* was cut (1 inch pieces) and then ground for 5 minutes using the mixer-grinder.
Pre 2	Fresh *A. sessilis* was blanched by soaking the samples in hot water at a temperature of 95 °C for one minute and then immediately dipping it in cold water at a temperature of 15 °C for two minutes (Chandrika *et al*., 2006). Then the samples were cut into pieces of one inch length, approximately.
Pre 3	Fresh *A. sessilis* was blanched following the procedure in Pre 2 and then ground for 5 minutes using the mixer-grinder.
Pre 4	Fresh *A. sessilis* was ground using mortar and pestle.
Pre 5	Fresh *A. sessilis* was dried in an oven for six hours at a temperature of 40 °C and then ground manually.

intervals. The average of duplicated experiments was used for the analysis.

RESULTS AND DISCUSSION

Optimum extraction conditions

Optimum solvent volume to A. sessilis ratio

Figure 1 indicates that the maximum chlorophyll concentration was obtained at a solvent to *A. sessilis* ratio of 5 mL/g. However, the extracted weight of chlorophyll was found to be increasing up to a ratio of 8 mL/g. This may be due to the dual effect of increase in the rate of extraction and dilution of the solution. Up to a solvent to *A. sessilis* ratio of 5 mL/g, the rate of extraction is significantly higher than the dilution. However the dilution effect dominates above 5 mL/g.

Therefore, considering the economy of operation, 5mL/g was selected as the optimum solvent to *A. sessilis* ratio, under 30 °C operating temperature and a time duration of 300 minutes. The corresponding concentrations obtained for chlorophyll *a*, *b* and *a* plus *b* were 87.34, 41.33 and 128.67 µg/mL, respectively with a chlorophyll *a* to *b* ratio of 2.12, and the weight of chlorophyll *a* plus *b* was 643.36 µg/g.

Optimum temperature of extraction

The concentration of chlorophyll *a* and *b* in the extract with various extraction temperatures is shown in Figure 2. Chlorophyll extraction improves with the increase in temperature up to 50 °C. The degradation of chlorophyll may become prominent at high temperatures and prolong extraction. Therefore 50 °C was selected as the optimum temperature with extraction time not exceeding 2 hours.

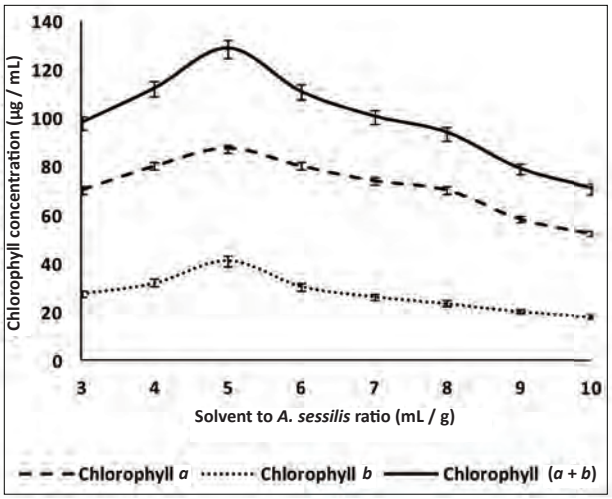

Figure 1: Variation of solvent volume on the concentration of chlorophyll *a*, *b* and *a* + *b*

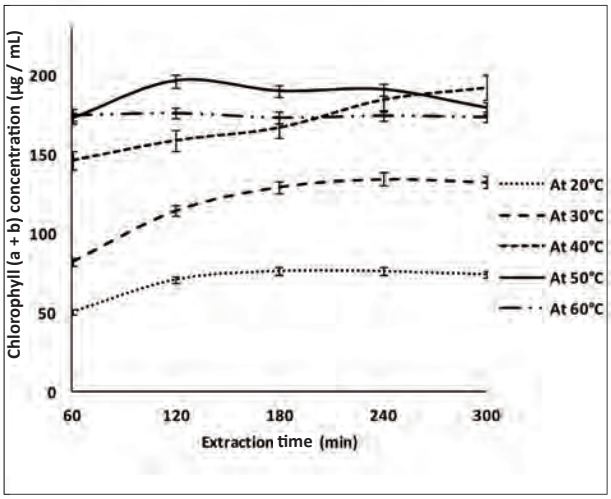

Figure 2: Effect of extraction temperature on concentration of chlorophyll *a* + *b*; solvent to *A. sessilis* ratio 5 mL/g

Optimum time for extraction

According to Figure 3, chlorophyll concentration increased gradually at the beginning and reached a constant value. After 45 minutes of extraction, the increase of chlorophyll *a* plus *b* became less significant and hence the optimum extraction time was selected as 45 minutes. The yield of chlorophyll *a* and chlorophyll *b* at 45 minutes of extraction was 659.5 and 261 µg per gram of fresh *A. sessilis*, respectively. The corresponding values on dry basis were 3.879 and 1.535 µg per milligram of dry *A. sessilis*, respectively.

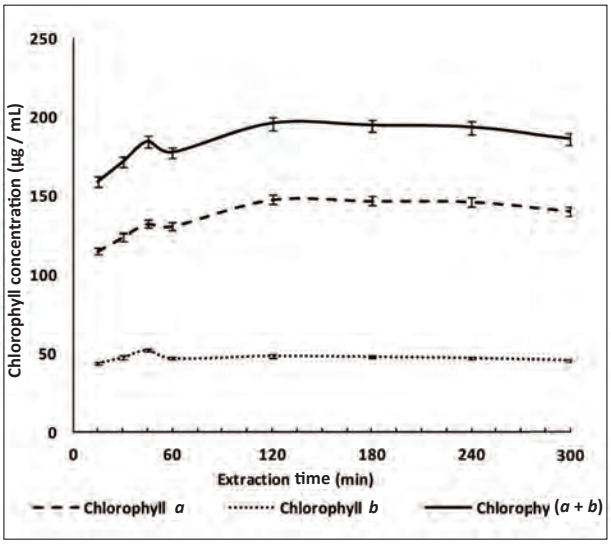

Figure 3: Effect of extraction time on chlorophyll concentration; temperature 50 °C, solvent to *A. sessilis* ratio 5 mL/g

Comparison of the yield of chlorophyll extracted from *A. sessilis* with other plants

Hojnik *et al*. (2007) have studied the isolation of chlorophyll from stinging nettle (*Urtica dioica* L.) using different preservation techniques and storage conditions. The yield was found to be 0.8 mg of chlorophyll per gram of fresh nettle at a temperature of 20 °C and an extraction time of one hour with a solvent to nettle ratio of 10 L/kg (Hojnik *et al.*, 2007). Comparatively, the yield of chlorophyll *a* plus *b* was 0.9205 mg of chlorophyll per gram of fresh *A. sessilis.*

Cubas *et al*. (2008) used N,N-dimethylformamide to extract chlorophyll in green beans (*Phaseolus vulgaris* L.) of cultivar Emerite. They have reported a maximum chlorophyll content of 13.3 mg of chlorophyll per 100 g of fresh green beans (for five extractions, a 90 minute extraction time and 1 minute homogenisation time) (Cubas *et al.*, 2008). The contents of chlorophyll *a* and chlorophyll *b* were found to be 6.4 and 6.8 mg per 100 g of fresh green beans, respectively. Furthermore Khalyfa *et al*. (1992) have reported that the extraction of chlorophyll from 100 g of fresh spinach leaves with methanol resulted in 38 mg of chlorophyll *a* and 13 mg of chlorophyll *b*, while extraction with acetone resulted 61 mg of chlorophyll *a* and 29 mg of chlorophyll *b* (Khalyfa *et al.*, 1992). Comparatively, the amount of chlorophyll *a*, *b* and *a* plus *b* extracted from *A. sessilis* were 65.95, 26.1 and 92.05 mg of chlorophyll per 100 g of fresh *A. sessilis*, respectively. Although the amount of chlorophyll *b* extracted from *A. sessilis* was slightly lower than the amount extracted from spinach using acetone, both the amounts of chlorophyll *a* and *a* plus *b* extracted from *A. sessilis* were higher than the reported values for green beans and spinach.

Barnes *et al*. (1992) have compared DMSO and acetone as solvents for the extraction of chlorophyll from several lichens and higher plants. The highest amount of chlorophyll extraction was observed from the lichen *Ramalina farinacea* collected from El Robledo, and the higher plant *Trifolium repens* out of the plants under investigation in their study using both the solvents (Barnes *et al.*, 1992). The highest chlorophyll content found from the lichens and higher plants in this study and the comparative values for chlorophyll extracted from *A. sessilis* are given in Table 3. The results suggest that *A. sessilis* contains a considerably higher amount of chlorophyll compared to other higher plants and vegetables.

Mass transfer model for chlorophyll extraction

Mass transfer of chlorophyll from *A. sessilis* to solvent was modelled by assuming that the rate of mass transfer from the solid surface to the liquid was the limiting factor. The following general mass transfer equation was used (Geankoplis, 2002).

$$N_a = V\frac{dC_a}{dt} = AK_L(C_{as} - C_a) \quad ...(4)$$

Where;

N_a - Rate of solute dissolving to the solution (mol/s)
A - Surface area of particles
K_L - Mass transfer coefficient
C_a - Concentration of solute at time *t*
C_{as} - Saturation solubility of solute in solution
V - Volume of the solution

Table 3: Comparison of the chlorophyll content of *A. sessilis* with some of the plant materials used by Barnes *et al*. (1992)

Plant material	Solvent used	Chlorophyll content (µg/mg dry plant material)		
		Chl *a*	Chl *b*	Chl *a* and *b*
Ramalina farinacea	DMSO	2.2	0.53	2.73
Ramalina farinacea	Acetone	1.26	0.47	1.73
Trifolium repens	DMSO	2.486 ± 0.11	1.085 ± 0.11	3.571 ± 0.17
Trifolium repens	Acetone	2.55 ± 0.15	1.112 ± 0.15	3.662 ± 0.16
A. sessilis	Acetone	3.879	1.535	5.414

By integrating,

$$\frac{C_{as} - C_a}{C_{as} - C_{a0}} = e^{-\left(\frac{K_L A}{V}\right)t} \quad ...(5)$$

Where, C_{a0} is the initial solute concentration.

Since $C_{a0} = 0$;

$$C_a = C_{as} - C_{as} \times e^{-\left(\frac{K_L A}{V}\right)t} \quad ...(6)$$

Where, $\frac{K_L A}{V} = K$ is a constant as all the samples were prepared from the same material, the solvent volume was kept constant and the extraction process was carried out at a constant temperature. Finally the mass transfer equation is simplified to,

$$C_a = C_{as} \times \left(1 - e^{-Kt}\right) \quad ...(7)$$

The experimental results given in section 'Optimum temperature of extraction' were smoothed with Loess (quadratic) method using MatLab™. The smoothed concentrations of chlorophyll *a* and *b* were plotted against the extraction time and these data were used in curve fitting tool of MatLab™ to find the values of C_{as} and K in mass transfer model corresponding to each temperature. Figure 4 shows both the predicted [using equation (7)] and smoothed experimental concentrations of chlorophyll *a*. Similar trends were observed for chlorophyll *b* as well. The correlation coefficients R^2 (coefficient of determination) and sum of squares due to error (SSE) values corresponding to the plots for each temperature are given in Table 4. The R^2 and SSE values for temperatures 20 and 30 °C indicate better correlations of actual and theoretical data. For temperatures above 30 °C, the predicted and actual data were significantly different. This may be due to the degradation of chlorophyll at elevated temperatures, which was not accommodated in the mass transfer model.

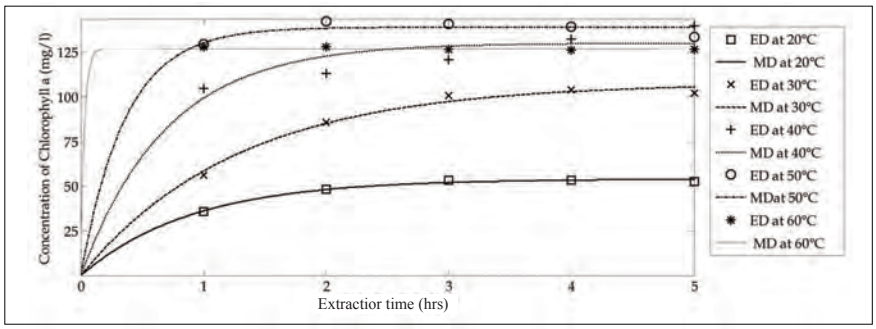

Figure 4: Comparison of experimental (ED) and model (MD) data of concentration of chlorophyll *a* with extraction time at five different temperatures

Table 4: Correlations of actual and predicted data

Temperature °C	Type	R^2 value	SSE
20	Chl *a*	0.9856	3.322
	Chl *b*	0.9133	3.974
30	Chl *a*	0.9836	2.648
	Chl *b*	0.6945	3.859
40	Chl *a*	0.6415	261.6
	Chl *b*	0.5256	39.32
50	Chl *a*	0.5635	49.81
	Chl *b*	0.7059	5.336
60	Chl *a*	Minus value	2.796
	Chl *b*	Minus value	0.2055

Table 5 shows the coefficients of mass transfer model at 20 and 30 °C. The increase of *K* values with the increase in temperature agrees with the general characteristic of leaching behaviour for most of the solvent extraction processes.

Table 5: Coefficients of mass transfer model

Temperature °C	Type	C_{as} (µg/mL)	K ($\times 10^{-4}$)
20	Chl *a*	54.06	3.075
	Chl *b*	23.13	3.189
30	Chl *a*	107.6	4.954
	Chl *b*	29.68	5.842

Determination of the effective storage condition for *A. sessilis*

The weight of extracted chlorophyll *a* plus *b* obtained for different storage conditions are presented in Figure 5. The loss of chlorophyll is significantly low with refrigeration as compared to other storage conditions. The results indicate no significant difference between refrigeration at 15 and 5 °C. Drying also reduces the loss of chlorophyll in long term storage, as compared to storing in ambient conditions. Moisture content of the dried samples at 40 and 70 °C were found to be 84 and 55 %, respectively. This may be the reason for better preservation of chlorophyll after drying at 70 °C as compared to drying at 40 °C.

Effective method of pre processing of *A. sessilis*

Figure 6 depicts that mechanical grinding (Pre 1) has given the highest yield, while grinding by mortar and pestle (Pre 4) has given the second highest yield. Pre processing may result in the degradation of chlorophyll and this effect is more significant with blanching (Pre 2 and 3) and drying (Pre 5). Further, the extraction of chlorophyll from *A. sessilis* may be promoted by disintegration of cell walls as in the case of grinding (Pre 1 and 4). Mechanical grinding (Pre 1) has given more yield than grinding by mortar and pestle (Pre 4) because of the better disintegration of cells by the motor driven grinding.

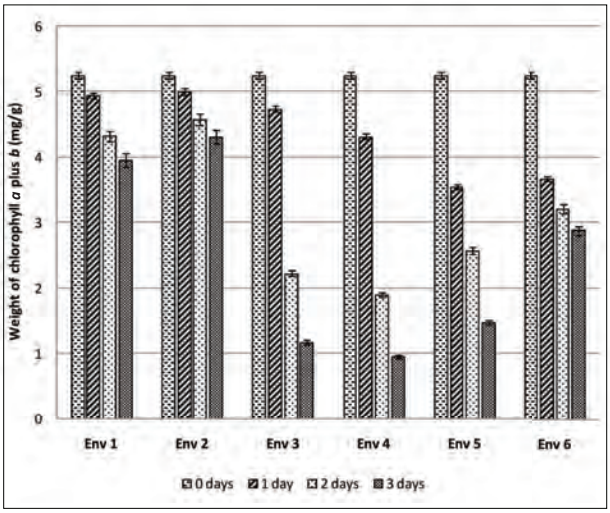

Figure 5: Effect of storage condition on the yield of chlorophyll *a* + *b*. The weight is presented in milligrams per 1 g of fresh *A. sessilis*

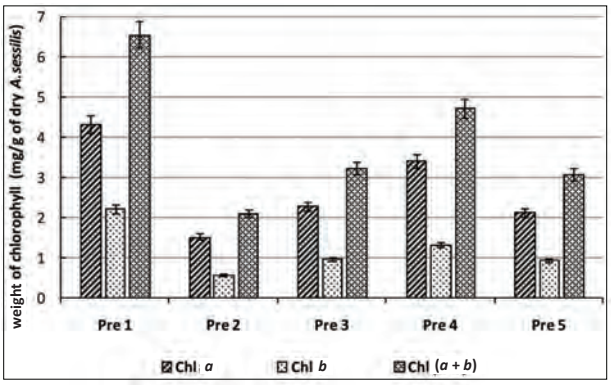

Figure 6: Weight of chlorophyll *a*, *b* and *a* + *b* for different pre-processing methods

Degradation kinetics of chlorophyll extracted from *A. sessilis*

Chlorophyll degradation follows a first-order model (Hayakawa & Timbers, 1977; Lichtenthaler, 1987; Canjura *et al*., 1991; Steet & Tong, 1996; Ryan-Stoneham & Tong, 2000; Koca *et al*., 2007; Erge *et al*., 2008; Rudra *et al*., 2008; Seema & Keshav, 2010) and the effect of temperature on the rate constant can be adequately

described by the Arrhenius equation (Lichtenthaler, 1987; Ryan-Stoneham & Tong, 2000; Koca *et al*., 2007). A similar model was used to describe the degradation of chlorophyll extracted from *A. sessilis* at four different temperatures; 15, 30, 40 and 50 °C.

The first order kinetic model,

$$\ln \frac{C}{C_0} = -kt \qquad ...(8)$$

where,

C - The concentration at any time
C_0 - The concentration at time zero
k - Rate constant at the reaction temperature
t - Time

The Arrhenius equation,

$$\ln k = \ln A_0 - \left(\frac{E_0}{R}\right) = \frac{1}{T} \qquad ...(9)$$

Where,

k - Rate constant
A_0 - Pre-exponential constant
E_0 - Activation energy
R - Gas constant temperature in Kelvin

The experimental results and the model data for the normalised concentrations of chlorophyll *a* and *b* at 15 °C is shown in Figure 7. Similar trends were observed for temperatures of 30, 40 and 50 °C. Since the experimental data were well in agreement with the model data, rate

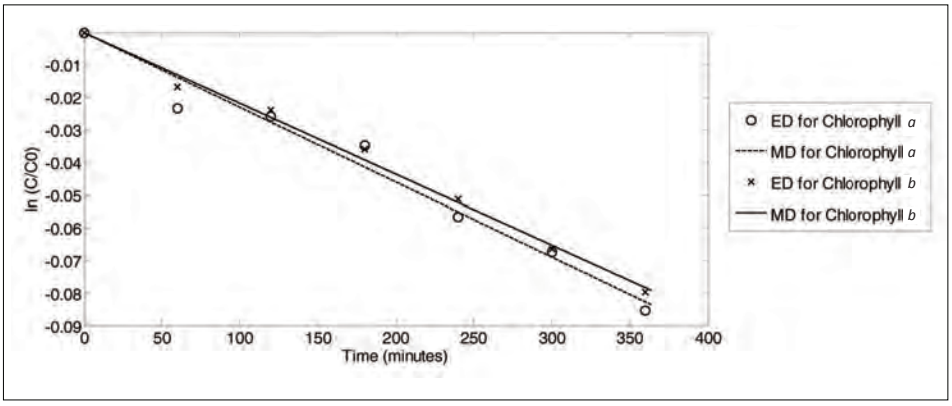

Figure 7: The relationship between the concentration of chlorophyll and time at 15 °C (ED - experimental data, MD - model data)

constant k was calculated. The calculated rate constant values at four different temperatures have been tabulated in Table 6 with the corresponding R^2 values.

Table 6: The rate constants at various temperatures

	Temperature (°C)	*k*	R^2 value
Chl *a*	15	0.0002296	0.9991
	30	0.0003053	0.9993
	40	0.0003506	0.9997
	50	0.0004079	0.9995
Chl *b*	15	0.0002175	0.9995
	30	0.0002777	0.9991
	40	0.0003278	1.0
	50	0.0003669	0.9995

The plot of the logarithm of rate constant against 1/T shows a good correlation (Figure 8) with the Arrhenius equation with a minimum R^2 value of 0.9985. Therefore the values for A_0 and E_0 were calculated based on those data (Table 7). The E_0 values listed in Table 7 are for solutions of chlorophyll and that may be the reason for having very low values in comparable to the values found in literature on pastes and purees.

Table 7: Activation energy and A_0 of chlorophyll

	Activation energy (kcal/mol)	A_0
Chl *a*	3.0143	0.0449
Chl *b*	2.78	0.0282

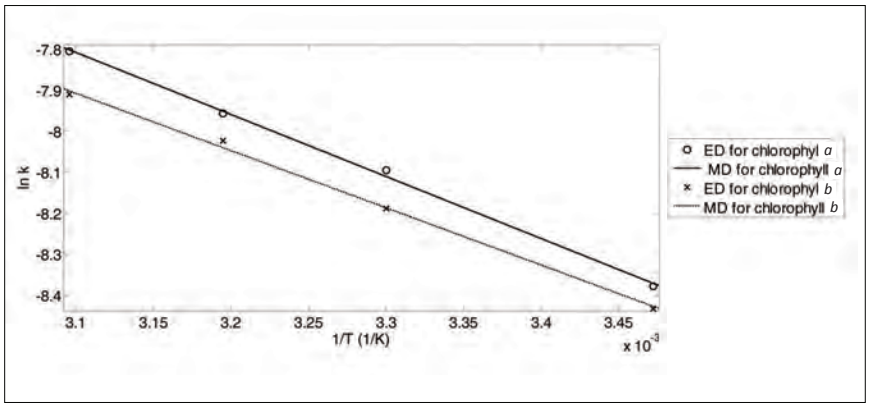

Figure 8: The relationship between the rate constant and the temperature (ED - experimental data, MD - model data)

CONCLUSION

The optimum operating conditions for the extraction of chlorophyll from *A. sessilis* using 80 % (v/v) acetone were found to be: solvent volume to *A. sessilis* weight ratio of 5:1 mL/g, extraction temperature of 50 °C and extraction time of 45 minutes.

Comparison with other higher plant materials and vegetables suggests that *A. sessilis* is a good source for the extraction of chlorophyll. Under the above mentioned optimum conditions, the maximum extractable amount of chlorophyll *a* and chlorophyll *b* were 659.5 and 261 µg per gram of fresh *A. sessilis,* respectively.

General mass transfer equation adequately describes the chlorophyll extraction using acetone as the solvent at temperatures up to 30 °C but fails above this temperature due to degradation effects. Kinetic studies on the degradation of chlorophyll extracted from *A. sessilis* confirmed that chlorophyll degradation follows the first order kinetics and the temperature dependence of the rate constant can be modelled by the Arrhenius equation.

The study on the effect of storage conditions suggests that fresh *A. sessilis* retain the chlorophyll content without considerable loss under ambient conditions for one day. Refrigeration and/or freezing are better alternatives when compared with drying, for lengthy storage times. Mechanical disintegration of *A. sessilis* by grinding was found to be a better pre-processing technique as compared to blanching and drying for obtaining higher yields in the extraction of chlorophyll.

Acknowledgement

The authors express their gratitude to the financial support given by the Senate Research Council of the University of Moratuwa, Sri Lanka.

REFERENCES

1. Barnes J.D., Balaguer L., Manrique E., Elvira S. & Davison W. (1992). A re-appraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. *Environmental and Experimental Botany* **32**(2): 85 – 100.
2. Canjura F.L., Schwartz S.J. & Nunes R.V. (1991). Degradation kinetics of chlorophylls and chlorophyllides. *Journal of Food Science* **56**(6): 1639 – 1643. DOI: http://dx.doi.org/10.1111/j.1365-2621.1991.tb08660.x
3. Chandrika G., Savenberg U. & Jansz E.R. (2006). *In vitro* accessibility of β-carotene from cooked Sri Lankan green leafy vegetables and their estimated contribution to vitamin A requirement. *Journal of the Science of Food and Agriculture* **86**: 54 – 61. DOI: http://dx.doi.org/10.1002/jsfa.2307
4. Chernomorsky S.A. & Segelman A.B. (1988). Biological activities of chlorophyll derivatives. *New Jersey Medicine* **85**(8): 669 – 673.
5. Chernomorsky S., Segelman A. & Poretz R.D. (1999). Effect of dietary chlorophyll derivatives on mutagenesis and tumour cell growth. *Teratogenesis Carcinogenic and Mutagenesis* **19**: 313 – 322.
6. Cubas C., Lobo M.G. & Gonzalez M. (2008). Optimization of the extraction of chlorophylls in green beans (*Phaseolus vulgaris* L.) by N,N-dimethylformamide using response surface methodology. *Journal of Food Composition and Analysis* **21**: 125 – 133. DOI: http://dx.doi.org/10.1016/j.jfca.2007.07.007

7. Dashwood R.H., Breinholt V. & Bailey V. (1991). Chemo preventive properties of chlorophyllin: inhibition of aflatoxin B1 (AFB1)-DNA binding *in vivo* and anti-mutagenic activity against AFB1 and two heterocyclic amines in the *Salmonella* mutagenicity assay. *Carcinogenesis* **12**(5): 939 – 942. DOI: http://dx.doi.org/10.1093/carcin/12.5.939
8. Erge H.S., Karadeniz F., Koca N. & Soyer Y. (2008). Effect of heat treatment on chlorophyll degradation and colour loss in green peas. *GIDA* **33**(5): 225 – 233.
9. Fernandes T.M., Gomes B.B. & Lanfer-Marquez U.M. (2007). Apparent absorption of chlorophyll from spinach in an assay with dogs. *Innovative Food Science and Emerging Technologies* **8**: 426 – 432. DOI: http://dx.doi.org/10.1016/j.ifset.2007.03.019
10. Ferruzzi M.G. & Blakeslee J. (2007). Digestion, absorption, and cancer preventive activity of dietary chlorophyll derivatives. *Nutrition Research* **27**: 1 – 12. DOI: http://dx.doi.org/10.1016/j.nutres.2006.12.003
11. Ferruzzi M.G., Bohm V., Courtney P.D. & Schwartz S.J. (2002). Antioxidant and antimutagenic activity of dietary chlorophyll derivatives determined by radical scavenging and bacterial reverse mutagenesis assays. *Journal of Food Science* **67**: 2589 – 2595. DOI: http://dx.doi.org/10.1111/j.1365-2621.2002.tb08782.x
12. Francis F.J. (2000). *Wiley Encyclopedia of Food Science and Technology*, 2nd edition, pp. 391. John Wiley and Sons, New York, USA.
13. Gayathri B.M., Balasuriya K., Gunawardena G.S.P.D.S., Rajapakse R.P.V.J. & Dharmaratne H.R.W. (2006) Toxicological studies of the water extract of green leafy vegetable sessile joy weed (*Alternanthera sessilis*). *Current Science* **91**(11): 1517 – 1520.
14. Geankoplis C.J. (2002). *Transport Processes and Unit Operations*, 3rd edition, pp. 725 – 726. Prentice-Hall, New Delhi, India.
15. Hayakawa K. & Timbers G.E. (1977). Influence of heat treatment on the quality of vegetables: changes in visual green colour. *Journal of Food Science* **42**(3): 778 – 781. DOI: http://dx.doi.org/10.1111/j.1365-2621.1977.tb12601.x
16. Hojnik M., Skerget M. & Knez Z. (2007). Isolation of chlorophylls from stinging nettle (*Urtica dioica* L.). *Separation and Purification Technology* **57**: 37 – 46. DOI: http://dx.doi.org/10.1016/j.seppur.2007.02.018
17. Jalalpure S.S., Agrawal N., Patil M.B., Chimkode R. & Tripathi A. (2008). Antimicrobial and wound healing activities of leaves of *Alternanthera sessils* (linn). *International Journal of Green Pharmacy* **2**(3): 141 – 144.
18. Jayaweera D. (1981). *Medicinal Plants (Indigenous and Exotic) used in Ceylon*. The National Science Council of Sri Lanka, 47/5, Maitland Place, Colombo 07.
19. Jones I.D., White R.C. & Gibbs E. (1963). Influence of blanching or brining treatments on the formation of chlorophyllides, pheophytins, and pheophorbides in green plant tissue. *Journal of Food Science* **28**(1): 437 – 439. DOI: http://dx.doi.org/10.1111/j.1365-2621.1963.tb00223.x
20. Jordan F.L. (1998). *Chlorophyll Based Fuel Additive for Reducing Pollutant Emissions*. US Patent 5826369.
21. Kamat J.P., Boloor K.K. & Devasagayam T.P.A. (2000). Chlorophyllin as an effective antioxidant against membrane damage *in vitro* and *ex vivo*. *Biochemica Biophysica Acta* **1487**: 113 – 127.
22. Khalyfa A., Kermasha S. & Alli I. (1992). Extraction, purification and characterization of chlorophylls from spinach leaves. *Journal of Agricultural and Food Chemistry* **40**: 215 – 220. DOI: http://dx.doi.org/10.1021/jf00014a010
23. Koca N., Karadeniz F. & Burdurlu S.H. (2007). Effect of pH on chlorophyll degradation and colour loss in blanched green peas. *Food Chemistry* **100**(2): 609 – 615. DOI: http://dx.doi.org/10.1016/j.foodchem.2005.09.079
24 Lichtenthaler H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic membranes. *Methods in Enzymology* **148**: 350 – 382.
25. Macias-Sanchez M.D., Mantell C., Rodriguez M., De Martinez I.O.E., Lubian L.M. & Montero O. (2007). Supercritical fluid extraction of carotenoids and chlorophyll *a* from *Synechococcus* sp. *Journal of Supercritical Fluids* **39**: 323 – 329. DOI: http://dx.doi.org/10.1016/j.supflu.2006.03.008
26. Madrid R. & Madrid J.M. (1990). Los colorantes en la alimentacion. *Alimentacion, Equipos y Tecnologia* **9**(01): 185 – 191.
27. Marquez U.M.L. & Borrmann D. (2009). Chlorophylls. *Handbook of Natural Colorants* (eds. T. Bechtold & R. Mussak), pp. 247. John Wiley and Sons, UK. DOI: http://dx.doi.org/10.1002/9780470744970.ch15
28. Matney T.S. (1980). Antimutagenic activities of common vegetables and their chlorophyll content. *Mutation Research* **77**: 245 – 250.
29. Porra R.J., Thompson W.A. & Kriedemann P.E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents; verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochemica Biophysica Acta* **975**: 384 – 394.
30. Rudra S.G., Sarkar B.C. & Shivhare U.S. (2008). Thermal degradation kinetics of chlorophyll in pureed Coriander leaves. *Food and Bioprocess Technology* **1**(1): 91 – 99. DOI: http://dx.doi.org/10.1007/s11947-007-0016-z
31. Ryan-Stoneham T. & Tong C.H. (2000). Degradation kinetics of chlorophyll in peas as a function of pH. *Journal of Food Science* **65**(8): 1296 – 1302. DOI: http://dx.doi.org/10.1111/j.1365-2621.2000.tb10600.x
32. Sarkar D., Sharma A. & Talukder G. (1994). Chlorophyll and chlorophyllin as modifiers of genotoxic effects. *Mutation Research* **318**: 239 – 247.
33. Sartory D.P. & Grobbelaar J.U. (1984). Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia* **114**: 177 – 187. DOI: http://dx.doi.org/10.1007/BF00031869

34. Seema & Keshav A. (2010). Thermal degradation of coriander leaves: kinetic modelling. *International Journal of Chemical Sciences* **8**(5): S568 – S577.
35. Shoaf W.T. & Lium B.W. (1976). Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. *Limnology and Oceanography* **21**(6): 926 – 928. DOI: http://dx.doi.org/10.4319/lo.1976.21.6.0926
36. Simon D. & Helliwell S. (1998). Extraction and quantification of chlorophyll *a* from freshwater green algae. *Water Research* **32**(7): 2220 – 2223.
37. Steet J.A. & Tong C.H. (1996). Degradation kinetics of green colour and chlorophylls in peas by colourimetry and HPLC. *Journal of Food Science* **61**(5): 924 – 928. DOI: http://dx.doi.org/10.1111/j.1365-2621.1996.tb10903.x
38. Sweeney J.P. & Martin M. (1958). Determination of chlorophyll and pheophytin in broccoli heated by various procedures. *Journal of Food Science* **23**(6): 635 – 647. DOI: http://dx.doi.org/10.1111/j.1365-2621.1958.tb17615.x
39. Thompson R.C., Tobin M.L., Hawkins S.J. & Norton T.A. (1999). Problems in extraction and spectrophotometric determination of chlorophyll from epilithic microbial bio-films: towards a standard method. *Journal of the Marine Biological Association of the United Kingdom* **79**(3): 551 – 558. DOI: http://dx.doi.org/10.1017/S0025315498000678
40. Wang X.F., Xiang J., Wang P., Koyama Y., Yanagida S., Wada Y., Hamada K., Sasaki S. & Tamiaki H. (2005). Dye-sensitized solar cells using a chlorophyll *a* derivative as the sensitizer and carotenoids having different conjugation lengths as redox spacers. *Chemical Physics Letters* **408**: 409 – 414. DOI: http://dx.doi.org/10.1016/j.cplett.2005.04.067
41. Wellburn A.R. (1994). The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology* **144**: 307 – 313.