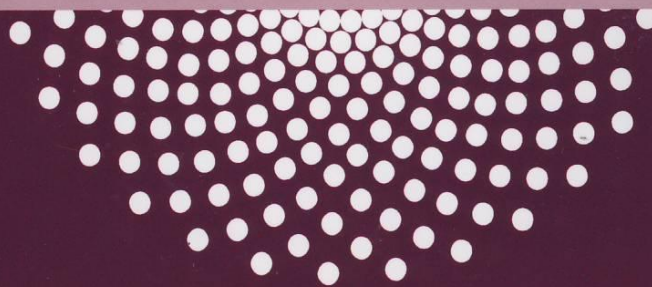


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2nd Humboldt Kolleg in Conjunction with International Conference on Natural Sciences 2014, HK-ICONS 2014

Editors:

**Roy Hendroko Setyobudi, Hugo Scheer,
Leenawaty Limantara, Yuzo Shioi,
Leszek Fiedor, Tatas H.P. Brotosudarmo
and Monika N.U. Prihastyanti**

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Preface

- L. Limantara.
- The Condition of *Uncaria* C
- A. Rauf, Rahmawaty, A.
- Hepatoprotective Effects of
- A. Dwijayanti, A. Frethe
- Coral Diseases in Panjang I
- A. Sabdono, P.H. Sawor
- The Routes of Administrati
- A.D. Wijayanti, G.D. Sa
- Apoptosis in Mice Liver C
- A.A. Maramis, M. Ami
- An α -Glucosidase Inhibitor
- A.P.G. Macabeo, P.Y.M
- Isolation and Characterizat
- A.P. Subroto, C. Utomo
- Microwave Assisted Extra
- A.C. Kumoro, I. Hartat
- Hydrothermal Carbonation
- A.T. Mursito, A. Yuliy
- Biosolid Management Op
- A. Ghimire, R. Sen, A.
- Identifying Sumatran Peat
- A. Wibowo, H. Sloterd
- Pathotyping of Local Isol
- A. Haryanto, M. Purw
- Process Improvement of I
- Avicenna, M. Mel, S.I
- Preliminary Evaluation o
- C.J. Hui, M.N.U. Prib
- Inhibition of the Non-Me
- C. Lienau, S. Konzuel
- Design of Expression Ca
- C. Utomo, A.P. Subro
- Micropropagation of Em
- C.L.M. Marbun, N. T
- Numerical Simulation an
- Wave Energy Conversio
- D.T. Ba, N.D. Anh, P
- Thermal Stability of α -P
- D. Markovic, S. Böhm
- Expression Analysis of
- D.A. Listiawan, R. T
- The Level of Glutamic A
- Rr. E. Susetyarini . . .
- Genotypic Characteriza
- E.A. Corcolon, A.C.
- Oxygen Ion-Conductivi
- Biodiesel-Fuel Cells
- F. Rahmawati, W. Fa



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Procedia Chemistry 14 (2015) iii–v

Procedia
Chemistry

Contents

Preface	1
L. Limantara	1
The Condition of <i>Uncaria Gambir</i> Roxb. as One of Important Medicinal Plants in North Sumatra Indonesia	3
A. Rauf, Rahmawaty, A.Z. Siregar	3
Hepatoprotective Effects of <i>Acalypha Indica</i> and <i>Centella Asiatica</i> in Rat's Liver Against Hypoxia	11
A. Dwijayanti, A. Frerhery, N.S. Hardiany, E.H. Purwaningsih	11
Coral Diseases in Panjang Island, Java Sea: Diversity of Anti-Pathogenic Bacterial Coral Symbionts	15
A. Sabdon, P.H. Sawonua, A.G.D. Kartika, J.M. Amelia, O.K. Radjasa	15
The Routes of Administration of Amikacin as Consideration in Reptile Therapy	22
A.D. Wijayanti, G.D. Satria, A.E.T.H. Wahyuni	22
Apoptosis in Mice Liver Cells Caused by Formalin-containing Food: Normalization of HSP70 Overexpression by Chlorophyllin	27
A.A. Maramis, M. Amin, Sumarno, A.D. Corebima	27
An α -Glucosidase Inhibitor from <i>Drepananthus Philippinensis</i>	36
A.P.G. Macabeo, P.Y.M. Rubio, G.J.D. Alejandro, M. Knorn	36
Isolation and Characterization of Oil Palm Wrinkled 1 (WR1) Gene	40
A.P. Subroto, C. Utomo, C. Darmawan, Z.A. Tanjung, T. Liwang	40
Microwave Assisted Extraction of Dioscorin from Gadung (<i>Dioscorea Hispida</i> Dennst) Tuber Flour	47
A.C. Kumoro, I. Hartati	47
Hydrothermal Carbonation of K-Rich Ash, Value Added Energy Engineering and CO ₂ Mineral Sequestration	56
A.T. Mursito, A. Yuliyanti, Jakah	56
Biosolid Management Options in Cassava Starch Industries of Thailand: Present Practice and Future Possibilities	66
A. Ghimire, R. Sen, A.P. Annachatre	66
Identifying Sumatran Peat Swamp Fish Larvae through DNA Barcoding, Evidence of Complete Life History Pattern	76
A. Wibowo, H. Sloterdijk, S.P. Ulrich	76
Pathotyping of Local Isolates Newcastle Disease Virus from Field Specimens by RT-PCR and Restriction Endonuclease Analysis	85
A. Haryanto, M. Purwaningrum, S. Verawati, S.H. Irianingsih, N. Wijayanti	85
Process Improvement of Biogas Production from Anaerobic Co-digestion of Cow Dung and Corn Husk	91
Avicenna, M. Mel, S.I. Ihsan, R.H. Setyobudi	91
Preliminary Evaluation of the Pigments Content from <i>Rhodobacter Sphaeroides</i> at Stages during Photosynthetic Growth	101
C.J. Hui, M.N.U. Prihastyanti, T.H.P. Brotosudarmo	101
Inhibition of the Non-Mevalonate Isoprenoid Pathway by Reverse Hydroxamate Analogues of Fosmidomycin	108
C. Lienau, S. Konzuch, T. Gräwert, B. Illarionov, A. Bacher, M. Fischer, N. Tanaka, T. Kurz	108
Design of Expression Cassettes for Inverted Repeat and Intron-Spliced Inverted Repeat of PATE Gene Silencing	117
C. Utomo, A.P. Subroto, C. Darwaman, T. Liwang	117
Micropropagation of Embryogenic Callus of Oil Palm (<i>Elaeis Guineensis</i> Jacq.) Using Temporary Immersion System	122
C.L.M. Marbun, N. Toruan-Mathius, Reflini, C. Utomo, T. Liwang	122
Numerical Simulation and Experimental Analysis for a Linear Trigonal Double-Face Permanent Magnet Generator Used in Direct Driven Wave Energy Conversion	130
D.T. Ba, N.D. Anh, P.V. Ngoc	130
Thermal Stability of α -Phycocyanin	138
D. Markovic, S. Böhm, K.-H. Zhao, H. Scheer	138
Expression Analysis of Transcription Factors Involved in Chloroplast Differentiation	146
D.A. Listiawan, R. Tanoue, K. Kobayashi, T. Masuda	146
The Level of Glutamic Acid in the Semen of Male White Rat (<i>Ratus Norvegicus</i>) after Being Treated with Tannin of <i>Pluchea Indica</i>	152
Rr. E. Susetyarini	152
Genotypic Characterization of Turmeric (<i>Curcuma Longa</i> L.) Accessions from Mindanao, Philippines Using RAPD Markers	157
E.A. Corcolon, A.C. Laurena, M.L. Dionisio-Sese	157
Oxygen Ion-Conductivity and Chemical Stability of Ceria Based-Electrolyte in Composite with Sodium Carbonate as Electrolyte for Direct Biodiesel-Fuel Cells	164
F. Rahmawati, W. Fajriati, E. Herald, D.G. Syarif	164

Function of Tetrapyrroles, Regulation of Tetrapyrrole Metabolism and Methods for Analyses of Tetrapyrroles	
H. Schlicke, A. Richter, M. Rothbart, P. Brzezowski, B. Hedtke, B. Grimm	17
Biliproteins and their Applications in Bioimaging	
H. Scheer, X. Yang, K.-H. Zhao	17
Mathematical Model of the Hydrotropic Microwave Assisted Extraction of Anti-Malarial Agent from <i>Andrographis Paniculata</i>	
I. Hartati, L. Kurniasari, Y. Anas	18
Composition of Photosynthetic Pigments in a Red Alga <i>Kappaphycus Alvarezii</i> Cultivated in Different Depths	
Indriatmoko, Heriyanto, L. Limantara, T.H.P. Brotsudarmo	19
Separation of Photosynthetic Pigments by High-performance Liquid Chromatography: Comparison of Column Performance, Mobile Phase, and Temperature	
Indriatmoko, Y. Shioi, T.H.P. Brotsudarmo, L. Limantara	20
Potentially Antihyperglycemic from Biomass and Phycocyanin of <i>Spirulina Fusiformis</i> Voronikhin by in Vivo Test	
I. Setyaningsih, M. Bintang, N. Madina	21
Flower Bracts of Temulawak (<i>Curcuma Xanthorrhiza</i>) for Skin Care: Anti-Acne and Whitening Agents	
I. Batubara, I. Julita, L.K. Darusman, A.M. Muddathir, T. Mitsunaga	21
Analysis on the Chlorophyll Content of Commercial Green Leafy Vegetables	
L. Limantara, M. Dettling, R. Indrawati, Indriatmoko, T.H.P. Brotsudarmo	22
Identification, Isolation and Antioxidant Activity of Pheophytin from Green Tea (<i>Camellia Sinensis</i> (L.) Kuntze)	
L. Kusmita, I. Puspitaningrum, L. Limantara	23
In Vitro Selection of <i>Jatropha Curcas</i> Linn. Hybrids Using Polyethylene Glycol to Obtain Drought Tolerance Character	
Maftuchah, A. Zainudin	23
Application of Simple Multispectral Image Sensor and Artificial Intelligence for Predicting of Drought Tolerant Variety of Soybean	
M.A.S. Adhiwibawa, Y.E. Setiawan, Y. Setiawan, K.R. Prilianti, T.H.P. Brotsudarmo	24
Whole Cell Hydrolysis of Sardine (<i>Sardinella Lemuru</i>) Oil Waste Using <i>Mucor Circinelloides</i> NRRL 1405 Immobilized in Poly-Urethane Foam	
M.G.M. Purwanto, M.V. Maretha, M. Wahyudi, M.T. Goelton	25
Sensory Characteristics of Seasoning Powders from Overripe Tempeh, a Solid State Fermented Soybean	
M.D.P.T. Gunawan-Puteri, T.R. Hassanein, E.K. Prabawati, C.H. Wijaya, A.N. Mutukumira	26
Indigenous Herbal Recipes for Treatment of Liver Cirrhosis	
M.P.B. Wijayagunawardane, C.U.B. Wijerathne, C.B. Herath	27
Financial Prospects in the Application of Decanter System for the Treatment of Sludge Wastewater in Water Treatment Plant System	
Mohajit	27
Detection of Gamma-Irradiated Mutant of Rodent Tuber (<i>Typhonium Flagelliforme</i> Lodd.) in Vitro Culture by RAPD Molecular Marker	
N.F. Sianipar, Ariandana, W. Maarisit	28
Performance of Tetraphenylsulfonato Disilane in Catalytic Transesterification of Crude Palm Oil and Esterification of Fatty Acids with Secondary Alcohols	
N. Bangun, W. Manullang, L. Panggabean, S.B. Sembiring, R. Simangunsong, P. Bali, F.R. Panjaitan	29
The Potency of Guava <i>Psidium Guajava</i> (L.) Leaves as a Functional Immunostimulatory Ingredient	
N. Laily, R.W. Kusumaningtyas, I. Sukarti, M.R.D.K. Rini	30
Formulating Two Steps Loan Scheme to Support Emission Reduction Projects in Indonesia	
N. Syaifudin, H. Amir, S. Wianwihat	30
The Performance of <i>Jatropha Curcas</i> Linn. Capsule Husk as Feedstocks Biogas in One Phase Anaerobic Digestion	
P.G. Adinurani, S.R. Hendroko, S.K. Wahono, A. Nindita, M. Mairziwan, A. Sasmito, Y.A. Nugroho, T. Liwang	31
Bioremediation of Aged Petroleum Oil Contaminated Soil: From Laboratory Scale to Full Scale Application	
Q. Helmy, R. Laksmono, E. Kardena	32
Integrated Geographic Information System and Global Positioning System for Mapping of Forest Plants in Supporting Natural Resources Protection	
Rahmawati, E.K. Sari, A. Syofyan, A. Rauf	33
Peptide-derived from Seahorse Exerts a Protective Effect Against Cholinergic Neuronal Death in <i>in vitro</i> Model of Alzheimer's Disease	
R. Pangestuti, S.-K. Kim	34
Encapsulation of Brown Seaweed Pigment by Freeze Drying: Characterization and its Stability during Storage	
R. Indrawati, H. Sukowijoyo, Indriatmoko, R.D.E. Wijayanti, L. Limantara	35
Genetic Diversity of <i>Celosia</i> Variants in East Java Based on Polyphenol Oxidase-PPO Genes	
R. Mastuti, E.L. Arumingtyas, A.A. Fatinah	36
Potential of Ciplukan (<i>Physalis Angulata</i> L.) as Source of Functional Ingredient	
R.W. Kusumaningtyas, N. Laily, P. Limandha	36
Preliminary Study the Potency of Macroalgae in Yogyakarta: Extraction and Analysis of Algal Pigments from Common Gunungkidul Seaweeds	
R. Haryatfrehni, S.C. Dewi, A. Meilianda, S. Rahmawati, I.Z.R. Sari	37
Removal of Lead and Copper from Contaminated Water Using EAPR System and Uptake by Water Lettuce (<i>Pistia Stratiotes</i> L.)	
R.S. Putra, F. Cahyana, D. Novarita	38
Biological Purification System: Integrated Biogas from Small Anaerobic Digestion and Natural Microalgae	
Salafudin, R.H. Setyobudi, S.K. Wahono, A. Nindita, P.G. Adinurani, Y.A. Nugroho, A. Sasmito, T. Liwang	38
Serial Endothelial Cell Count of Donor Corneal Buttons in Optisol-GS	
S.A.B. Sibayan, Ma. C.P. Garcia-Arenal, K.D. Corpus, J.R.G. Manlongat, M.B.B. Ibañez IV, Ma. D.B. Padilla, J.U. Dy Liacco	39
Vitamin A Fortification Effort	
S.A. Tanumihardjo	17
Second Generation Bioethanol	
S.S. Harsono, Salahuddin	17
Adaptation of the Photosynthesis	
T.H.P. Brotsudarmo, L. L	18
The Chlorophyll <i>b</i> Reductase	
T. Jia, H. Ito, A. Tanaka	19
Heavy Metals in Water of Str	
T.M. Palapa, A.A. Marami	20
Effects of Pigment-Protein F	
Nervous Necrosis (VNN) Inf	21
U. Yanuhar	21
A Survey of Photosynthetic F	
W. Miyata, T. Suzuki, B.E	21
Effects of Cyclohexane/Acete	
W. Rahmalia, J.-F. Fabre,	22
Anti-Cancer Effect of Kaffir	
W.A. Sri Tunjung, J. Cina	23
<i>Agrobacterium Tumefaciens</i>	
Early Flowering	23
Y.A. Purwestri, R.D.K. Sa	24
The Photosensitizer Stabiliti	
Y.A. Handoko, F.S. Rond	25
Status of Drug Discovery Re	
Y.H. Tapilatu	26
Sweet Potato Production for	
Y. Widodo, S. Wahyuning	27
Peroxidase Enzyme Activit	
(<i>Xanthomonas Axonopodis</i> f	27
Y. Yanti	28
Partial Oxidation of Methan	
Y.K. Krisnandi, B.A.P. Pt	29
	30
	30
	31
	32
	33
	34
	35
	36
	36
	37
	38
	38
	39

Vitamin A Fortification Efforts Require Accurate Monitoring of Population Vitamin A Status to Prevent Excessive Intakes	171	S.A. Tanumihardjo	398
Second Generation Bioethanol from Arabica Coffee Waste Processing at Smallholder Plantation in Ijen Plateau Region of East Java	176	S.S. Harsono, Salahuddin, M. Fauzi, G.S. Purwono, D. Soemarno, Kissinger	408
<i>is Paniculata</i>	186	T.H.P. Brotsudarmo, L. Limantara, Heriyanto, M.N.U. Prihastyanti	414
Adaptation of the Photosynthetic Unit of Purple Bacteria to Changes of Light Illumination Intensities	186	T.H.P. Brotsudarmo, L. Limantara, Heriyanto, M.N.U. Prihastyanti	414
The Chlorophyll <i>b</i> Reductase NOL Participates in Regulating the Antenna Size of Photosystem II in <i>Arabidopsis Thaliana</i>	193	T. Jia, H. Ito, A. Tanaka	422
Heavy Metals in Water of Stream Near an Amalgamation Tailing Ponds in Talawaan – Tatelu Gold Mining, North Sulawesi, Indonesia	202	T.M. Palapa, A.A. Maramis	428
Effects of Pigment–Protein Fraction from <i>Nannocloropsis Oculata</i> on TNF α and IL-6 which Act as an Anti-inflammatory Against Viral Nervous Necrosis (VNN) Infection	211	U. Yanuhar	437
A Survey of Photosynthetic Pigments from Surface to Oligotrophic Deep Seawater in Suruga Bay, Japan	216	W. Miyata, T. Suzuki, B.E. Casareto, Y. Suzuki, Y. Shioi	444
Effects of Cyclohexane/Acetone Ratio on Bixin Extraction Yield by Accelerated Solvent Extraction Method	225	W. Rahmalia, J.-F. Fabre, Z. Mouloungui	455
Anti-Cancer Effect of Kaffir Lime (<i>Citrus Hystrix</i> DC) Leaf Extract in Cervical Cancer and Neuroblastoma Cell Lines	232	W.A. Sri Tunjung, J. Cinatl Jr., M. Michaelis, C.M. Smales	465
<i>Agrobacterium Tumefaciens</i> Mediated Transformation of <i>rolC::Hd3a-GFP</i> in Black Rice (<i>Oryza Sativa</i> L. cv. Cempo Ireng) to Promote Early Flowering	239	Y.A. Purwestri, R.D.K. Sari, L.N. Anggraeni, A.B. Sasongko	469
The Photosensitizer Stabilities of Tookad [®] on Aggregation, Acidification, and Day-light Irradiation	246	Y.A. Handoko, F.S. Rondonuwu, L. Limantara	474
Status of Drug Discovery Research Based on Marine Organisms from Eastern Indonesia	256	Y.H. Tapilatu	484
Sweet Potato Production for Bio-Ethanol and Food Related Industry in Indonesia: Challenges for Sustainability	263	Y. Widodo, S. Wahyuningsih, A. Ueda	493
Peroxidase Enzyme Activity of Rhizobacteria-introduced Shallots Bulbs to Induce Resistance of Shallot towards Bacterial Leaf Blight (<i>Xanthomonas Axonopodis</i> pv <i>Allii</i>)	270	Y. Yanti	501
Partial Oxidation of Methane to Methanol over Heterogeneous Catalyst Co/ZSM-5	285	Y.K. Krisnandi, B.A.P. Putra, M. Bahtiar, Zahara, I. Abdullah, R.F. Howe	508
	295		
	301		
	308		
	316		
	326		
Supporting Natural Resources	334		
of Alzheimer's Disease	343		
	353		
	361		
	367		
on Gunungkidul Seaweeds	373		
(<i>a Stratiotes</i> L.)	381		
	387		
J.U. Dy Liacco	394		



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HK-ICONS 2014Analysis on the Chlorophyll Content of Commercial
Green Leafy VegetablesLeenawaty Limantara^{a*}, Martin Dettling^{a,b}, Renny Indrawati^a,
Indriatmoko^a, Tatas Hardo Panintingjati Brotosudarmo^a^aMa Chung Research Center for Photosynthetic Pigments, Universitas Ma Chung, Villa Puncak Tidar N1, Malang 65151, Indonesia^bSRH Distance Education University Riedlingen, Lange Strasse 19, Riedlingen 88499, Germany

Abstract

The objectives of the present study were to evaluate the chlorophyll content of green leafy vegetables found commercially and carry out a comparative investigation between in vivo and in vitro data. The chlorophyll of green leafy vegetable can be used as visible parameters of the quality of vegetables during storage, since it will be degraded gradually along with post-harvest senescence. Therefore, the development of reliable in vivo chlorophyll measurement should be advantageous rather than visual observation for the purpose of quality control and product sortation. Here, the existence of chlorophylls in ten green leafy vegetables were reported as SPAD values of a handheld SPAD-502 chlorophyll meter and % N of an Agriexpert CCN-6000 nitrogen meter (in vivo data), as well as total peak area data of HPLC measurement for chlorophyll *a* and *b* after exhaustive extraction using methanol (in vitro data). Both in vivo and in vitro measurement gave comparable grouping of vegetables with high and low content of chlorophyll. Moreover, correlation plots between SPAD values and total peak area of HPLC showed adequate linear correlation ($R^2 > 0.7$), revealing the potency of in vivo observation for the prediction of actual chlorophyll content in commercial leafy vegetables. SPAD values and % N presented strong linear relationship ($R^2 > 0.9$), in which SPAD-meter performed better detection at very low values. The calibration curve for each species of vegetable should be substantial to overcome the limiting factors of in vivo observation, such as leaf size, tissue thickness, and variation of chloroplast distribution.

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Keywords: Chlorophyll; HPLC; leafy vegetable; nitrogen meter; SPAD

*Corresponding author. Tel.: +62 341 550 171; fax: +62 341 550 175.

E-mail address: leenawaty.limantara@machung.ac.id

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Nomenclature

SPAD	Soil Plant Analysis Development
HPLC	High Performance Liquid Chromatography
R^2	coefficient of determination or goodness-of-fit of linear regression
sp.	species
subsp.	subspecies
var.	variety
s	second (60 s = 1 min; 60 min = 1 h)

1. Introduction

Nowadays, consumption of green leafy vegetables has been increasing, especially as a counterbalance of the growing number of degenerative diseases. Several bioactive compounds in vegetables are including vitamins, minerals, antioxidants, as well as the pigments (chlorophylls and carotenoids). In the living plants, chlorophylls play an important role as primary photosynthetic pigment to capture light energy from the sun. Composed together with carotenoids (the accessory photosynthetic pigments) in pigment-protein complexes, it exhibits colour appearance which is specific for each plant leaf and even used as parameter of maturity, quality, and freshness of food crops^{1,2}. The colour is extremely important because it defines the appearance of the vegetables and influences consumer choice³. However, this minimally processed vegetables are most kept under low temperature preservation (usually 4 °C to 10 °C, with 95 % to 100 % relative humidity) and sold within one week. During that period, the physiological processes occur and particularly cause loss of colour due to degradation of leaf pigments or tissue browning. Hence the quality and freshness of commercially sold vegetable can be monitored by measuring its chlorophyll contents⁴.

The content of chlorophylls can be determined photometrically following extraction of the pigments using an organic solvent, such as acetone or dimethyl formamide, or else by means of handheld device based on light-emitting diodes and silicon photodiode receptor that measures leaf transmittance in the red (650 nm) and infrared (950 nm) regions of the electromagnetic spectrum⁵. The transmittance values are used by the device to derive a relative SPAD meter values that is proportional to the amount of chlorophyll in the sample. The former method is considered as *in vitro* measurement which is well established and accurate, but time-consuming, destructive, and requires the use of toxic or flammable chemicals. On the other hand, the latter provides an alternative *in vivo* method for the measurements of relative leaf chlorophyll levels that overcome these disadvantages, but it is less accurate, not applicable for small or thick leaf, influenced by light condition, and produces only predictive value.

There have been numerous studies which evaluated the correlation between *in vitro* chlorophyll data with its *in vivo* data, based on SPAD value as well percentage of nitrogen in leaf, by using available handheld instruments. The correlation between SPAD value and % N was mostly found as strong linear function, while the strong relationship ($R^2 \sim 0.9$) between SPAD value and *in vitro* chlorophyll concentration has been previously proposed to follow exponential⁶ or second-order polynomial function^{5,7}. Most of the studies employed the leaves of growing plant at certain medium or light set up, but there are only a few numbers of investigations which employed post-harvested samples. Here, the correlation between *in vivo* and *in vitro* data of the chlorophyll content of ten green leafy vegetables purchased from three different supermarkets at Malang, East Java, Indonesia, was observed. The objectives of this study were to (i) identify the range of the SPAD value and % N for ten selected green leafy vegetables on their condition in the market; and (ii) find out the distribution of the feasibility of *in vivo* measurement among ten selected leafy vegetables, compared to their *in vitro* data.

2. Materials and methods

2.1. Plant materials

Green leafy vegetables. All samples were purchased from local markets. The procedure for SPAD value analysis. The whole exper

2.2. *In vivo* assay

The relative chlorophyll meter (Konica Minolta, Japan) side of the leaves was always taken. Each leaf, based on its total area, and the average value was taken from several spots of measurement each leaf. The chlorophyll in the leaf, and

2.3. *In vitro* assay

Liquid nitrogen was placed in a porcelain mortar and added to the sample for 10 s with inert atmosphere. The sample was injected to the HPLC system of photosynthetic pigments. The HPLC (Japan) equipped with photodiode array software (Shimadzu, Japan) was used for separation. The mobile phase (v/v) as mobile phase. The detection wavelength was 400 nm to 700 nm.

2.4. Data analysis

The figures were created using Microsoft Excel 2013. The data were

3. Results and discussion

Ten green leafy vegetables were selected except basil and sword-leaved. After purchasing, samples were immediately conducted under the shade to avoid interference which may affect the extraction and HPLC procedure.

Fig. 1 shows the SPAD values of the average of SPAD value for ten green leafy vegetables. Chinese cabbage pak-choi and pak-choi has darker green color, while pak-choi has darker green color. The SPAD value showed low SPAD value

2. Materials and methods

2.1. Plant materials

Green leafy vegetables were collected from three different supermarkets in Malang area (East Java, Indonesia). All samples were purchased early in the morning, transported into laboratory and directly measured by in vivo procedure for SPAD value and % N. Then, the samples were stored at -18 °C deep freezer for further in vitro analysis. The whole experiment was accomplished during May 2014 until June 2014.

2.2. In vivo assay

The relative chlorophyll content (SPAD value) and % N were determined by means of SPAD-502 chlorophyll meter (Konica Minolta, Japan) and nitrogen meter Agriexpert CCN 6000 (Satake, Japan), respectively. The adaxial side of the leaves was always placed toward the emitting window of the instrument and major veins were avoided. Each leaf, based on its total leaf area, was marked in five up to 14 representative points for in vivo measurements, and the average value was calculated. As for basil, due to its small size, seven leaves were used with two up to three spots of measurement each. This method was intended to overcome the influence of non-uniform distribution of chlorophyll in the leaf, and hence producing more representative data.

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Liquid nitrogen was poured to accurately weighted 0.2 g of leaf samples, and then the frozen leaf was ground in a porcelain mortar and added with 3 mL methanol (Merck, Germany). The extraction was continued by using Vortex for 10 s with inert atmosphere. After that, the extract was filtered (0.22 µm) and injected to HPLC instrument. Each sample was injected to the HPLC in triplicate. All steps were carried out under dim light to prevent any degradation of photosynthetic pigments. The chromatography was performed on a Shimadzu LC20AD system (Shimadzu, Japan) equipped with photo diode array detector and CTO 20A column oven. LC Solution chromatographic software (Shimadzu, Japan) was used for data processing and evaluation. The RP-18e column (Chromolith, Germany) was used for separation, using methanol (Merck, Germany) and acetonitrile (Merck, Germany) of 50 : 50 (v/v) as mobile phase. The flow rate was adjusted to 0.5 mL · min⁻¹ and detection was performed over the range 400 nm to 700 nm.

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The figures were created from numeric data and plotted using Origin 7 Software (OriginLab) and Microsoft Excel 2013. The data were expressed as the mean ± standard error of the mean (SEM).



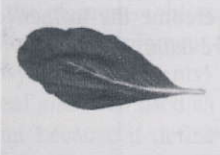



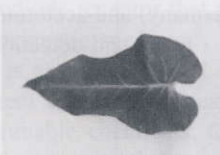
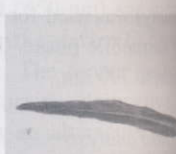

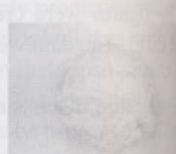
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Ten green leafy vegetables were successfully collected from three different supermarkets in Malang (Table 1), except basil and sword-leaf lettuce which were not available in the third supermarket during this time of research. After purchasing, samples were subsequently subjected to in vivo and in vitro measurement. All measurements were conducted under the same light condition in order to eliminate data variation caused by difference of light interference which may lead to pigment degradation or any disturbance in instrumentation. Identical in vitro extraction and HPLC procedure was similarly employed for all samples.

Fig. 1 shows the SPAD values of ten green leafy vegetables purchased from three supermarkets in Malang. The average of SPAD values ranges from the lowest 2.7 ± 0.8 of head cabbage (Kbs) up to the highest 52.4 ± 3.7 of Chinese cabbage pak-choi (Pch). The SPAD value is proportional to the relative amount of chlorophyll content and consistent with their colour appearance, in which the head cabbage has light green leaves, whereas Chinese cabbage pak-choi has darker green foliage. Higher SPAD value signifies higher chlorophyll content. Three vegetables showed low SPAD value (below 30), i.e. head cabbage (Kbs), green leaf lettuce (Sel), and sword-leaf lettuce (Sak).

On the other hand, seven other vegetables have SPAD values which are greater, supposing that the vegetables are rich in chlorophyll. The variation of SPAD values among the same species can be influenced by the time of harvest, the nature heterogeneity among species⁸, and the difference in growth conditions which may lead to a redistribution of chloroplasts within mesophyll cells⁹.

Table 1. The ten species of leafy vegetables collected from three local markets in Malang, East Java, Indonesia.

No	Vegetables (Local name, scientific name)	Pictures	No	Vegetables (Local name, scientific name)	Pictures
1	Chinese kale (Kailan, <i>Brassica oleracea</i> var. <i>alboglabra</i> Bailey)		6	Green leaf lettuce (Selada, <i>Lactuca sativa</i> L.)	
2	Chinese flowering cabbage (Chaisim or sawi hijau, <i>Brassica rapa</i> L. var. <i>parachinensis</i> (L. H. Bailey) Hanelt)		7	Chinese cabbage pak-choi (Pakcoy, <i>Brassica rapa</i> L. subsp. <i>chinensis</i>)	
3	Spinach (Bayam, <i>Amaranthus</i> <i>spinosus</i> L.)		8	Basil (Kemangi, <i>Ocimum</i> <i>citriodorum</i> Vis.)	
4	Water spinach (Kangkung, <i>Ipomoea</i> <i>aquatica</i> Forsk)		9	Sword-leaf lettuce (Siomak, <i>Lactuca sativa</i> L. var. <i>augustana</i>)	
5	Cassava leaf (Daun Singkong, <i>Manihot</i> <i>utilissima</i> Pohl)		10	Head cabbage (Kubis, <i>Brassica oleracea</i> L. var. <i>capitata</i>)	

Abbreviation: Chinese kale (Kai), Chinese flowering cabbage (Cha), spinach (Bay), water spinach (Kan), cassava leaf (Sin), green leaf lettuce (Sel), Chinese cabbage pak-choi (Pch), basil (Kgi), sword-leaf lettuce (Sak), head cabbage (Kbs).

Furthermore, nitrogen meter displays a direct value of nitrogen percentage (% N) which presents on the leafy vegetables. The characteristic of nitrogen percentages was comparable to SPAD values, varying from $0.8\% \pm 0.1\%$ for green leaf lettuce (Sel) up to $4.8\% \pm 0.4\%$ for Chinese cabbage pak-choi (Pch) (Fig. 1). Unfortunately, the nitrogen meter value of head cabbage (Kbs) was exempted due to its unmeasurable thick leaf and pale colour. Seven leafy vegetables, composed as the previous discussion of SPAD value, indicated the presence of nitrogen from

$3.9\% \pm 0.2\%$ up to $4.8\% \pm$
and sword-leaf lettuce (Sak)

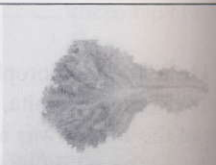
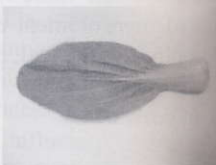



Fig. 1. In vivo an
Abbreviat

The measurement by me
of certain wavelengths whi
calculations in order to pro
within a short time after sar
comparison purpose. In the
vegetable after extraction
measurements, which are pr
the result of in vivo measu
total peak area < 200 00
Nevertheless, the other sev
revealing that in vitro detec

Fig. 2. In vitro analysis of t
chlorophyll a and b r

water, supposing that the vegetables are influenced by the time of harvest, conditions which may lead to a redistribution

va, Indonesia.

Vegetables (scientific name)	Pictures
Spinach (<i>Spinacia oleracea</i> L.)	
Water spinach (<i>Ipomea aquifolium</i> L.)	
Choy sum (<i>Brassica rapa</i> L.)	
Spinach (<i>Spinacia oleracea</i> L.)	
Head cabbage (<i>Brassica oleracea</i> L.)	

spinach (Kan), cassava leaf (Sin), green leaf lettuce (Sel), water spinach (Kai), choy sum (Sak), head cabbage (Kbs).

nitrogen (% N) which presents on the leafy vegetables. SPAD values, varying from 0.8 % ± 0.1 % for water spinach (Kai) to 4.8 % ± 0.4 % for choy sum (Pch) (Fig. 1). Unfortunately, the thick leaf and pale colour of head cabbage (Kbs) indicated the presence of nitrogen from

0.8 % ± 0.2 % up to 4.8 % ± 0.4 % which is fairly higher than those of head cabbage (Kbs), green leaf lettuce (Sel), and sword-leaf lettuce (Sak) whose values were less than 2.4 % ± 0.2 %.

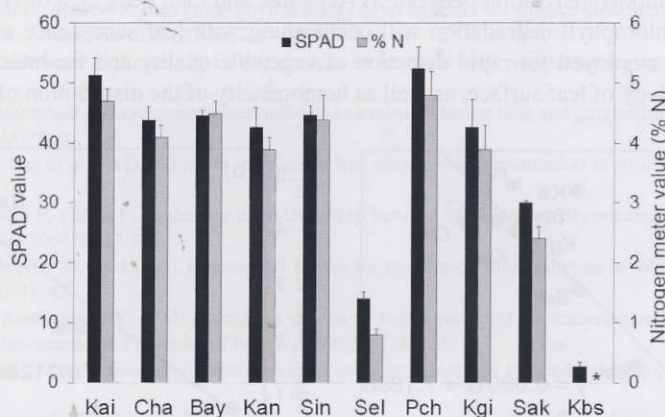


Fig. 1. In vivo analysis of green leafy vegetables by means of SPAD-502 chlorophyll meter and nitrogen meter. Abbreviations were defined in Table 1. *: Head cabbage (Kbs) was unmeasurable by the nitrogen meter.

The measurement by means of chlorophyll and nitrogen meter is based on the principle of detecting absorbance of certain wavelengths which were sent through the leaf tissue. Both systems use particular algorithms for internal calculations in order to produce in vivo data. This method is non-destructive, cheaper, and can be accomplished within a short time after sample collection. However, in vitro quantification is often needed for any verification and comparison purpose. In the present study, HPLC was employed to quantify chlorophyll content of each green leafy vegetable after extraction using organic solvent. Fig. 2 shows data of total peak area, obtained from HPLC measurements, which are proportional to the actual amount of chlorophylls present in the leaf. It was consistent with the result of in vivo measurements using chlorophyll and nitrogen meter, three vegetables showed an average of total peak area < 200 000, i. e. head cabbage (Kbs), green leaf lettuce (Sel), and sword-leaf lettuce (Sak). Nevertheless, the other seven vegetables were not in the same order as observed through in vivo measurement, revealing that in vitro detection is somehow needed for actual data verification.

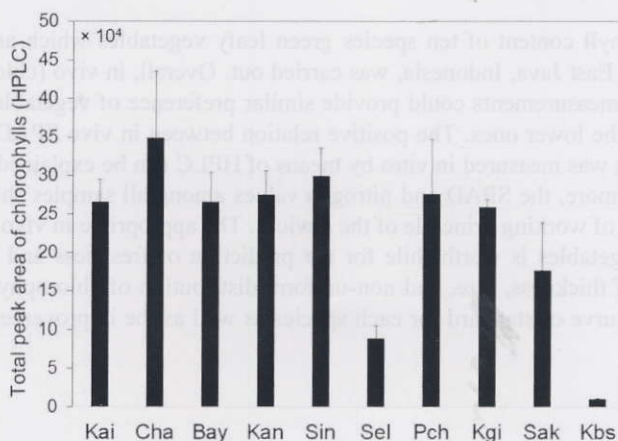


Fig. 2. In vitro analysis of the methanol extract of green leafy vegetables by means of HPLC, expressed as total peak area of chlorophyll a and b monitored at 400 nm to 700 nm. Abbreviations were defined in Table 1.

In order to find out the distribution of the feasibility of in vivo measurement among ten selected leafy vegetables, compared to their in vitro data, the data plot between total peak area of chlorophylls (HPLC) and SPAD value was generated (Fig. 3a). The best-fit regression was obtained as linear correlation with goodness of fit ($R^2 > 0.7$), revealing the presence of moderate relation between SPAD value and total peak area of HPLC for chlorophyll *a* and *b*. In green vegetables, chlorophyll degradation will occur along with leaf senescence after harvest^{3,10}. Therefore, chlorophyll meter can be employed for rapid detection of vegetable quality and freshness. Several limiting factors are the thickness, morphology of leaf surface, as well as homogeneity of the distribution of chlorophyll in leaves.

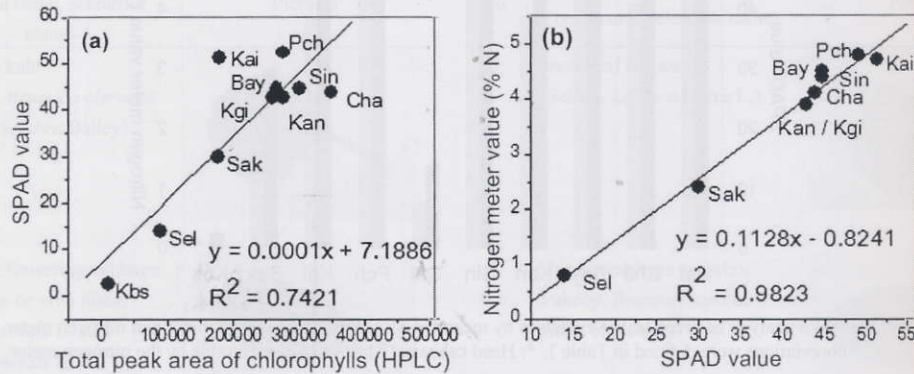


Fig. 3. Data plots of SPAD values versus total peak area of chlorophyll *a* and *b* obtained from HPLC measurement (a), and nitrogen meter versus SPAD values (b). The equation of the linear regression line and its coefficient of determination (R^2 -value) is given in the figure. Abbreviations were defined in Table 1.

In addition, appreciable linear fit ($R^2 = 0.98$) was found in the positive correlation among in vivo measurement (Fig. 3b). This finding is comparable with the result of Johan et al.⁶ and Qihua et al.⁵, in which a strong linear relationship was observed between SPAD and nitrogen meter values. This is acceptable since the development of nitrogen meter is technically based on the chlorophyll meter (SPAD-502)⁶. However, beside the influence of leaf thickness, the SPAD-502 may be more reliable at very low values, since the lowest SPAD value of Kbs was not recorded by nitrogen meter.

4. Conclusion

Investigation on chlorophyll content of ten species green leafy vegetables which are common in daily diet of local community in Malang, East Java, Indonesia, was carried out. Overall, in vivo (chlorophyll meter and nitrogen meter) and in vitro (HPLC) measurements could provide similar preference of vegetables having significantly high content of chlorophyll than the lower ones. The positive relation between in vivo SPAD values and total peak area of chlorophyll *a* and *b* which was measured in vitro by means of HPLC can be explained as 74 % of the variation in a linear relationship. Furthermore, the SPAD and nitrogen values among all samples showed strong relationship of 98 % based on the similarity of working principle of the devices. The appropriate in vivo detection of photosynthetic pigments on green leafy vegetables is worthwhile for the prediction of freshness and nutritional quality. Several limiting factors, such as leaf thickness, size, and non-uniform distribution of chlorophyll, can be overcome by the development of calibration curve of standard for each species as well as the improvement of measuring capacity of the instrumentation.

Acknowledgements

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Among ten selected leafy vegetables, HPLC and SPAD value was with goodness of fit (R^2) > 0.7. Area of HPLC for chlorophyll *a* and freshness after harvest^{3,10}. Therefore, several limiting factors of chlorophyll in leaves.



measured from HPLC measurement (a), and its coefficient of determination

variation among in vivo measurement (b) and its coefficient of determination (c), in which a strong linear relationship is acceptable since the development of SPAD value, beside the influence of leaf senescence, the lowest SPAD value of Kbs was not

which are common in daily diet of leafy vegetables (chlorophyll meter and nitrogen content). Leafy vegetables having significantly high SPAD values and total peak area explained as 74 % of the variation in HPLC. Samples showed strong relationship of SPAD value and in vivo detection of photosynthetic activity and nutritional quality. Several limiting factors of chlorophyll, can be overcome by the improvement of measuring capacity of

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Analysis on the Chlorophyll Content of Commercial Green Leafy Vegetables

Leenawaty Limantara^{a*}, Martin Dettling^{a,b}, Renny Indrawati^a,
Indriatmoko^a, Tatas Hardo Panintingjati Brotosudarmo^a

^aMa Chung Research Center for Photosynthetic Pigments, Universitas Ma Chung, Villa Puncak Tidar N1, Malang 65151, Indonesia

^bSRH Distance Education University Riedlingen, Lange Strasse 19, Riedlingen 88499, Germany

Abstract

The objectives of the present study were to evaluate the chlorophyll content of green leafy vegetables found commercially and carry out a comparative investigation between in vivo and in vitro data. The chlorophyll of green leafy vegetable can be used as visible parameters of the quality of vegetables during storage, since it will be degraded gradually along with post-harvest senescence. Therefore, the development of reliable in vivo chlorophyll measurement should be advantageous rather than visual observation for the purpose of quality control and product sortation. Here, the existence of chlorophylls in ten green leafy vegetables were reported as SPAD values of a handheld SPAD-502 chlorophyll meter and % N of an Agriexpert CCN-6000 nitrogen meter (in vivo data), as well as total peak area data of HPLC measurement for chlorophyll *a* and *b* after exhaustive extraction using methanol (in vitro data). Both in vivo and in vitro measurement gave comparable grouping of vegetables with high and low content of chlorophyll. Moreover, correlation plots between SPAD values and total peak area of HPLC showed adequate linear correlation ($R^2 > 0.7$), revealing the potency of in vivo observation for the prediction of actual chlorophyll content in commercial leafy vegetables. SPAD values and % N presented strong linear relationship ($R^2 > 0.9$), in which SPAD-meter performed better detection at very low values. The calibration curve for each species of vegetable should be substantial to overcome the limiting factors of in vivo observation, such as leaf size, tissue thickness, and variation of chloroplast distribution.

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Keywords: Chlorophyll; HPLC; leafy vegetable; nitrogen meter; SPAD

* Corresponding author. Tel.: +62 341 550 171; fax: +62 341 550 175.
E-mail address: leenawaty.limantara@machung.ac.id

Nomenclature

SPAD	Soil Plant Analysis Development
HPLC	High Performance Liquid Chromatography
R^2	coefficient of determination or goodness-of-fit of linear regression
sp.	species
subsp.	subspecies
var.	variety
s	second (60 s = 1 min; 60 min = 1 h)

1. Introduction

Nowadays, consumption of green leafy vegetables has been increasing, especially as a counterbalance of the growing number of degenerative diseases. Several bioactive compounds in vegetables are including vitamins, minerals, antioxidants, as well as the pigments (chlorophylls and carotenoids). In the living plants, chlorophylls play an important role as primary photosynthetic pigment to capture light energy from the sun. Composed together with carotenoids (the accessory photosynthetic pigments) in pigment-protein complexes, it exhibits colour appearance which is specific for each plant leaf and even used as parameter of maturity, quality, and freshness of food crops^{1,2}. The colour is extremely important because it defines the appearance of the vegetables and influences consumer choice³. However, this minimally processed vegetables are most kept under low temperature preservation (usually 4 °C to 10 °C, with 95 % to 100 % relative humidity) and sold within one week. During that period, the physiological processes occur and particularly cause loss of colour due to degradation of leaf pigments or tissue browning. Hence the quality and freshness of commercially sold vegetable can be monitored by measuring its chlorophyll contents⁴.

The content of chlorophylls can be determined photometrically following extraction of the pigments using an organic solvent, such as acetone or dimethyl formamide, or else by means of handheld device based on light-emitting diodes and silicon photodiode receptor that measures leaf transmittance in the red (650 nm) and infrared (950 nm) regions of the electromagnetic spectrum⁵. The transmittance values are used by the device to derive a relative SPAD meter values that is proportional to the amount of chlorophyll in the sample. The former method is considered as *in vitro* measurement which is well established and accurate, but time-consuming, destructive, and requires the use of toxic or flammable chemicals. On the other hand, the latter provides an alternative *in vivo* method for the measurements of relative leaf chlorophyll levels that overcome these disadvantages, but it is less accurate, not applicable for small or thick leaf, influenced by light condition, and produces only predictive value.

There have been numerous studies which evaluated the correlation between *in vitro* chlorophyll data with its *in vivo* data, based on SPAD value as well percentage of nitrogen in leaf, by using available handheld instruments. The correlation between SPAD value and % N was mostly found as strong linear function, while the strong relationship ($R^2 \sim 0.9$) between SPAD value and *in vitro* chlorophyll concentration has been previously proposed to follow exponential⁶ or second-order polynomial function^{5,7}. Most of the studies employed the leaves of growing plant at certain medium or light set up, but there are only a few numbers of investigations which employed post-harvested samples. Here, the correlation between *in vivo* and *in vitro* data of the chlorophyll content of ten green leafy vegetables purchased from three different supermarkets at Malang, East Java, Indonesia, was observed. The objectives of this study were to (i) identify the range of the SPAD value and % N for ten selected green leafy vegetables on their condition in the market; and (ii) find out the distribution of the feasibility of *in vivo* measurement among ten selected leafy vegetables, compared to their *in vitro* data.

2. Materials and methods

2.1. Plant materials

Green leafy vegetables were collected from three different supermarkets in Malang area (East Java, Indonesia). All samples were purchased early in the morning, transported into laboratory and directly measured by *in vivo* procedure for SPAD value and % N. Then, the samples were stored at -18 °C deep freezer for further *in vitro* analysis. The whole experiment was accomplished during May 2014 until June 2014.

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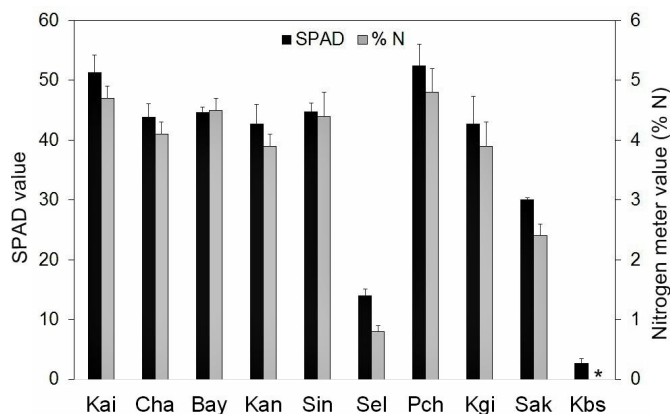


Fig. 1. In vivo analysis of green leafy vegetables by means of SPAD-502 chlorophyll meter and nitrogen meter. Abbreviations were defined in Table 1. *: Head cabbage (Kbs) was unmeasurable by the nitrogen meter.

The measurement by means of chlorophyll and nitrogen meter is based on the principle of detecting absorbance of certain wavelengths which were sent through the leaf tissue. Both systems use particular algorithms for internal calculations in order to produce in vivo data. This method is non-destructive, cheaper, and can be accomplished within a short time after sample collection. However, in vitro quantification is often needed for any verification and comparison purpose. In the present study, HPLC was employed to quantify chlorophyll content of each green leafy vegetable after extraction using organic solvent. Fig. 2 shows data of total peak area, obtained from HPLC measurements, which are proportional to the actual amount of chlorophylls present in the leaf. It was consistent with the result of in vivo measurements using chlorophyll and nitrogen meter, three vegetables showed an average of total peak area < 200 000, i. e. head cabbage (Kbs), green leaf lettuce (Sel), and sword-leaf lettuce (Sak). Nevertheless, the other seven vegetables were not in the same order as observed through in vivo measurement, revealing that in vitro detection is somehow needed for actual data verification.

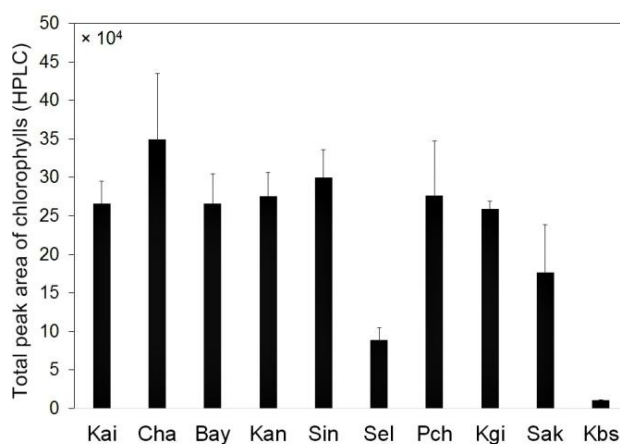


Fig. 2. In vitro analysis of the methanol extract of green leafy vegetables by means of HPLC, expressed as total peak area of chlorophyll a and b monitored at 400 nm to 700 nm. Abbreviations were defined in Table 1.

In order to find out the distribution of the feasibility of in vivo measurement among ten selected leafy vegetables, compared to their in vitro data, the data plot between total peak area of chlorophylls (HPLC) and SPAD value was generated (Fig. 3a). The best-fit regression was obtained as linear correlation with goodness of fit (R^2) > 0.7, revealing the presence of moderate relation between SPAD value and total peak area of HPLC for chlorophyll *a* and *b*. In green vegetables, chlorophyll degradation will occur along with leaf senescence after harvest^{3,10}. Therefore, chlorophyll meter can be employed for rapid detection of vegetable quality and freshness. Several limiting factors are the thickness, morphology of leaf surface, as well as homogeneity of the distribution of chlorophyll in leaves.

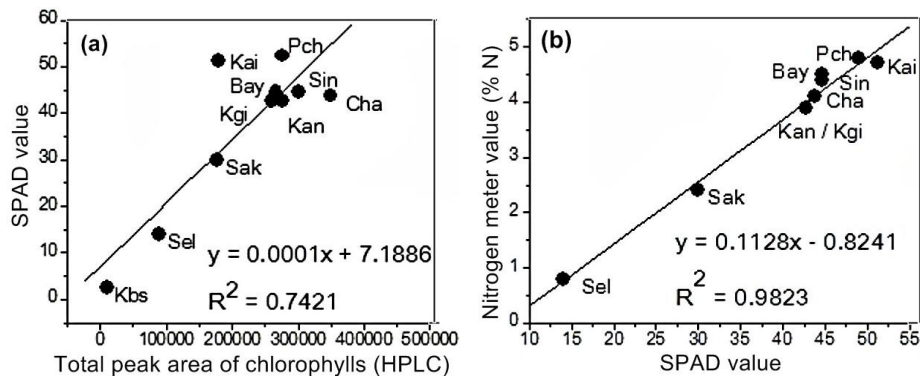


Fig. 3. Data plots of SPAD values versus total peak area of chlorophyll *a* and *b* obtained from HPLC measurement (a), and nitrogen meter versus SPAD values (b). The equation of the linear regression line and its coefficient of determination (R^2 -value) is given in the figure. Abbreviations were defined in Table 1.

In addition, appreciable linear fit ($R^2 = 0.98$) was found in the positive correlation among in vivo measurement (Fig. 3b). This finding is comparable with the result of Johan et al.⁶ and Qihua et al.⁵, in which a strong linear relationship was observed between SPAD and nitrogen meter values. This is acceptable since the development of nitrogen meter is technically based on the chlorophyll meter (SPAD-502)⁶. However, beside the influence of leaf thickness, the SPAD-502 may be more reliable at very low values, since the lowest SPAD value of Kbs was not recorded by nitrogen meter.

4. Conclusion

Investigation on chlorophyll content of ten species green leafy vegetables which are common in daily diet of local community in Malang, East Java, Indonesia, was carried out. Overall, in vivo (chlorophyll meter and nitrogen meter) and in vitro (HPLC) measurements could provide similar preference of vegetables having significantly high content of chlorophyll than the lower ones. The positive relation between in vivo SPAD values and total peak area of chlorophyll *a* and *b* which was measured in vitro by means of HPLC can be explained as 74 % of the variation in a linear relationship. Furthermore, the SPAD and nitrogen values among all samples showed strong relationship of 98 % based on the similarity of working principle of the devices. The appropriate in vivo detection of photosynthetic pigments on green leafy vegetables is worthwhile for the prediction of freshness and nutritional quality. Several limiting factors, such as leaf thickness, size, and non-uniform distribution of chlorophyll, can be overcome by the development of calibration curve of standard for each species as well as the improvement of measuring capacity of the instrumentation.

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