A STUDY ON THE EXTRACTION OF LUTEIN FROM SELECTED LOCALLY GROWN VEGETATION

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To Thompson and family

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ABSTRACT

A study on the extraction of lutein from selected locally grown vegetation was carried out as the first step in developing the most suitable method of producing the lutein. Lutein is an antioxidant, important for preventing age related macular degeneration (ARMD) from occurring especially for person approaching the age of 60 years. The first part of the study was focused on screening some potential raw materials to determine the most suitable source of lutein which offers the highest yield and easily assessable. Two main extraction methods, namely the conventional method and the Soxhlet method, were investigated using the chosen raw material. In evaluating the extraction methods, some important factors affecting the extraction process were investigated. These include the standing or exposure time, solids to solvent ratio, and variation in different types of solvents. The effect of these parameters on the concentration of the extracted lutein, and the rate of extraction were examined. In this study, it was found that in both the conventional and soxhlet method of extractions, acetone yielded the highest amount of lutein compared to other solvents used in the study. In general, the amount of lutein extracted was proportional to the standing time and the raw materials to solvent ratio. In all the operating conditions, maximum achievable lutein concentration was present, after which a plateau condition was observed. The maximum achievable lutein concentration was dependent on the operating conditions. As expected, the rate of mass transfer was higher in soxhlet extraction in comparison to the conventional method, with a magnitude of approximately 5 folds. Recognizing the emerging of ultrasound technology in enhancing many unit operations, a feasibility study was also conducted to gauge its capability in improving the extraction of lutein. It was found that the ultrasound extraction method offers a much improved technique with mass transfer rate equivalent to 4000 folds that of the conventional method, and 40 folds that of the soxhlet extraction method, and with much increased product yield.

ABSTRAK

Suatu kajian ke atas penyarian lutein daripada tanaman tempatan telah dijalankan sebagai langkah pertama dalam membangunkan cara yang paling sesuai untuk menghasilkan lutein. Lutein suatu *antioxidant*, penting untuk mengelakkan penyakit *Age Related Macular Degenerations* (ARMD) daripada berlaku khasnya bagi seseorang yang lebih daripada 60 tahun. Bahagian pertama kajian ini menumpukan pemilihan bahan mentah yang mempunyai potensi untuk menentukan sumber yang mempunyai hasil yang paling tinggi and senang diperoleh. Dua kaedah utama untuk penyarian iaitu cara conventional dan cara menggunakan Soxhlet telah dikaji dengan menggunakan sumber yang diperoleh daripada bahagian satu. Dalam pengajian cara-cara penyarian ini, faktor-faktor penting yang mempengaruhi proses ini telah dikaji. Ini termasuk tempoh masa penyarian, nisbah bahan mentah kepada pelarut dan perbezaan pelbagai pelarut. Kesan-kesan parameter ini dikaji ke atas kepekatan lutein dan kecepatan pemindahan jisim dalam keadaan mantap. Di dalam kajian ini, didapati aseton menghasilkan lutein yang paling tinggi berbanding dengan pelarut yang lain dalam kedua-dua cara pengurasan. Amnya, lutein yang dihasilkan adalah berkadar kepada tempoh masa penyarian dan pecahan bahan mentah kepada pelarut. Di dalam kesemua keadaan operasi, terdapat konsentrasi maksimum yang boleh dicapai yang kemudiannya diikuti dengan keadaan *plateau*. Konsentrasi maksimum lutein yang boleh didapati bergantung kepada keadaan operasi. Seperti yang dijangka, kepantasan pemindahan jisim adalah lebih tinggi untuk kaedah Soxhlet berbanding dengan conventional sebanyak lebih kurang 5 kali ganda. Dengan pengiktirafan kaedah penyarian dengan ultrasonik dalam banyak unit operasi, suatu kajian telah dijalankan untuk melihat kebolehannya dalam memperbaiki penyarian lutein. Adalah didapati cara penyarian dengan ultrasonik merupakan suatu cara penyarian yang jauh lebih baik dengan kepantasan pemindahan jisim yang lebih kurang 4000 kali ganda berbanding dengan cara convensional dan 40 kali ganda berbanding dengan cara Soxhlet dan hasilnya yang juga lebih tinggi.

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LIST OF ABBREVIATIONS

°C	-	Degree celcius
°F	-	Degree Farenheit
psi	-	Per square inch
kg	-	Kilogrammes
g	-	Grammes
%	-	Percentage
kHz	-	Kilohertz
MHz	-	Megahertz
hr	-	Hour
D_{AB}	-	The diffusivity of solute in solvent in m^2/s
c_{A1}	-	Concentration of solute in solvent at point 1 in $kgmol/m^3$
c_{A2}	-	Concentration of solute in solvent at point 2 in $kgmol/m^3$
z_2-z_1	-	The diffusion distance in m
N'_A	-	Flux of A in kgmol/s
A	-	Surface area of particles in m^2
ε	-	The open void fraction
τ	-	The factor which corrects for the path longer than (z_2-z_1)
ε_D	-	The eddy mass diffusivity in m^2/s
P	-	Molar polarization
m	-	Metre
cm	-	Centimetre
L	-	Litre
ml	-	Mililitre
s	-	Second

µg/ml	-	Microgramme per mililitre
µg/ml.s	-	Microgramme per mililitre second
RSS	-	Residual Sum of Squares
R2	-	A measurement of the proportion of variation in the data points
ARMD	-	Age Related Macular Disease

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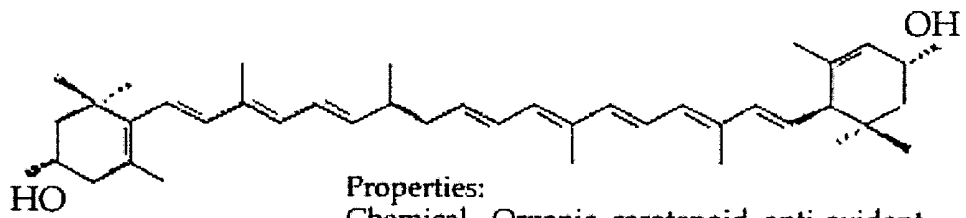
CHAPTER I

INTRODUCTION

1.1 Research Background

Lutein as illustrated in Figure 1.1 is an important carotenoid, a class of light-absorbing chemical pigments that serve as precursors to vitamin A. It belongs to the family of Vitamin A. The chemical name for vitamin A and its family are "retinol" and "retinoids," which express their relationship to the retina of the eye.



Properties:
Chemical - Organic, carotenoid, anti-oxidant
Physical - yellow colored slightly hydroscopic crystalline solid
Formula - $C_{40}H_{52}O_2$
Molecular weight - 568.88

Figure 1.1 Chemical Structure of Lutein (www.luteinfo.com)

Lutein is one of two primary pigments found in the central part of the retina (the other being zeaxanthin), which helps filter out damaging light. Lutein's antioxidant property may help protect the outer retina, which is rich in polyunsaturated fats, from light-induced free radicals.

Macular degeneration is the leading cause of irreversible blindness in adults. A recent study showed that people with the highest intakes of certain carotenoids from foods had 43% less risk for macular degeneration than those with the lowest intakes.

1.2 Lutein Research

Lutein and zeaxanthin are the major determinants in macular pigment optical density. Dietary lutein and zeaxanthin accounted for the greatest amount of macular pigment density variance, according to researchers at Indiana University School of Medicine and Ophthalmology (http://www.luteininfo.com).

In 1996, researchers at Florida International University examined the levels of lutein in people with Age Related Macular Degeneration (ARMD) versus those without. By extracting eyes from cadavers of individuals with and without ARMD, the researchers found that those with the disease had lower levels of lutein in the macular region than those who did not. Researchers at the DVA Medical Center-North Chicago found that antioxidant supplements may have helped prevent progression of ARMD among patients, providing strong evidence that ARMD is a nutrition-responsive disease.

In 1995, Dr. Max Snodderly of the Schepens Eye Institute of Harvard University

analyzed the biochemical role of lutein in ARMD. Snodderly found that blue light is particularly damaging to the eye, and can cause photo-oxidation in the macular region, leading to lipid peroxidation, which is highly toxic to the retina. He concluded that lutein and zeaxanthin inhibit blue light damage by absorbing the blue light and inhibiting photo-oxidation. In 1994, Dr. Johanna Seddon of Harvard University published a study in the Journal of the American Medical Association that examined the effect of consumption of specific carotenoids on ARMD prevalence. The study found that the highest correlation of disease prevention was associated with an intake of 6 mg per day of lutein, which led to a 43 percent lower prevalence of disease. The study also recommended a diet high in lutein to lower the risk of contracting ARMD.

In 1993 to 1995 two studies, by Khachik and Bone, addressed whether lutein is converted to zeaxanthin. Lutein content is on average seven to ten times higher than zeaxanthin in fruit and vegetables. Khachik found that lutein can be converted to zeaxanthin in blood serum. Similarly, Bone also found that lutein can be converted to zeaxanthin. It appears that human metabolism converts lutein to the proper amount of zeaxanthin. Therefore, lutein is the key carotenoid for providing the proper amount of lutein and zeaxanthin.

1.3 Other Health Benefits of Lutein

Research indicates that because of its antioxidant properties, lutein consumption may play a role in maintaining the health of the eyes, heart and skin as well as the breasts and cervix in women. Ongoing research by internationally recognized skin cancer experts from Scripps Memorial Hospital in La Jolla, California, demonstrates the presence of lutein and other antioxidants in the skin, with the hypothesis that antioxidant levels can actually protect the skin from sun damage and the onslaught of the aging process. Further

supporting evidence from a 1998 paper in the *Journal of Dermatology* found that lutein and beta-carotene seemed effective in protecting the cells from UVA damage. Lutein is also found in HDL, or 'good' cholesterol and researchers think it may prevent LDL cholesterol from oxidizing. Evidence from a 1994 study in *Circulation* suggested that one of the reasons the French have a low risk of heart disease is that they consume foods high in lutein content like spinach and collard greens.

1.4 The Importance of The Research

From the literature review, alot of information has been gathered regarding the importance of lutein on the human health. One is able to obtain lutein from various sources such as spinach, cabbage and other leafy vegetables. As 6 mg per day of lutein is needed to prevent ARMD we may not get enough from our daily consumption. So, besides from the food intake, one can also obtain lutein from food supplement as an alternative. However, not much publication has been seen in the world in the production of lutein such as the extraction and the purification of lutein. Kemin Foods of United States of America produces purified FloraGLO Lutein . In fact, FloraGLO Lutein is the only purified and market-proven lutein available for the vitamin and dietary supplement market.

The interest of lutein is still new to the world. It only started aggresively in the 90's. With respect to Malaysia, as far as the publication on the extraction and purification of lutein is concerned, there is still nil. This is the main reason the research on the extraction of lutein is proposed here in the study. Moreover, research shows that most of the abundance of lutein can be found in locally grown vegetation and one should make full use of the nation heritage to create things which are beneficial to the human race. There are lots of potential to be explored here and chances to contribute to the

advancement of human health. Therefore, it would seem to be like a vacuum in the Malaysian research to overlook this field.

In understanding the extraction process of lutein, this research is undertaken. So, as a first step in venturing to this field the screening process is carried out to examine the best suitable raw material for the study. Upon identifying the suitable raw material for the work, the research is focused on the main unit operation of the lutein production, i.e. the extraction process. The identification of the best method of extracting the lutein would be the prerequisite for the development of the lutein production on a large scale.

1.5 Objective

In accordance to the matter discussed above, the objective of this study is therefore to establish a suitable extraction process for lutein from locally grown vegetable.

1.6 Research Scope

This study was divided into seven main parts:

1) Screening Process which is a process which screened through various raw materials to find the best suited raw material to be used in this study.

2) To study two methods of extraction which is the conventional method and Soxhlet extraction method. From literature studies, all possible methods of extracting carotenoids were screened through and examined to look for the options to use for the extraction process of lutein. From here, the two types of methods chosen were examined through a series of experiments to look for the best extraction process.

3) To study the effect of various solvents on the rate of extraction and the percentage of yield.Various solvents were used for each method and the effects were examined and related empirically.

4) To study the effect of the duration of the extraction process. It is expected that the duration of the extraction process will influence the amount of lutein extracted. Here, a study is done to find the optimum duration.

5) To study the effect of the ratio of raw material to the amount of solvent. It is expected that the ratio of raw material to the amount of solvent will influence the performance of the extraction. The amount of raw material offers the degree of lutein availability. The amount of solvent on the other hand influence the driving force of the extraction.

6) To study the feasibility of applying ultrasonic fields in assisting the extraction of lutein. There are alot of claims made on the capability of ultrasonic application in assisting extraction. In this area of research, preliminary experiments were carried out to allow an initial study on the feasibility of this method on extraction of lutein.

7) Development of an empirical model. From the results gathered in this study, analysis on the transport processes taking place in the extraction of lutein will be undertaken. The outcome of the analysis results in a development of an empirical model of the process.

8. The empirical models developed are acceptable in the range of the operating Conditions where the propotion of variation is more than 90%.

6.2 Recommendations

Based on the study conducted, below are some recommendations for further studies.

1. Ultrasonic extraction shows a promising method of extraction which should be further investigated since it is able to offer high product yield at a much faster rate of production.

2. Ethanol and other consumable solvent should be used for future lutein extraction study in order to expand the applicability of the product.

3. Since lutein and other carotenoids are sensitive to sunlight, it is recommended that future extraction study should be conducted in a dark room or fume cupboard to examine its effectiveness.

4. Antioxidant Activity study should also be conducted to examine the effect of process parameters on the quality of the lutein produced.

5. Perform a pilot scale study to evaluate the economical point of view of this research.

REFERENCE

A.R. Mangles et al (1993) " Lutein, Phytonutrient with Burgeoning Utility" Journal of the American Dietic Assoc, Vol. 93:284-296, USA.

Ausich L.Rodney et al. (1995) " Process for the formation, isolation and purification of lycopene crystals" U.S Patent: 5,858,700

Ausich L.Rodney et al. (1997) " Process for the isolation and purification of comestible xanthophyll crystals from plants" U.S Patent: 5,648,564

Britton G. et al. (1995) "Carotenoids" Birkhauser Verlag, Bassel.

Britton, G., Liaaen-Jensen, S. dan Pfander, H. (1995). "Carotenoids Vol 1A: Isolation and Analysis." Birkhauser Verlag, Bassel .

Britton, G., Liaden-Jensen, S. dan Pfander, H. .(1996). "Carotenoids Vol 2: Synthesis." Birkhauser Verlag, Bassel.

Brink, S., Wright, A.R dan Newman, R.J. (1994) "Greens over Carrots for Vision." *U.S.News & World Report.* **117**. pg 97.

Brown, P.R (1990) "Analytical Chemistry" Vol 62 : p 995

Coulson, J.M. et al (1991) "Chemical Engineering, 4th edition" Pergamon Press, London.

Cunniff P. (1999) "Official Methods of Analysis at AOAC International, 16th Edition" Association of Official Agricultural Chemist.

Dicesare J.L et al (1981) "Am lab" No 13 : p 52

Ensminger, D. (1973). "Ultrasonics:The Low and High Intensity Applications." Marcel Dekker Inc, New York.

Fackelmann, K.A. (1994). "Nutrients May Prevent Blinding Disease." *Science News*.**146**. pg 310.

Fassendan R.J and Fessenden J.S. (1990) "Organic Chemistry, 4th Edition" Cole Publishing Co., California.

Fogler H.Scott. (1986) " Elements of chemical reaction engineering" Prentice-Hall, USA.

Geankoplis, J.C.(1995) "Transport Processes and Unit Operations" Prentice-Hall International, Inc., Singapore.

Goodwin T.W. (1980) " The biochemistry of the carotenoids" Volume 1, Chapman and Hall, London, New York.

Gallant R.W. (1968) "Physical Properties of Hydrocarbons" Gulf Publication, Houston.

Haliwell, B., Aeschbach, R., Lologer, J. dan Aruoma, O.I. (1995). "Natural Antioxidants:An Overview." in. F. Shahidi. "Natural Antioxidants:Chemistry Health Effects and Application". *AOCS Press, Zllinois*. hlm 1-11

Hanspeter, P. (1992). "Carotenoids : an Overview"." dlm Packer, L. "Carotenoids: Chemistry, Separation, Quantitation and Antioxidant." Methods in Enzymology Vol 213, Academic Press, United States of America.

Hart, DJ. (1995). "Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK" *Food Chem* 1995;54:101-111

Hines, A.L and Maddox, R.N. (1985) "Mass Transfer: Fundamentals and Applications" Prentice Hall, New Jersey.

Houghton J. Peter and Raman Amala.(1998) "Laboratory handbook for the fractionation of natural extracts" Chapman & Hall, UK.

Jagannath G. (1989) "Biochemistry of Vitamin A" CRC Press, Boca Raton.

Jeana G. (1991) " Pigments in Vegetables: chlorophylls and carotenoids" Chapman and Hall, London.

Khacik F and Frederick. (1994) " Process for isolation, purification, and recystallization of lutein from saponified marigold oleoresin and uses thereof" U.S. Patent: 5,382,714

Khachik, F. (2001). "Process for Extraction and Purification of Lutein, Zeaxanthin and Rare Carotenoids from Marigold Flowers and Plants." (U.S. Patent:6,262,284).

Kull D. dan Pfander, H. (1995). "Isolation and Identification of carotenoids from the Petals of rape (Brassica Napus)." *Journal of Agricultural & Food Chemistry.* **43.** pg 10-12.

Landrum T.J et al (1999) "Analysis of Zeaxanthin Distribution within individual human retinas" Methods in Enzymology Vol 299, Academic Press, San Diego.

Lide, D.R. (1995) "Handbook of Organic Solvent" CRC Press, Boca Raton.

Madley H. Rebecca. (2000) " Seeing Is Believing" Nutraceuticals World, May/June 2000, Rodman Publishing Corporation, USA.

Macrae, R. (Ed) (1988). " HPLC in food analysis, 2nd edition" Food science and Technology, Academic press, London.

Mason, T.J. (1990). "Sonochemistry:The Uses of Ultrasound in Chemistry" The Royal Society of Chemistry, Cambridge.

Marcus Yizhak (1998) "The Properties of Solvents" John Wiley & Sons Inc, USA.

McClements, D.J. (1995). "Ultrasonic in Food Processing." Elsevier Science B.V., Netherlands

Mclure W.F (1994) "Analytical Chemistry" Vol 66 : p 44

Newell W. Frank. (1996) " Ophthalmology; principles and concepts, 8th edition" Mosby-year book, USA.

Ong S.H. and Tee E.S. (1992) " Natural Sources of Carotenoids from Plants and Oils" Methods in Enzymology Vol 213, Academic Press, San Diego.

Packer. L and C. Colman (1999) " The Antioxidant Miracle", John Wiley & Sons Inc, USA.

Rousseuau, R.W. (1987) "Handbook of Separation Process Technology" John Wiley, New York.

Sas B.J et al (1997) "Method for the conversion of xanthophylls in plant material" U.S Patent: 5,876,782.

Schweitzer, P.A. (1997) " Handbook of Separation Technique for Chemical Engineers, 3rd Edition" McGraw-Hill, New York.

Simpson, R.C and Brown, P.R (1987) "Journal of Chromatography" No 400 : p 297

Skoog, D.A et al (1992) "Fundamentals of Analytical Chemistry, 6th Ed" Saunders College Publishing, New York.

Solomons and Graham T.W. (1996) "Organic Chemistry, 6th Edition" John Wiley, New York.

Sommerburg, O., Keunen, J.E.E., Bird, A.C. dan Kuijk, F.J.G. (1998). "Fruits and Vegetables That are Sources for Lutein and Zeaxanthin: The Macular Pigment in Human Eyes." *British Journal of Opthalmology*. **82**. hlm 907-910.

Steinmetz, K.A. dan Potter, J.D. (1991). "Vegetables, Fruit and Cancer" dlm Erik, J.M. Konings and Harry, H. S. Roomans . "Evaluation and Validation of an LC Method for the Analysis of Carotenoids in Vegetables and Fruit." *Journal of Food Chemistry*. **59**. hlm 599.

Synder,L.R Etal (1983) "Analytical Chemistry" Vol 55 : p 1412-30

Tee E.S. (1988) "Nutrient Composition of Malaysian Food" Institute of Medical Research, Kuala Lumpur.

Tee E.S. (1988) "Carotenoids and Retinoids in Human Nutrition" Institute of Medical Research, Kuala Lumpur.

Tess R.W (1973) "Solvents Theory and Practice" American Chemical Society,

Washington.

Treybal, R.E. (1980) "Mass Transfer Operations, 3rd Edition" Mc Graw Hill, New York.

Warburg.O & Negelein.E (1923), Z. Physik,Chem.106.191

http://kerouac.pharm.uky.edu/asrg/hplc/applications.html

http://macweb.acs.usm.maine.edu/chemistry/newton/Chy251_253/Lectures/Solvents/Solvents.html

http://mothernature.com/ency/Supp/Lutein.asp

http://onsager.bd.psu.edu/~jircitano/polar.html

http://sul-server-2.stanford.edu/byauth/burke/solpar/solpar2.html

http://techdev.freeyellow.com/parameters.html

http://www.chem.qmw.ac.uk/iupac/gtpoc/PQ.html

http://www.chem.ox.ac.uk/icl/dermot/mechanism1/lecture3/cross.html

http://www.erowid.org/psychoactives/faqs/faq_otc_solvents.shtml

http://www.kemin.com/html/release_spinach.html

http://www.kemin.com/html/lutein.html

http://www.leafy-greens.org/greens/kale.html

http://www.mse.iastate.edu/microscopy

http://www.netwizards.net/wap/vydac/vitamins.htm

http://www.ocuwel.com/case12.html

http://www.solublesolutions.com/solvselect.html

http://www.sos.bangor.ac.uk/netstudy/o3p23overheads/separartion3.htm

http://www.umr.edu/~jstoffer/Solution/solparaexp.html

http://www.vitaminexpress.com/lutein.htm