



Extraction and Quantification of Marigold Lutein Using Different Solvent Systems

R. Surendranath^{1#}, M. Ganga², M. Jawaharlal^{3*}, K. Anitha¹

Dept of Floriculture and Landscaping, Horticultural College and Research Inst, Tamil Nadu Agricultural University, Coimbatore, India.

[#]Dean, Horticultural College and Research Inst for Women, Trichy, Tamil Nadu Agricultural University, Coimbatore, India.

*Corresponding author's E-mail: hortsuren@gmail.com

Accepted on: 08-03-2016; Finalized on: 31-03-2016.

ABSTRACT

Carotenoids (or pigments) are additives that can enhance the colour of animal products. Because hens cannot synthesize these pigments, they must be provided in the feed. Lutein is one such important member of carotenoid (xanthophyll) family widely present in marigold flower, spinach; kale used as functional food as it has vivid applications from poultry to ophthalmology-pharmacy industry. Lutein and its isomer zeaxanthin are the oxidation products of carotenoids in plants. Both lutein and its stereoisomer zeaxanthin are distinguished from other carotenoid compounds based on the chemical composition of hydroxyl group attachments to their structures.

Keywords: Carotenoids, marigold lutein, solvent systems.

INTRODUCTION

Carotenoids (or pigments) are additives that can enhance the colour of animal products. Because hens cannot synthesize these pigments, they must be provided in the feed^{1,2}. Lutein is one such important member of carotenoid (xanthophyll) family widely present in marigold flower, spinach; kale^{3,4} used as functional food as it has vivid applications from poultry to ophthalmology-pharmacy industry. Lutein and its isomer zeaxanthin are the oxidation products of carotenoids in plants. Both lutein and its stereoisomer zeaxanthin are distinguished from other carotenoid compounds based on the chemical composition of hydroxyl group attachments to their structures^{5,6}.

Below is the molecular structure of lutein (C₄₀H₅₆O₂) and zeaxanthin (C₄₀H₅₆O₂) molecules, the latter accounting for 2-9% of the total xanthophyll content. Chemically it is 3, 3'-dihydroxy-β-carotene; a non-vitamin A yellow plant pigment of the carotenoid family. It occurs in all green plants and also in many flower petals.

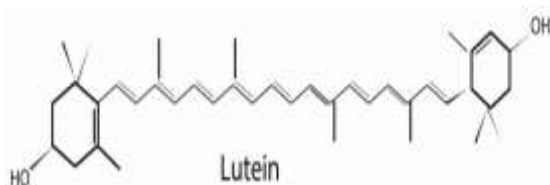


Figure: The above is the structure of lutein molecule. It is an isoprenoid structure with hydroxyl groups attached to the cyclic end groups. C₄₀H₅₆O₂ by IUPAC it is named as β, ε-carotene-3,3'-diol.

Lutein can be sourced from a plethora of plant origins and among them marigold is the most prominent⁷. Some of the prominent sources are listed in table below. It is considered as a potent antioxidant thus preventing the

AMD (Age-related Macular Degeneration) disorders of eyes^{8,9}. In nature antioxidants are available in a wider diversity and they play a significant role by scavenging upon ROS (Reactive Oxygen Species). Scientific studies indicate that free lutein, unlike lutein esters, is the active compound in the human body that is deposited in the serum, eye and other tissues of the body and may be responsible for the reduction of risk of age-related macular degeneration (AMD) and increasing macular pigment density^{8,9}. In infants, lutein is also known to protect skin from UV damage and prevents cardiovascular hardening caused by aging, coronary heart disease and cancer¹⁰.

Lutein and its sister isomer zeaxanthin are the only macular pigments that prevent UV damage by the virtue of their rich antioxidant activity. Its antioxidant activity is to protect outer retina, which is rich in polyunsaturated fats, from light induced free radicals.

Animals do not possess the potential to synthesize those pigments naturally^{11,12}.

Different extraction techniques for lutein pigment from fresh marigold flowers (the most commonly available source in tropical India) have been proposed and demonstrated by numerous authors. Lutein extraction has been multifarious as different authors have devised and described their own techniques which include, soxhlet extraction¹³, enzyme mediated extraction^{14,15}, methanol (80%) based extraction¹⁶, supercritical fluid extraction method¹⁷ and HSCCC (High Speed Counter Current Chromatography)^{18,19}.

Considering the diversity of the above techniques and the difficulties involved, the following experiment was performed so as to check the efficiency of the cheapest and best technique (preferably Soxhlet extraction) with different solvent systems.

Table1: Some of the prominent sources of lutein pigment along with their lutein content.

S. No.	Sample	Lutein (mg/kg)	Reference
1.	Marigold Flower petals	9000	20,17
2.	<i>Spinacia oleracea</i>	640	21
3.	<i>Commelina benghalensis</i>	1100-1200	22
4.	Kale	1310	23
5.	<i>Chlorella vulgaris</i>	24mg/L of culture	19,24
6.	Rice (Kavuni)	900	25
7.	Maize (cob silk)	39	26

MATERIALS AND METHODS

Chemicals used

A wide array of commonly available solvents of HPLC grade namely hexane, ethyl acetate, ethanol, dichloromethane, methanol (carbinol), acetonitrile, chloroform, tetrahydrofuran (THF), Benzene, Diethyl ether, isopropanol, isobutanol, acetone, xylene and propanol were used. Standard lutein was obtained from Natural Remedies, Bangalore.

Sample preparation

In the present study, 0.5 g of dried marigold petal powder (cv. AVT-2 obtained on good will from marigold fields of AVT, Sathyamangalam) was taken in aluminium foil covered 50 ml eppendorf tubes.

Each tube was filled with 15ml of the desired solvent as mentioned in the Table 1 below.

The solution was initially shaken for 15-20 min and allowed to stand at room temperature for an hour.

Later all the samples were centrifuged @ 10,000 rpm, filtered using Whatmann filter paper No. 1 and the solution collected was kept in room temperature.

Observation was taken every 0th, 9th, 18th, 27th hour. The TLC test was conducted on 0th hour to get the R_f values in comparison to lutein standard.

Lutein detection

Thin Layer Chromatography (TLC) test was conducted to detect presence of lutein pigment in the extracts.

Sample and the standard lutein dissolved in the same solvent system was loaded in the same TLC sheet as a single dot side by side and were allowed to run in Hexane (70): Ethyl Acetate (30) mobile phase system. TLC was allowed to run until the mobile phase reached 3/4th of the TLC length. The R_f was calculated using the formula:

$$R_f = \frac{\text{spot travelled}}{\text{Length of mobile phase travelled}}$$

Measurement of absorbance

As evidenced from^{27,28} the absorbance was measured using UV-VIS at 446nm from 0th hour to 27th hour at intervals of every 9 hours was recorded. The concentration of the sample was calculated using the formula:

$$\text{Concentration of lutein}(\mu\text{g/g of sample}) = \frac{A \times V(\text{ml}) \times \text{Dilution factor}}{\text{Absorption coefficient} \times W(\text{g})\text{of sample}}$$

RESULTS AND DISCUSSION

The experiment was conducted to work out the most suitable solvent system that can be harnessed for extraction of marigold lutein (preferably embedded in oleoresin) and also to understand its relative stability at room temperature post extraction.

Such a work especially by using a robust array of solvent has never been performed for marigold lutein extraction.

Though a time stability assay has been performed⁷ for lutein extracted using different plant sources, it has been evaluated with only a few solvents.

In the present study, 15 different solvents were utilized for the extraction efficiency and the relative stability (indicated as the concentration of lutein) at different time intervals.

TLC test was conducted at the 0th hour to identify the lutein presence in coherence with the standard dissolved in the same solvent system.

The R_f values obtained are presented in table 1, similar and approximate values were obtained in TLC of the samples thus indicating that the dark orange-yellow band represented the presence of lutein in the samples extracted. In the case of ethanol though the solubility of lutein is moderate (300mg/L)²⁹ a very faint band was obtained from the samples and didn't matched (0.817) with the standard thus indicating a poorer extraction capacity of lutein from marigold powder. Since no corresponding bands were obtained for dichloromethane, benzene, isopropanol, isobutanol and xylene it can be inferred that these solvents have poorer extraction potential of lutein from marigold petal powder. But the use of dichloromethane³⁰ and diethyl ether³¹ post oleoresin extraction and saponification for lutein purification has been sufficed and standardized.

Another hypothesis that can be sufficed here is that since dichloromethane and diethyl ether have been used for extraction directly from marigold meal powder some other carotenoids and compounds would have dissolved much faster compared to lutein thus reducing its affinity for lutein solubility. But its use in post saponification and further purification has been effective since the availability of lutein relatively increases compared to other unwanted compounds.

Table 1: R_f values for lutein standard and sample in different solvent systems

S. No.	Solvent	R _f value for standard	R _f value from sample
S1	Hexane	0.8	0.79
S2	Ethyl acetate	0.875	0.848
S3	Ethanol	0.971	0.817
S4	Dichloromethane	0.759	No good band detected
S5	Methanol (carbinol)	0.937	0.942
S6	Acetonitrile	0.93	0.95
S7	Chloroform	0.602	0.62
S8	Tetrahydrofuran (THF) + 250ppm BHT	0.834	0.82
S9	Benzene	0.737	Very faint band/ No corresponding band detected
S10	Diethyl ether	0.823	0.901
S11	Isopropanol (Propan-2-ol)	0.976	No corresponding band detected
S12	Isobutanol	0.969	No corresponding band detected
S13	Acetone	0.870	0.896
S14	Xylene	0.810	0.915
S15	Propanol (Propan-1-ol)	0.972	0.94

Table 2: Concentration of lutein (µg/g) in different solvents as affected by different standing time kept in room temperature

S. No.	Solvent	Time (hr)			
		0	9	18	27
S1	Hexane	6.298	6.247	1.89	1.037
S2	Ethyl acetate	4.2	5.015	2.399	2.323
S3	Ethanol	1.523	2.399	2.183	1.064
S4	Dichloromethane	6.35	4.394	2.947	1.002
S5	Methanol (carbinol)	0.372	0.516	0.236	0.16
S6	Acetonitrile	0.289	2.009	1.729	0.362
S7	Chloroform	6.797	3.476	2.337	1.144
S8	Tetrahydrofuran (THF) + 250ppm BHT	4.056	6.119	4.975	2.929
S9	Benzene	0.947	6.753	4.422	2.718
S10	Diethyl ether	4.414	5.156	5.921	4.413
S11	Isopropanol (Propan-2-ol)	7.7	3.076	1.694	1.666
S12	Isobutanol	2.669	4.655	4.401	2.421
S13	Acetone	6.592	10.866	7.028	5.381
S14	Xylene	8.841	6.795	2.78	4.146
S15	Propanol (Propan-1-ol)	8.547	4.736	3.433	3.435

Table 2 and Fig 1 represents the effectiveness of different solvents for extraction of lutein from marigold petal powder as influenced under different standing times. From the data it is evident that for most of the solvents a standing of time up to 9 hours was sufficient for maximum extraction of the carotenoid.

Among all the solvents, acetone gave the highest values thus indicating its effectiveness for extraction of lutein from marigold⁷ and is also supported from the evidence of TLC experiment performed. The other solvents hexane, dichloromethane, isopropanol, propanol, xylene gave higher lutein yields at 3 hours stand by thus indicating their effectiveness for use as extraction solvents. But taking into consideration of solvent characters like

volatility, toxicity, viscosity, stability and availability; hexane and diethyl ether can be considered as better solvent systems for extraction. The use of hexane is much higher as is evident with a plethora of literature available³⁰⁻³⁵ but this can be contradictory to the findings of Craft & Soares, 1992²⁹ who mentioned a mediocre solubility of lutein in hexane (20mg/L). Tetrahydrofuran (THF) and Benzene gave a higher lutein yield after 9 hours of standby time^{36,37} but their use is limited by the caution that they can form harmful peroxides and are carcinogenic in nature.

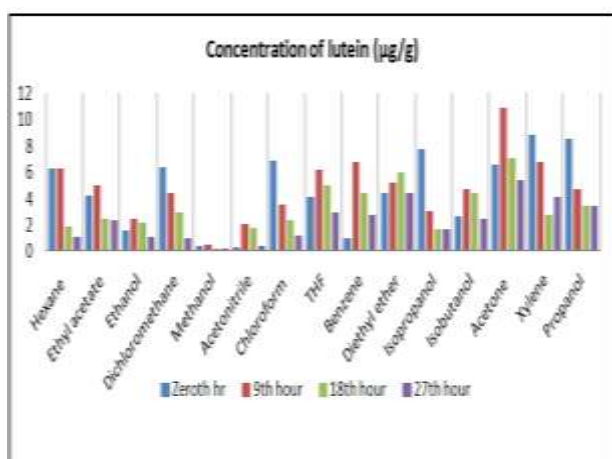


Figure 1: Graphical representation of the concentration of lutein ($\mu\text{g/g}$) obtained using different solvents at different standing hours

The use of any suitable antioxidant like Butylated Hydroxyl Toluene (BHT) is mentioned in many literatures^{38,34} during carotenoid extraction and in the present experiment performed it is clearly evident that there is degradation of lutein pigment post 9 hours of standby in most of the solvents thus tacitly indicating the importance of such antioxidants during extraction.

CONCLUSION

The present experiment was performed with the objective of identifying a suitable solvent system for extraction of xanthophyll from dried marigold petal powder. Different solvents have been employed among which hexane, acetone, xylene, THF, chloroform was found to be suitable for extraction which was further confirmed by TLC tests but the selection of solvents for extraction are subjected to toxicity, availability and viscosity characteristics. Further, the concentration of lutein extracted ranged from ca. 6.5 to 7.5 $\mu\text{g/g}$ of petal powder and this is in accordance with most of the available literature exploring marigold as potential source of lutein pigment. With regard to standby time required for extraction, 9 hours at room temperature was found to be highly effective for most of the solvents beyond which degradation was observed which can be due to the relative instability of lutein at high temperatures or quicker volatility of some solvents from the tubes.

Acknowledgement: The authors would like to thank Faculty of Horticulture, TNAU, Coimbatore for providing all the necessary chemicals and lab facilities.

REFERENCES

- 1) Lokaewmanee K, Yamauchi K, Komori T, Saito K. Enhancement of yolk color in raw and boiled egg yolk with lutein from marigold flower meal and marigold flower extract. *Journal of Poultry Science*, 48, 2011, 25–32. doi: 10.2141/jpsa.010059.
- 2) Voutilainen S. Carotenoids and cardiovascular health. *J. Clin. Nutr.* 83(6), 2006, 1265–1271.
- 3) Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am. J. Clin. Nutr.* 62, 1995, 1448S–1461S.
- 4) Subczynski WK, Wisniewska A, Widomska J. Location of macular xanthophylls in the most vulnerable regions of photoreceptor outer-segment membranes. *Arch. Biochem. Biophys.* 504, 2010, 61–66.
- 5) Lee BL, New AL, Ong CN. Simultaneous determination of tocotrienols, tocopherols, retinol, and major carotenoids in human plasma. *Clin. Chem.* 49, 2003, 2056–2066.
- 6) Jenkins MY, Mitchell GV, Grundel E. Natural tocopherols in a dietary supplement of lutein affect tissue distribution of tocopherols in young rats. *Nutr. Cancer.* 37, 2000, 207–214.
- 7) Hajare R, Ray A, Shreya C, Tharachand MN, Avadhani M, Selvaraj IC. Extraction and Quantification of Antioxidant Lutein from Various Plant Sources. *Int. J. Pharm. Sci. Rev. Res.*, 22(1), 2013, 152–157.
- 8) Bone RA, Landrum JT, Cains A. Optical density spectra of the macular pigment *in vivo* and *in vitro*. *Vis. Res.* 32, 1992, 105–110.
- 9) Koushan K, Rusovici R, Li W, Lee R, Ferguson R and Chalam KV. The Role of Lutein in Eye-Related Disease. *Nutrients*, 5, 2013, 1823–1839. doi: 10.3390/nu5051823.
- 10) Slattery ML. Carotenoids and colon cancer. *The American journal of clinical nutrition.* 71(2), 2000, 575–582.
- 11) Batista AP, Raymundo A, Sousa I, Empis J, Rheological characterization of coloured oil-in-water food emulsions with lutein and phycoerythrin added to the oil and aqueous phases, *Food Hydrocolloids*, 20, 2006, 44–52.
- 12) Sasaki M, Yuki K, Kurihara T, Miyake S, Noda K, Kobayashi S, Ishida S, Tsubota K, Ozawa Y, Biological role of lutein in the light-induced retinal degeneration, *Journal of nutritional biochemistry*, 23, 2012, 423–429.
- 13) Kadam PV, Laxman BC, Balasaheb SR, Yuvraj NR and Janardlian PM. Pharmacognostic, physicochemical and photochemical, investigation of *Tagetes erecta* Lin. flowers (Asteraceae). *Journal of Biological & Scientific Opinion*, 1(1), 2013, 21–24.
- 14) Delgado-Vargas, F. and O. Paredes-Lopez. Enzymatic treatment to enhance carotenoid content in dehydrated marigold flower meal. *Plant Foods for Human Nutrition*, 50, 1997, 163–169.
- 15) Mauricio Mora-Pale, Sandra Perez-Mungua J, Jessica C, Gonzalez-Mejra, Jonathan SD, Eduardo B. The lipase-catalyzed hydrolysis of lutein diesters in non-aqueous

- media is favoured at extremely low water activities. *Biotechnol. Bioeng.* 98, 2007, 535–542. doi:10.1002/bit.21417
- 16) El-Sayed, Abdel-Aal and Iwona R. Antioxidant Properties of High-Lutein Grain-Based Functional Foods in comparison with Ferulic Acid and Lutein. *Am. J. Biomed. Sci.*, 5(2), 2013, 109-125; doi: 10.5099/aj130200109.
 - 17) Sujith, APA, Hymavathi TV and Yasoda Devi P. 2010. Supercritical Fluid Extraction of Lutein Esters from Marigold Flowers and their Hydrolysis by Improved Saponification and Enzyme Biocatalysis. *World Academy of Science, Engineering and Technology*, 4, 2010, 01-24.
 - 18) Wei Y, Zhang T, Xu G and Ito Y. Application of CCC for the separation of lutein from a crude extract of marigold flower petals, *journal of liquid chromatography & related technologies*, 26(9-10), 2003, 1659-1669.
 - 19) Li Hua-Bin, Feng Chen, Tian-You Zhang, Fu-Quan Yang, Guo-Qing Xu. Preparative isolation and purification of lutein from the microalga *Chlorella vulgaris* by high-speed counter-current chromatography (HSCCC). *Journal of Chromatography A*, 905, 2001, 151–155.
 - 20) Pratheesh, VB, Benny N and Sujatha CH. Isolation, stabilization and characterization of xanthophyll from marigold flower-*Tagetes erecta* L. *Modern Applied Science*, 3(2), 2009, 19-28.
 - 21) Kadeem EJ. Identification and Quantitative Estimation of Lutein in Iraqi *Spinacia oleracea* Family *Chenopodiaceae* by Using Chromatographic Methods. *Baghdad Science Journal*, 8(1), 2011, 96-102.
 - 22) Vatsala, TM, Rekha R. An Efficient Method for Extracting Lutein from Indian Medicinal Plant *Commelina benghalensis*. A comparative Study On Solvents Efficiency. *Indian Journal of Science and Technology*. 6(2), 2013, 3999-4005.
 - 23) Lefsrud MG and Kopsell DA, Kpose DE and Celentano JC. Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. *Hort Science*. 40(7), 2005, 2026-2030.
 - 24) Cordero BF, Obratzsova I, Couso I, Leon R, Vargas MA, Rodriguez H. Enhancement of Lutein Production in *Chlorella sorokiniana* (Chlorophyta) by Improvement of Culture Conditions and Random Mutagenesis. *Marine Drugs*. 9(9), 2011, 1607-1624.
 - 25) Valarmathi R, Raveendran M, Robin S and Senthil N. Unravelling the nutritional and therapeutic properties of 'Kavuni' a traditional rice variety of Tamil Nadu. *Journal of Plant Biochemistry and Biotechnology*, 24(3), 2015, 305-315.
 - 26) Kuhn S, Dias PF, Oglari JB, Maraschin M. Brazilian Maize Landraces Silks as Source of Lutein: An Important Carotenoid in the Prevention of Age-Related Macular Degeneration. *Food and Nutrition Sciences*, 3, 2012, 1609-1614. <http://dx.doi.org/10.4236/fns.2012.311210>
 - 27) Wang M. Antioxidant activity, mutagenicity/anti-mutagenicity, and clastogenicity/anti-clastogenicity of lutein from marigold flowers. *Food and Chemical Toxicology*, 44(9), 2006, 1522-1529.
 - 28) Helrich K. A. O. A. C. Official Methods of Analysis. 15th ed. Arlington, Virginia, 1990, 1048–1049.
 - 29) Craft, NE and Soares JH Jr. Relative solubility, stability and absorptivity of Lutein and B-carotene in organic solvents. *J. Agric. Food Chem.* 40, 1992, 431-434.
 - 30) Arunkumar R, Prashanth KVH, Baskaran V. Promising interaction between nanoencapsulated lutein with low molecular weight chitosan: characterization and bioavailability of lutein *in vitro* and *in vivo*, *Food Chemistry* 2013, doi: <http://dx.doi.org/10.1016/j.foodchem.2013.02.10>
 - 31) Boonnoun P, Opaskonkun T, Prasitchoke P, Goto M and Shotipruk A. Purification of Free Lutein from Marigold Flowers by Liquid Chromatography. *Engineering Journal*. 16(5), 2012 doi:10.4186/ej.2012.16.5.145
 - 32) Navarrete-Bolanos JL, Hugo J, Enrique B, Ramiro R, Octavio P. Improving Xanthophyll Extraction from Marigold Flower Using Cellulolytic Enzymes. *J. Agric. Food Chem.* 52, 2004, 3394-3398.
 - 33) Sarkar CR, Bagmita B, Lima D, Bhabesh Ch G. An efficient condition of Saponification of Lutein ester from marigold flower. *Annals of Biological Research*, 3(3), 2012, 1461-1466.
 - 34) Rao, JR and Reddy GBS. Extraction of lutein from marigold meal. 2004. Patent No. US 2004/0267033A1.
 - 35) Swaminathan S and Kunhiraman PM. Isolation and purification of marigold carotenoids from marigold flowers. 2009, Patent No. US76, 22, 599 B2.
 - 36) Khachik F. Process for isolation, purification, and recrystallization of lutein from saponified marigold oleoresin and uses thereof. 1995, Patent No. US 53, 82, 714.
 - 37) Khachik F. Process for extraction and purification of lutein, zeaxanthin and rare carotenoids from marigold flowers and plants. 2001, Patent No. US6, 262, 284 B1.
 - 38) Peng ZY, Li Chunlei, Ella Yang, Xu Jianguo. Method for preparing lutein using marigold. 2010, Chinese patent No.: CN101817772 A.

Source of Support: Nil, Conflict of Interest: None.