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RESEARCH ARTICLE

A Study on Optimization of Marigold Petal Yield, Pure Lutein, and Formulation of Free-Flowing Lutein Esters

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Abstract

The present study was conducted with the main objective to optimize petal yield from important marigold cultivars in West Bengal, and to standardize isolation of lutein from petals because these dietary xanthophylls are known to reduce the risks of age-related macular degeneration (AMD) and cataracts. Six cultivars were studied *viz*. African marigold-Double (AFM-D), African marigold-Single (AFM-S), African marigold-Orange (AFM-O), French marigold-Orange (FRM-O), French marigold-Double (FRM-D), and LC (Local type), which withstand typical environmental conditions of northern West Bengal and produce flowers that do not vary in color and are relatively unaffected by pests and diseases. Lutein esters were extracted from milled marigold petals using n-hexane. However, overall performance showed that LC is superior to the other cultivars. Pure lutein was obtained after saponification with 50% KOH. The re-crystallized lutein was characterized by UV-VIS, IR spectroscopy, and HPLC. A free-flowing lutein ester was also formulated. This free-flowing lutein ester was found to be suitable to undergo commercialization or subsequent processing. An economic method for petal yield and isolation of lutein was thus standardized.

Key words : HPLC, lutein, lutein esters, marigold, rice-flour, *Tagetes erecta* L.

Introduction

Lutein is an oxycarotenoid/xanthophyll, chemical formula $C_{40}H_{56}O_2$, having molecular weight 568.88 and containing two cyclic end groups with the basic C40 isoprenoid structure (Rice-Evanset al. 1997). Lutein's supremacy over β-carotene increases the demand for lutein and lutein ester globally. Lutein and zeaxanthin are the only carotenoids absorbed (much smaller proportion) in the blood stream after ingestion which accumulates afterwards in the human retina (Landrum and Bone 2001). Lutein absorbs high-energy blue light from sunlight and protects the retina by filtering out blue light and so has been recognized as a major constituent of the macular pigment of the human retina. Age-related macular degeneration (AMD) accounts for 8.7% of all cases of blindness globally and is considered as the leading cause of irreversible blindness in developed countries (Wong et al. 2006). In India, data revealed that prevalence of AMD ranged from 1.8 to 4.7% (Nirmalan et al. 2004). In addition, dietary lutein has been also studied as an agent of cancer prevention and immune accelerator (Chew et al. 1996). It also acts as an active antioxidant to quench active radicals. Recent studies have shown that lutein has systemic anti-inflammatory (Izumi-Nagai et al. 2007) properties which noticeably decreases circulating levels of the complement factor CFD, C5a, and C3d levels, which might play a role to control the inflammatory pathway of the innate immune system (Tian et al. 2013). The diets rich in lutein might significantly reduce the risk of chronic diseases, such as heart disease, cancer, and age-related eye diseases. In addition to this, lutein administration has led to beneficial effects in various models of experimental inflammation such as endotoxin induced uveitis (Jin et al. 2006), experimental age-related macular degeneration (Izumi-Nagai et al. 2007), retinal ischemia (Liet al. 2009), and diabetic retinopathy (Muriach et al. 2006). The consideration of lutein as a vitamin has also been discussed, standing on the fact that meets the three requirements to be

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considered as an essential nutrient: (a) it cannot be synthesized by human metabolism and must be ingested; (b) the consumption of a lutein-deprived diet has been proven to cause blindness in primates; (c) the dysfunction can be reverted by the reintroduction of lutein either dietary or as a supplement, as long as the condition does not become irreversible (Semba and Dagnelie 2003). Hence, the uses of extracts containing lutein in the formulation of nutritional supplements have gained increasing popularity for the prevention of AMD and also as an antioxidant.

Although lutein is a common carotenoid found in most fruits and vegetables, zeaxanthin is present only in small quantities in most fruits and vegetables (Mangels et al. 1993; Sommerburg et al. 1998). Marigold flower petals contain high levels of lutein (of the order 10 g kg^{-1}) and no significant levels of other carotenoids (Verghese 1998). In marigold flower petals, lutein is chemically bound to various types of fatty acids such as lauric, mystric, and palmitic acids. The lutein fatty acid esters are converted to free lutein upon saponification of the marigold extract (Khachik 1995). Commercially, lutein isolated from marigold flowers was first used in chicken feed to provide a yellow color to the skin of broilers and yolks of layers. Currently, lutein (5 to 50%) was obtained from the petals of marigold after an extraction process which yields oleoresins. The oleoresins are obtained mostly in the diester form. Crystalline lutein is very difficult to handle and, hence, is commonly sold as suspensions of the carotenoid in corn or safflower oils. The oleoresin has been found to be a good antioxidant but the plastic-flow caking character of oleoresin is a massive problem.

In this study, we have attempted to formulate the oleoresin in an economic free flow state. Native marigold extracts supply more than 95% of the esterified lutein available (Breithaupt et al. 2002). A solvent method, following saponification, has been widely used for the extraction of free lutein from marigold (Larsen and Christensen 2002). However, the free lutein is susceptible to degradation (Oliver et al. 1998). We mixed oleoresin with rice flour to give a free flow state which was stable up to six months at 27°C. Moreover, we report for the first time from north-east India the results in terms of optimization of both petal and lutein from important marigold cultivars in support of a potential agri-based industry.

Materials and Methods

All the chemicals were purchased from Merck India and used without further purification. All other reagents and solvents were of analytical grade. UV-1700 Spectrometer (Jasco, Tokyo, Japan) and FTIR-8300 spectrophotometer (Shimadzu, Japan) were used for characterization of complexes.

Preparation of soil and plantation

A total of six cultivars of the marigold (*Tagetes erecta* L.) plants viz. African marigold- Double (AFM-D), African marigold-Single (AFM-S), African marigold-Orange (AFM-O), French marigold-Orange (FRM-O), French marigold-Double (FRM-D), and LC (Local type), were grown in an experimental field within the premise of the Centre of Floriculture and Agro-Business Management (COFAM), University of North Bengal. The land was thoroughly cleaned and measurements were done as per layout of beds and paths in between the beds. Cleaning was followed by repeated plowing. Plowed land was left to exposure of sunlight and air for 4 - 7 days. The required soil pH range 5.5 - 6.0 was attained by the addition of CALRICH/LIME @ 140 g M^{-2} to soil and was also drenched with Bilzeb (contact)/Bavistin (systemic) @ 4.0 g L^{-1} to prevent soil-borne diseases. Well decomposed FYM (cowdung) @ 0.57 - 0.65 tons ha^{-1} was mixed thoroughly in the last plow to enrich the soil fertility. The raised beds were prepared for plantation of marigold with the following dimension: length, 9.75 m; width, 1.04 m; height, 0.18 m; bed-to-bed distance, 0.38 m. Planting was done in alternate rows. Plantlets were treated with Bavistin @ 2.5 g L^{-1} for 30 min before planting. Plant to plant spacing (P-P) was 0.53 m and row to row spacing (R-R) was 0.38 m. The experiment was conducted in a total area of 0.017 ha having 12 raised beds. Both inorganic (more precisely semi-organic) and organic practices were tested separately. FYM @ 0.57 tons ha^{-1} was applied as initial dose. Following the initial dose, a second dose of inorganic fertilizers, N : P : K @ 1.0 : 1.5 : 0.7 (26 kg : 13 kg : 13 kg) per 0.677 ha was applied after 3 weeks (21 days) of planting. In fields, Urea-0.05 kg + SSP- 0.07 kg + MOP- 0.05kg was applied per m^2. One half of the total nitrogen used was applied as a top dressing just after 35 days of transplantation. Micronutrient was applied as foliar sprays. Tracel-2 solution [5.0 g L^{-1} in water] was sprayed once fortnightly for raising the quality of flowers. Well decomposed cow dung (FYM) was applied @ 0.65 tons ha^{-1} during land preparation. Dry Neemkhol-Mustard cake @ 0.07 kg M^{-2} (5 : 1) was applied as a top dressing at 2 weeks of planting. Liquid manure [cow dung solution (2.5%, w/v)] was sprayed at intervals of 10 days.

Preparation of dry petal meal

Fresh marigold flowers were picked from experimental trial plots and petals from all varieties of flowers were removed manually immediately after harvest. Fresh petals were given to direct sunlight separately on the ground and continuously exposed to sunlight for 10 days. After removal of 80% moisture, final drying was in Hot Air Oven (Digilab, India) maintained at 50 - 60°C. Dried petals were ground in the mixer grinder (Mahananda Mixer Grinder 230V,India). The speed of the blade was initially maintained at low and as the size of fragmented particles became shorter, the speed of the blade was increased and maintained till complete grinding. Thus, each batch of grinding took about 10 - 20 min; the grinding process was carried on until the dried marigold flower meal was crushed to a mesh size of less than 600 - 800 microns. After grinding, the powder was packed in a plastic pot and stored in a dark place at 25 ± 2°C.

Table 1. Foliage and flower performance of French, local, and African marigold cultivars

Table 1A. Foliage performance

Marigold cultivar	Average initial plant height (cm)	Av. no. of leaves (during planting)	Av. plant height (cm) after 25 days	No. of shoots/plant (average)	Av. height of mature plants (cm)	Av. canopy diameter (cm)
French	9.38 ± 1.3[b]	5.4 ± 1.51[a]	13.52 ± 4.26[a]	6.8 ± 1.3[a]	47.54 ± 10.61[ab]	19.8 ± 6.4[a]
Local	6.7 ± 1.43[a]	5.4 ± 1.14[a]	9.4 ± 1.19[a]	6.8 ± 0.89[a]	35.58 ± 5.9[a]	17.24 ± 0.97[a]
African	8.74 ± 11.98[ab]	4.6 ± 1.51[a]	25.08 ± 8.42[b]	14.8 ± 3.89[b]	57.5 ± 10.29[b]	27.46 ± 4.39[b]

Means in the same column with different letters are significantly ($P< 0.05$) different.

Table 1B. Flower performance

Marigold cultivar	No. of Flower buds/plant (1st Flush)	Flower diameter (cm)	No. of Flower buds/Plant (2nd Flush)	No. of Flower buds/Plant (3rd Flush)	No. of Flower buds/Plant (4th Flush)	Wet weight per 25 flowers (g)	Total flower production/plant (kg)
French	40 ±12.39[a]	5.7 ±1.38[b]	68 ±10.33[a]	50 ±20.86[a]	53.8 ±23.2[a]	154.5 ±51.06[b]	1.27 ±0.28[b]
Local	61 ±9.44[b]	2.54 ±0.16[a]	90 ±7.91[b]	55 ±11.14[a]	96 ±13.23[b]	65.1 ±17.66[a]	0.77 ±0.17[a]
African	43.6 ±8.59[a]	5.74 ±1.45[b]	77.4 ±13.9[ab]	52.6 ±7.02[a]	51.8 ±8.46[a]	129.1 ±16.22[b]	1.17 ±0.17[b]

Means in the same column with different letters are significantly ($P< 0.05$) different.

Extraction and purification of Lutein

Isolation of oleoresin: The extaction and purification was done by following 50 g of the dried powder was placed in an amber colored bottle with about 150 mL hexane and shaken and filtered through cotton filter and the process repeated thrice. The combined extract was evaporated in vacuum rotary evaporator at room temperature. The yield of the oleoresin as gum was about 11% (weight/weight). Spectroscopic and chromatographic analyses were done to characterize the oleoresin.

Saponification of oleoresin: The oleoresin was subjected to hydrolysis by stirring to mix one unit volume oleoresin with three times of isopropanol at 60°C, until a free-flowing solution was obtained. An aqueous 50% potassium hydroxide solution (equivalent to 20 - 25% of the Oleoresin) was added slowly and the solution was maintained at 60 - 65°C with constant stirring for 45 min. The progress of saponification reaction was monitored by TLC silica gel60 F254 (MERCK, Germany) (petroleum ether : acetone : diethyl amine, 2.0 : 0.8 : 0.2). The saponified mixture was allowed to cool to room temperature and then diluted up to 50% (volume/volume) with deionized water. After 60 min, the mixture was further diluted four times (v/v) with deionized water and centrifuged (REMI C 24, India). The orange-colored precipitate was collected and washed thrice with water and dried. The dried precipitate contained 5% moisture. This lutein was preserved in aluminium wraps for future studies.

Recrystallization: Recrystallization of purified lutein was done from dried acetone. This precipitate was found to be crystalline, having an orange color, and was characterized.

Characterization of lutein: The purified lutein was characterized by UV-VIS and IR spectroscopy. Sampling injector (Rheodyne Mod 7725, Sigma-Aldrich, India) was used for injection in HPLC (Waters 2487 Dual λ Absorbance Detector, Ireland) study. The C-18 Analytical Column (Waters Part No. 186000112, Ireland) (diameter of 4.6 mm, length of 250 mm, stuffing diameter of 5 µm) was used with a guard-column (diameter of 4.6 mm, length of 50 mm, stuffing diameter of 10 µm, methanol at the flow rate of 1.0 mL min^{-1} as eluent) and scanned at 445 nm. This recrystallized lutein was used as standard for measuring lutein content in other samples. The spectrum was taken and matched with standard spectra.

Formulation of oleoresin: Commercial white rice was ground (using a laboratory blender) to flour through a 0.4 mm screen. Oleoresin (20 g) was mixed well with 10 g rice flour in a mortar and pestle. The mixture was stored in a dark and sealed container at room temperature.

Study of shelf-life of the formulation: The lutein content in the formulation was recorded regularly for six months using the aforesaid UV-VIS spectroscopy and HPLC method.

Statistical analysis

Results are expressed as mean value ± standard deviations (S.D) of three replicates. A one-way ANOVA analysis was applied to the obtained data, as well as Duncan's multiple range test in order to establish the statistical significance of difference. Significance was tested at a 5% level. The software SPSS 15.0 for windows (SPSS Inc., Chicago, IL, USA) was used for this purpose.

Results and Discussion

Plant growth characteristics and yield of petals

Overall plant growth, floral characteristics, and petal yield (wet weight and dry weight) of different cultivars tested in the COFAM premise of the University of North Bengal. Growth and morphological data of six different cultivars of marigold grown in different experimental plots showed significant variation; changes observed in both vegetative and reproductive parts of the plants were evident in the data-

Table 2. Pests, diseases, and their control measures in marigold cultivation

Table 2A. Diseases and pests encountered, and control measures taken during marigold cultivation

Type of Infection	Name of the disease/pest	Cause/causal organism	Symptoms	Control measures
Fungal	Brown and black leaf spot	*Septoria* sp.	Circular to irregular tan to brown spots developed on leaves; circular brown spots were also found in the ventral surface	MANCOZEB @ 2.5 g L^{-1} was sprayed every 4-7 days incase of severe infection. Companion (Mancozeb + Matalaxyl) was also used @ 2.0 g L^{-1} as spray with an interval of 7-10 days.
	Blight	*Botrytis cinerea*	Emergence of brown to black spots on leaves eventually spreads to flower parts; incidence of this disease is significant during prolong phases of cloudy, humid and wet weather conditions.	Immediate removal of the affected plant parts. Avoidance of wetting flowers and buds during watering. Proper soil drenching and application of Dithan M-45 and MATCO @ 4.0 g L^{-1} and 2.0 g L^{-1} respectively every 5 days.
	Bud rot	Unidentified organism	Small necrotic brown spots on unopened flower buds followed by complete damage of flower buds; occurrence noted particularly during a prolong phase of high humidity and temperature conditions.	Spraying of single dose of COMPANION @ 1.5 g L^{-1} every 15 days.
Pest	Leathery and wrinkling of leaves	Red mite	Observed during commencement of flowers; plant sap being sucked by mites left the leaves to turn leathery and wrinkled; insects found in the midrib on the lower surface of leaves.	Spraying KELTHAN @ 2.0 mL L^{-1} of water every 7 days of interval; alternatively, THIODAN @ 1.5 mL L^{-1} every 10 days.
	Yellowing of leaves	White fly	Yellowing of leaves and gradual drying of the entire plants are the visible symptoms; defoliation and shedding of buds were noted in severe cases.	Spraying CONFIDOR @ 1.0 mL L^{-1} every 7 days.
	Stunted growth & curling of foliage	Aphid	Tiny insect causing profuse damage leading to stunted growth; lowering of plant growth with curling of foliage in few plants. With infestation of aphids, sticky exudations noted on new buds and leaves.	Better result obtained by spraying NUVAN @ 1.5 mL L^{-1} every 7 days.
	Shedding of leaves	Caterpillar	Caterpillar infection commences during flowering period; Sucking of cell sap from leaves and flowers results in earlier shedding of leaves and flower buds.	Spraying of DECIS @ 0.5 mL L^{-1} every 10 days.
Deficiency of micronutrients	Splitting of flower bud	Deficiency of micro-nutrients	Indiscriminate splitting of buds followed by dropping of immature flowers.	Foliar spray of TRACEL-2 @ 5.0 g L^{-1} of water every 10 days.

Table 2B. Assessment of the susceptibility to pests and diseases of marigold cultivars

Marigold cultivar	Incidence of diseases and pests	Overall health condition
French	Botrytis blight, aphid Red mite Brown and black leaf spot Blight Leaf blight	Susceptible to fungal attack of aerial parts and also susceptible to two pests.
Local	Leaf spot Red mite	Least susceptible to disease and pest.
African	Brown &black leaf spot White fly & brown, black leaf spot Splitting of flower bud Red mite Brown & black leaf spot	Vegetative and reproductive both susceptible to fungus and pest.

Table 3. Yield of wet and dry petal, oleoresin, and lutein per cultivar of marigold

Variety of flower	Color of flower	Weight of petal (per kg flower)	Weight of (sun dried) dry petal (per kg flower)	Oleoresin yield in gm (per 50 g marigold meal)	Lutein yield in gm (per 50 g marigold meal)
LC	Magenta bicolour	0.82 ± 0.017^{a}	0.14 ± 0.003^{d}	2.90 ± 0.1^{c}	1.30 ± 0.07^{b}
AFM-S	Semi double	0.75 ± 0.045^{ab}	0.14 ± 0.003^{cb}	2.40 ± 0.15^{b}	1.20 ± 0.15^{b}
AFM-D	Double yellow	0.81 ± 0.062^{a}	0.13 ± 0.004^{b}	2.00 ± 0.1^{a}	0.90 ± 0.05^{a}
AFM-O	Deep orange	0.78 ± 0.036^{a}	0.13 ± 0.005^{b}	4.30 ± 0.18^{d}	2.10 ± 0.1^{c}
FRM-D	Deep yellow	0.69 ± 0.055^{b}	0.12 ± 0.004^{a}	2.30 ± 0.12^{ab}	1.25 ± 0.076^{b}
FRM-O	Slightly orange	0.83 ± 0.045^{a}	0.14 ± 0.003^{c}	3.10 ± 0.15^{c}	1.40 ± 0.015^{b}

Means in the same column with different letters are significantly ($P < 0.05$) different.

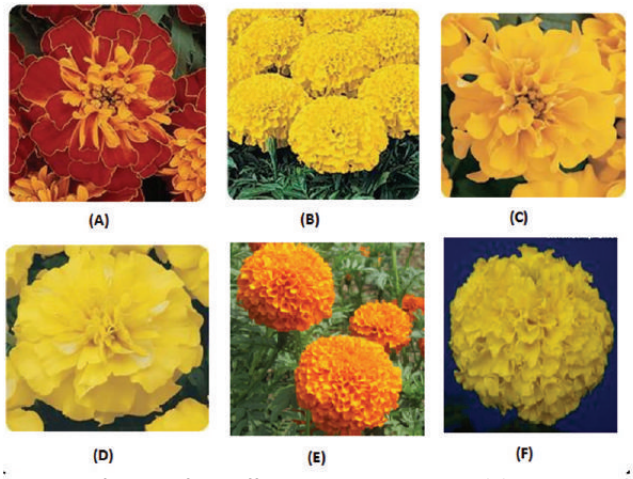

Fig. 1. The flowers of six different marigold cultivars. (A) Local type; (B) African marigold-Single; (C) French marigold-Double; (D) African marigold-Double; (E) African marigold-Orange; (F) French marigold-Orange.

Tables 1A & B. The average plant height of the African variety (57.5 cm) was significantly different from the local variety (35.58 cm). The leaves appeared in 2 - 3 days earlier in African marigold than the other two varieties. In terms of floral characteristics, the color of flowers in African marigold, French marigold, and local variety varied from orange to deep orange, orange to yellow, and magenta bicolor, respectively (Fig. 1).The average diameter of the full-bloomed flower was similar in French marigold and African marigold. Significant difference was also observed in a variety-wise flower wet-weight per plant; the highest and lowest values were recorded for African and local variety, respectively. It was also observed that the African variety was superior in terms of flower diameter and number of flowers per plant compared to the French and local cultivars. Local cultivar was found to be least susceptible to diseases and pests. The diseases and pests that were encountered during the cultivation and measures taken have been summarized in Tables 2A and B.

The petal and lutein yields of different varieties are shown in Table 3. Petal yield (wet-weight) kg^{-1} of marigold flower was least in FRM-D (French marigold variety that yielded deep yellow-colored flowers) compared to other five cultivars. Sun drying of loose petals contributed to de-hydrating to the extent of 81 - 84% in the three varieties. The data showed that the net recovery of petal dust from dried petal after grinding, sieving, and storage varied from 80 - 89%.

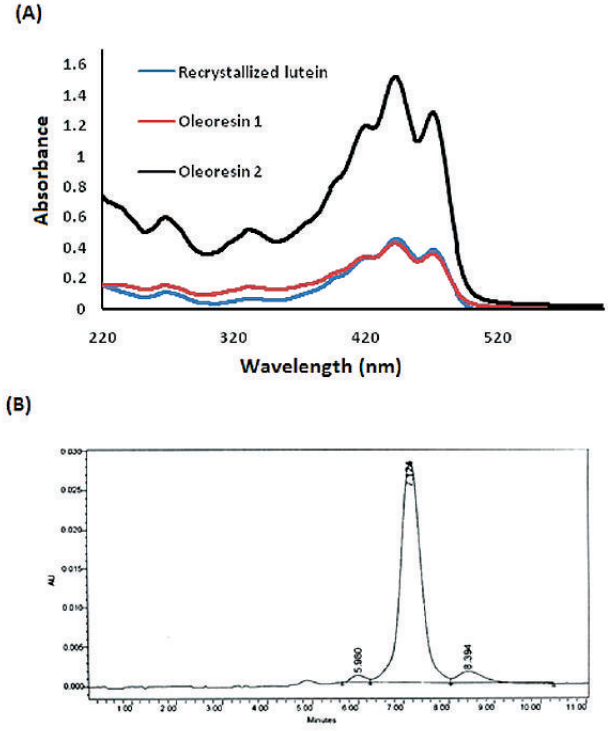

Fig. 2. UV-VIS spectra and HPLC profiles of marigold oleoresin and re-crystallized lutein. (A) UV-VIS spectra of oleoresin and re-crystallized lutein, (B) HPLC chromatogram of re-crystallized lutein.

Purification and characterization of lutein

The hydrolysis of oleoresin was checked by TLC and comparing the characteristics chromatogram of oleoresin and lutein. It was found that oleoresin gets hydrolyzed. In UV-Vis absorption spectra of lutein two characteristic absorption peaks were observed, λ_{max} at 445 nm and second at 472 nm (Khachik et al. 1997) as shown in Fig. 2A. The characteristic band at 962 cm^{-1} was also found in IR spectroscopy (Chen et al. 2009). The HPLC peaks at 445 nm on chromatogram were identified by retention times (7.12 min) for pure lutein (Sujith et al. 2012; Fig. 2B).

It was found that on the basis of the yield of the petals as well as weight of dry flower dust after grinding and sieving per kg of flower, LC variety's performance was better followed by AFM-Ovariety. But employing the simple HPLC method, the yield of lutein was highest from the petal dust of AFM-O. Thus AFM-O and LC could be preferred for lutein or lutein esters extraction in northern region of West Bengal.

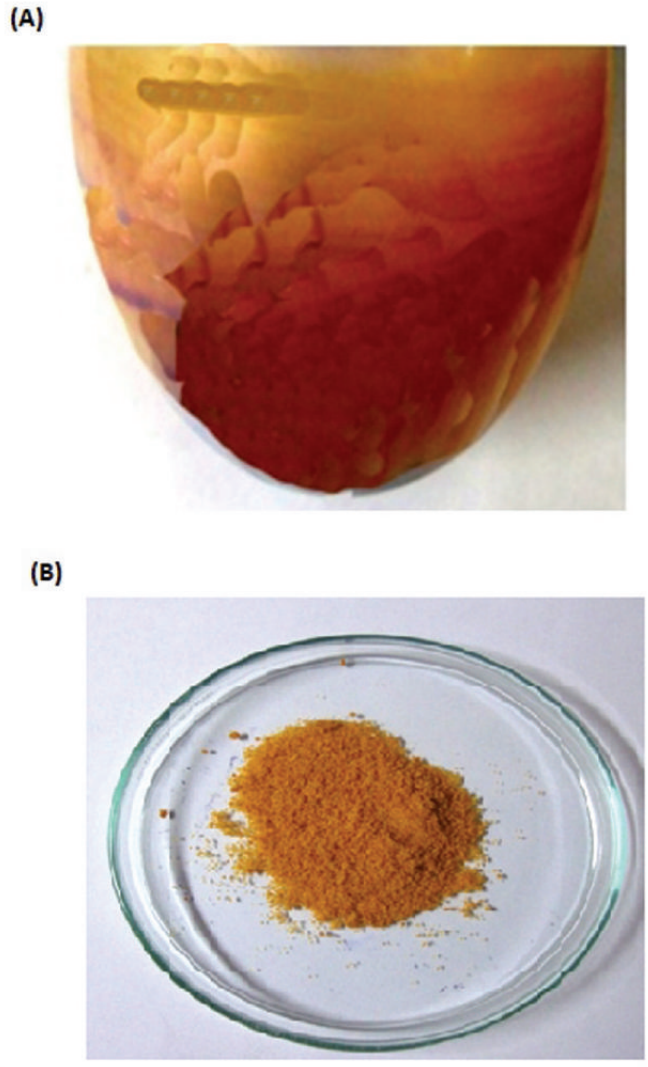

Fig. 3. Marigold oleoresin in absence and presence of rice-flour as anti-caking agent. (A) Marigold oleoresin in plastic-flow caking state, (B) Marigold oleoresin in free-flow state.

Formulation of lutein esters

The extracted marigold oleoresin, in the form of a solid or a paste, has a high viscosity at room temperature, and the content of lutein-fatty acid ester in it varied between 14 to 20%. Most of the commercially available marigold oleoresin suffers from major disadvantages like reduced flow ability due to stickiness (plastic-flow caking) and often losses of quality on prolonged storage. Caked and lump conditions cause packaging and performance problems. The ease of handling highly viscous marigold oleoresin is extraordinarily simplified by using rice flour. In this study, it was observed that rice flour when added to oleoresin has prevented ingredients from sticking together to permit a free flowing condition. The free flowing powder, orange in color, in comparison to the sticky oleoresin without addition of rice flour, is shown in Fig. 3. The UV-VIS spectra of this formulation taken after six months of storage in the sterile vials have confirmed the stability of the formulated oleoresin. The rationale for choosing rice flour as an anti-caking agent was because it is traditionally used in India as a food additive that prevents agglomeration and foster preservation of several homemade foods. Rice flour produced by grinding is used as a dusting or anticaking agent for refrigerated biscuit dough (Nishita and Bean 1982). It was reported that rice flour can also be used as an anti-caking agent to improve the flow ability of hygroscopic compounds like table salt (Akay et al. 2009). In addition to several uses of rice flour as an anti-caking agent, the present study proposes the specific use of rice flour to prevent plastic-flow caking of marigold oleoresin.

Conclusions

We can pertinently tell that the present work could orchestrate a platform for the floriculture and commercial preparation of high quality lutein from marigold in West Bengal particularly in the North Bengal region in the existing environment. For a more commercial approach, we have tried to formulate the lutein ester in a free-flow state. On the basis of the yield of the petals as well as weight of dry flower dust after grinding and sieving per kg of flower, the performance of LC variety was better over the other cultivars while AFM-O variety could be the second choice. This finding is important in terms of cultivation of the marigold varieties under the climatic conditions of North Bengal. An efficient method of isolating, purifying, and re-crystallizing substantially pure lutein from dried petals of marigold or marigold oleoresin has been standardized. On the basis of the standardized lab scale process, one can go for scale-up operations.

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References

Akay C, Ogur R, Korkmaz A, Gocgeldi E, Yaren H, Gulec M. 2009. Could rice be used as an anticaking agent in table salt? Int. J. Food Sci. Nutr. 60: 95-99

Breithaupt DE, Wirt U, Bamedi A. 2002. Differentiation between lutein monoester regioisomers and detection of lutein diesters from marigold flowers (*Tagetes erecta* L.) and several fruits by liquid chromatography-mass spectrometry. J. Agric. Food Chem. 50: 66-70

Chen XJ, Wu JG, Zhou SJ, Yang YJ, Ni XL, Yang J, Zhu ZJ,

Shi CH. 2009. Application of near-infrared reflectance spectroscopy to evaluate the lutein and β-carotene in Chinese kale. J. Food Compos. Anal. 22: 148-153

Chew BP, Wong MW, Wong TS. 1996. Effects of lutein from marigold extract on immunity and growth of mammary tumors in mice. Anticancer Res. 16:3689-3694

Izumi-Nagai K, Nagai N, Ohgami K, Satofuka S, Ozawa Y, Tsubota K, Umezawa K, Ohno S, Oike Y, Ishida S. 2007. Macular pigment lutein is anti-inflammatory in preventing choroidal neovascularization. Arterioscler. Thromb. Vasc. Biol. 27: 2555-2562

Jin XH, Ohgami K, Shiratori K, Suzuki Y, Hirano T, Koyama Y, Yoshida K, Ilieva I, Iseki K, Ohno S. 2006. Inhibitory effects of lutein on endotoxin-induced uveitis in Lewis rats. Invest. Ophthalmol. Vis. Sci. 47: 2562-2568

Khachik F. 1995. Process for isolation, purification, and recrystallization of lutein from saponified marigolds oleoresin and uses thereof. U.S. Patent 5,382,714, Jan 17

Khachik F, Bersnstein PS, Garland DL. 1997. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. Invest. Ophthalmol. Vis. Sci. 38: 1802-1811

Landrum JT, Bone RA. 2001. Lutein, zeaxanthin and macular pigment. Arch. Biochem. Biophys. 385: 28-40

Larsen E, Christensen LP. 2002. Simple saponication method for the quantitative determination of carotenoids in green vegetable. J. Agric. Food Chem. 50: 1071-1072

Li SY, Fu ZJ, Ma H, Jang WC, So KF, Wong D, Lo AC. 2009. Effect of lutein on retinal neurons and oxidative stress in a model of acute retinal ischemia/reperfusion. Invest. Ophthalmol. Vis. Sci. 50: 836-843

Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. 1993. Carotenoid content of fruits and vegetables: an evaluation of analytic data. J. Am. Diet. Assoc. 93: 284-296

Muriach M, Bosch-Morell F, Alexander G, Blomhoff R, Barcia J, Arnal E, Almansa I, Romero FJ, Miranda M. 2006. Lutein effect on retina and hippocampus of diabetic mice. Free Radic. Biol. Med. 41: 979-984

Nirmalan PK, Katz J, Robin AL, Tielsch JM, Namperumalsamy P et al. 2004. Prevalence of vitreoretinal disorders in a rural population of Southern India: The Aravind Comprehensive Eye Study. Arch. Ophthalmol. 122: 581-586

Nishita KD, Bean MM. 1982. Grinding methods: their impact on rice flour properties. Cereal Chem. 59: 46-49

Oliver J, Plaou A, Pon S. 1998. Semi-quantification of carotenoids by high performance liquid chromatorgraphy: saponification-induced losses in fatty foods. J. Chromatography A. 829: 393-399

Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. 1997.Why do we expect carotenoids to be antioxidants *in vivo*? Free Radic. Res. 26: 381-398

Semba RD, Dagnelie G. 2003. Are lutein and zeaxanthin conditionally essential nutrients for eye health? Med. Hypotheses 61: 465-472

Sommerburg O, Keunen JEE, Bird AC, van Kuijk FJGM. 1998. Fruits and vegetables that are sources of lutein and zeaxanthin: the macular pigment in human eye. Brit. J. Ophthalmol. 82: 907-910

Sujith PAA, Hymavathi TV, Yasoda DP. 2012. A Study on the different methods of preparation of lutein from supercritical fluid processed lutein esters. J. Nutr. Food Sci. 2: 154

Tian Y, Kijlstra A, van der Veen RLP, Makridaki M, Murray IJ, Berendschot TTJM. 2013. The effect of lutein supplementation on blood plasma levels of complement factor D, C5a and C3d. PLoS ONE 8: e73387

Verghese J. 1998. Focus on xanthophylls from *Tagetes erecta* L.the giant natural complex-I. Indian Spices 33: 8-13

Wong TY, Loon SC, Saw SM. 2006. The epidemiology of age-related eye diseases in Asia. Brit. J. Ophthalmol. 90: 506-511