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Isolation, Stabilization and Characterization of Xanthophyll from Marigold Flower- *Tagetes Erecta-L*

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Abstract

The present paper deals with the chemistry, isolation, separation, characterisation and stabilisation of the Marigold oleoresin and its application as a natural food colorant. Marigold (*Tagetes Erecta L*), an ornamental plant belonging to the composite family, has a rich source of natural antioxidant-Lutein. A natural pigment, xanthophylls offer an alternative to synthetic dyes as a food colorant, due to its non-toxicity. Chromatographic separations of saponified and unsaponified oleoresin were performed and Trans-Lutein identified as the major constituent. Well-preserved flowers exhibit a high yield of Xanthophyll content (105.19 g/Kg) in contrast to the unpreserved flower sample (54.87 g/Kg), emphasizing the significance of flower preservation in the extraction of xanthophyll. The stability and amount of xanthophyll also increased from 105.19 g/Kg to 226.88 g/Kg on saponification and subsequent purification with Ethylene Dichloride.

Keywords: Antioxidant Activity, Colorants, Extraction / Separation, HPLC, Natural Products, Pigments

1. Introduction

Since the early civilizations and in the beginning of the food industry, pigments –natural or synthetic, were used to give an attractive presentation, perception of freshness, taste, and quality of food. Saffron and other plant species were used to provide characteristic color and flavor in food. Today, natural colorants are emerging globally due to the perception of its safer and eco-friendly nature. Nowadays, a trend towards "naturalness" represents a challenge for food manufacturers, because of its pharmacological applications. Synthetic dyes received faster acceptability due to a wide range of applications in various fields like food (Torgils et al. 1998) , cosmetic (Calnan 1976), and more importantly in textile (Savarino et al. 1999 , Paisan et al. 2002) due to ease in dyeing, and reproducibility in shades and overall cost factor. Natural food colorants can be originally present in the foodstuff, or may be added as an extract to enhance the natural color. Quality of food is associated with many aspects -color, flavor, and texture, but color can be considered the most important of them, because of its appealing nature. Color as well gives the key to catalogue a food as safe, with good aesthetic and sensorial characteristics: the undesirable colors in meat, fruits, and vegetables warn us about a potential danger or at least of the presence of undesirable flavors, among other reactions. The food industry is therefore, interested in gaining a better understanding of color generation and color stabilization during the various steps of food processing. The processed food constitute 60- 65% of total food and the need for the food additives and its improved shelf life is also increasing. There are some 2500 chemicals that function as food additives that give rise to some 5000 trade name products on a worldwide basis (Scotter.M.J & Castle.L. 2004). However, the natural pigments that are permitted for human foods are very limited, and the approval of new sources is difficult, because the U.S. Food and Drug Administration (FDA) considers the pigments as additives, and consequently pigments are under strict regulations (Delgado-Vargas et al.2000).

A high quality of food and beverages is vital to our physical and mental well being. Of all the food additives in use, none gives rise to greater controversies than food colors. The readily available coloring matter based on natural products is of considerable importance since the United States have banned the use of synthetic coloring in foods. In ancient times tinted amaranth has been used to extract the coloring matter, which is hydrophilic in nature and was used for the dyeing of drinks, food and other products in Mexico, Bolivia and Ecuador (Gladis et al. 2000). In India and Mexico, for facial rouge the woman used amaranth juice. This pigment belongs to the group of betacyanines (Attoe. L.J. & Von Elbe, J.H. 1985, Mabry T.J & Drieiding, A.S.1968) and the betanine have been used as colorants in many types of food (Pasch, J. H. & von Elbe, J. H .1979). Some of the important plant pigments are carotenoids, Anthocyanins, and

betalains. Carotenoids are compounds comprised of eight Isoprenoid units (Ip) whose order is inverted at the molecule center (Delgado-Vargas et al.2000) (Figure: 1). Carotenoids are classified by their chemical structure as: (i) carotenes that are constituted by carbon and hydrogen; (ii) oxycarotenoids or xanthophylls that have carbon, hydrogen, and, additionally, oxygen. Also, Carotenoids have been classified as primary or secondary. Primary carotenoids group those compounds required by plants in photosynthesis (β-carotene, violaxanthin, and Neoxanthin), whereas secondary Carotenoids are localized in fruits and flowers (α-carotene, β -cryptoxanthin, zeaxanthin, antheraxanthin, capsanthin, capsorubin). Anthocyanins are the most important group of pigments, after chlorophyll, which is visible to the human eye (Harborne et al.1988). Chemically, Anthocyanins (Greek word *anthos* means a flower, and *kyanos*, dark blue) are flavonoids. Anthocyanins (Figure: 2) are substituted glycosides of salts of phenyl-2- benzopyrilium (anthocyanidins). Chemically, betalain definition embraces all compounds with structures based on the general formula (Figure 3) therefore, they are immonium derivatives of betalamic acid (Strack et al.1993).

Carotenoids are abundant in fruits and plants and are widely used an antioxidant and may be useful in the prevention of diseased including cancer. The consumption of lutein and zeaxanthin reduces 40 % of the age related macular degeneration (seddon 1994). The xanthophylls because of their yellow to orange-red coloration and natural occurrence in human foods, also finds its use as a food colorant. Therefore there exist a high demand for the significantly pure Xanthophyll that can be used as a food colorant and a nutrient supplement. Flowers such as *Tagetes* comprise different species about 33 in number, helenium, helianthus, sunflower, dandelion and many others. Of these, most concentrated source of xanthophylls is of the order 4500mg/lb (Verghese, 1998b) in the petals of *Tagetes Erecta* L (African marigold, Aztec marigold, Zempasuchil). Marigold flower petals are a significant source of the Xanthophyll and have a much higher concentration of this pigment compared to other plant materials (Verghese, 1998a). Marigold flower (*Tagetes Erecta* L) is a hardy annual branching herb about 60 to 90 cm tall and erect, grown in certain parts of India- Tamil Nadu, Andra Pradesh , Karnataka as well as in other parts of the World; and is extensively cultivated in temperate climate. It prefers nourishing soil of pH 7.0 to 7.5 with good water-holding capacity and well-drained fertile sandy loamy soil as well as a sunny climate. Mexican's use marigold flower as a traditional medicine and to Romans, an inexpensive food colorant, a substitute for saffron and it was they who introduced to other parts of Europe. Now a days, the marigold flower is exclusively used for the Malayali festival "Onam" in Kerala, India and all over the world to make "pookalam" (flower carpet) (Figure: 4), due to its exotic combination with other vibrant colored flowers.

Depending on the varieties, cultivar and horticulture practices, the yield of flower showed remarkable variations in number and in flower weight (from 11 to 30 ton/hectare). Although flower is made up of petals, calyx pedicle, seeds etc., approximately 40% to 50% of the flower consist of petals. Extraction studies of petals and total flower with hexane showed that only flower petals contain xanthophylls. Calyx contains chlorophyll which in-turn affects absorption of xanthophylls by broilers and layers (Verghese. J 1998b). Hence, only the petals are used for the isolation of oleoresin. The main coloring component of Marigold flower is lutein ($C_{40}H_{56}O_2$). Free Lutein hardly exists in the flower and it naturally occurs in the acylated form (Figure 5). The Lutein ester concentration in fresh Marigold flowers varies from 4 mg/Kg in greenish yellow flowers to 800 mg/Kg in orange brown flowers (Sowbhagya et al. 2004). Dark-colored flowers contain about 200 times more Lutein esters than the lightcolored flowers. Xanthophyll content varies in the range of 9 to 11 g/Kg. The concentration of Lutein varies in different shades of marigold flowers, viz.; greenish yellow to bright yellow and orange brown (Gregory et al.1986). Total Lutein esters have been reported to be in the range of 3.8 to 791 mg/Kg of flower (Sowbhagya et al. 2004). Lutein palmitate is the major ester in the flower. The other esters of lutein identified in the flower are dimyristate, myristate palmitate, palmitate sterate, and distearate (Table: 1). A purified extract of marigold petals mainly containing Xanthophylls dipalmitate is marketed as an ophthalmologic agent (Sowbhagya et al. 2004, Gau et. al. 1983). Lutein is stable in pH range 3 to 9. At extreme pH and in the presence of light, lutein undergoes isomerization resulting in color loss. Lutein structure consists of conjugated bonds, which when react with the oxygen present in air, cause oxidation to take place and lead to color loss. Oxidation products of xanthophylls are mono and di-epoxides, carbonyls, alcohols etc. and extensive oxidation results in bleaching of carotenoid pigments. To minimize color loss, it is safe to pack lutein-containing products in tin or opaque containers. Enzymes like lipoxygenase hasten oxidative degradation, which occurs by direct mechanisms. Enzymes first react with unsaturated or saturated fatty acids producing peroxides, which react with lutein xanthophylls and lead to oxidative degradation. "Blanching" (98^{o}C/5 min) exhibits an apparent increase in xanthophylls content due to inactivation of lipoxygenase and also enhances pigment extraction (Sowbhagya et al. 2004, Alam et al. 1968).

Marigold carotenoids have potential as a natural food colorant. The status of marigold, as a source of natural carotenoids, has been reviewed (Verghese. J 1998a, 1998b). Temperature, pH, light, activity in water is all factors which affects the stability of the pigment (Pasch, J. H. & von Elbe, J. H .1979). Stability of Xanthophyll pigment extracted from marigold has been studied by saponifying. Xanthophylls are usually esterified which produces additional analyses complications and requires both separation and identification. Saponification obtains less complex mixtures when only non-esterified pigments appear. Another advantage of saponification is chlorophyll destruction in the saponified samples (Delgado-Vargas et al.2000). Since commercial extracts are valued by their Xanthophyll and

trans-lutein content, concentration of lutein fatty acid esters in the extract can be enhanced by purification. Xanthophyll content can be boosted by phase partition between 70% and 90% of hexane, acetone, methanol and ethanol. When marigold extract was subjected to precipitation using isopropanol removes 65% of the lipids in the extract are removed. The precipitated fraction contained 51.3% lutein esters and a second precipitation from isopropanol-petroleum ether (80:20) resulted in a product of much higher purity, more than 65% pure (Sowbhagya et al. 2004). Since the availability of marigold flower is seasonal, flower preservation becomes vital in the extraction of marigold oleoresin. If flowers are not properly stored and preserved, Xanthophyll content will decrease. The extraction of xanthophylls (oleoresin) from marigold flower involves the following stages: ensilage, pressing and drying, Hexane extraction, and saponification. Of these, the ensilage is considered important in determining the efficiency of the overall process. Studies were conducted to obtain a relationship between the xanthophylls extraction yield and the efficiency of marigold flower ensilage (Navarrete-Bolanos et al. 2003). It was reported that during the ensilage, structural cell-wall degradation of the marigold petals by saprophytic microorganisms associated with the flower resulted in increased mass transfer during the extraction stage. Such an increase is associated with the level of cellulose synthesized by these microorganisms.

Numerous methods have been proposed in order to improve both xanthophylls extraction and purification efficiency (Ausich and Sanders 1997, Khachik 1995, Philip 1997). But many of them have the drawback of high temperature requirement and long processing times, which can result in the degradation and formation of unwanted isomeric components (Levi 2001, Madhavi & Kagan 2002). Enzymatic treatment has also been proposed as an alternative stage to enhance xanthophylls extraction from marigold flower (Delgado-Vargas. F.& Paredes-López. O. 1997, Navarrete-Bolanos JL.2004). But it too had practical limitations, due to the use of expensive commercial enzyme. It was reported that solid-state fermentation process of marigold flower showed improved yield efficiency (Soboleva 1978). Marigold extracts have been commercialized internationally and are used as additives for poultry feed, as they provide bright colors in egg yolks, skin, and fatty tissues. Marigold extract also finds application in coloring foods like edible oils, mustard and other salad dressings, cakes, ice cream, yogurt, and dairy products. The extract with only the purified form with a lutein content of known concentration and a pure crystalline lutein isolated from marigold flower is allowed for food use. *Tagetes* meal and extract has been listed with a color index of 75125, and it is allowed in chicken feed only to a maximum limit of 1% (Vernon-Carter 1996).

2. Material and methods

2.1 Reagents and equipment

All the reagents used were of A.R. grade or the best quality available and milli -Q water was used throughout in the analysis. A UV-Visible spectrophotometer with 1 cm quartz cell was used for the absorbance measurements and HPLC was used for the separation and analytical measurements.

2.2 Experimental

The method involved in this study was extraction, saponification, separation, identification, and quantification.

2.2.1 Isolation of Oleoresin- Extraction:

The raw sample- marigold flower was provided by Synthite Industrial chemicals Ltd, Kolenchery, Kerala, where the experimental part have been carried out. The experiment was carried out with two set of flowers (A1 & A2) - one stored without preservation and the other with proper preservation technique. For processing, the full-blown marigold flowers having minimum calyx portion are taken and was then laden in a room. It was then compressed and sprayed with lactic bacterial culture, covered with a layer of lime and covered with black tarpaulin. This will induce lactic acid fermentation under anaerobic condition. Under this condition, the material can be stored for 3 to 4 months. By this technique, it was reported that xanthophylls are stabilized and preserved. The water from the raw material was removed and then dried in a drier for 8 to 10 hour under controlled conditions at a temperature lower than 60- 65^{o}C to a moisture level of 8% to 10%. The dried flowers are powdered to a particle size of 0.5 mm and then extracted. Of the solvents, hexane, acetone, ether, isopropyl ether, methylenechloride, 1, 2- dichloroethane, chloroform, hexane- acetone and hexane- acetone-toluene; hexane was found to be the efficient solvent (Verghese.J 1998b) for better yield of xanthophylls. The powdered marigold flowers were then packed in a column and were eluted using analytical grade hexane under mild conditions (30°C, for 15 min). The extract (miscella) so obtained was distilled, under controlled conditions until the desired quantity of the solvent in the oleoresin was achieved. In order to prevent the degradation of xanthophylls, 0.1 to 0.3 % of ethoxyquin was mixed to the final product with stirring at a temperature less than 45^{o}C.

2.2.2 Saponification

Most carotenoids including xanthophyll are stable under alkaline treatments; thus, the use of methanolic solutions of potassium hydroxide is a common method of saponification, which de-esterifies the pigment to free xanthophylls, sometimes at room temperature or by heating. Saponification was accomplished with 40% methanolic KOH by 20 minute treatment at 56^{o}C. Enhanced stability of xanthophylls was obtained when the esters are partially saponified, then

neutralized with weak acid - acetic acid, propionic or lauric acid etc, so that the final product with pH greater than 8 contains about 10 to 20% by weight of unsaponified original xanthophylls esters. Oleoresins initially containing 12.5 % xanthophylls esters saponified to 85% and 100%, after 75 days of storage at 40^{o}C drops down to 5.2% and 33% respectively (Verghese.J 1998b) and this result established the merit of selective, incomplete saponification in conferring the stability. The alkali treated xanthophylls can be either incorporated to poultry feed itself or as the solution of the concentrate in vegetable oil and other oils.

2.2.3 Separation

Separation methods can be classified as non-chromatographic and chromatographic. Non-chromatographic method uses mainly phase partition, by using petroleum ether and aqueous methanol (90%) or ethylene dichloride (EDC) and chromatographic methods adsorbents used are sucrose, silica gel etc. The product obtained after extraction was then washed with EDC. The solvent was removed and dried.

2.2.4 Characterization

The most important technique in xanthophylls (carotenoid) analyses is UV-visible spectroscopy, which gives information about the presence of rings, carbonyl groups, and isomeric effects. In this analysis, absorption maxima, form, and fine structure of spectra are characteristic of the molecule's chromophore. The purity of compounds is obtained with a diode array detector (DAD), which makes the HPLC a versatile technique due to greater sensitivity, resolution, reproducibility, speed of analysis and flexibility to use at inert conditions.

2.3 Analysis of pigment

The oleoresin was analyzed by AOAC method to determine the total xanthophylls concentration and by HPLC-high-performance liquid chromatography (Hellish, 1990) to determine the profiles of the main components (Dietmar 2005). The xanthophyll esters obtained are unstable and are stabilized by saponification, which also resulted in the boosting of xanthophylls content. About 0.05g of oleoresin was transferred into a 100 ml amber colored volumetric flask and added 30 ml extractant (Hexane, 10 ml + Acetone, 7ml+ Absolute alcohol, 6ml + Toluene, 7ml). 2ml, 40% methanolic KOH was added and shaken for a minute. The flask was then kept in a water bath (56^{o}C) for 20 minutes. Cooled and kept in the dark for an hour. Added 30 ml of hexane, shaken for 1 minute and was then diluted with 10% Na_2SO_4 and kept in dark for an hour. The upper phase was 50 ml and with this part absorbance and chromatographic analysis were carried out.

2.3.1 Spectrophotometric method

0.5 ml of the upper phase was transferred into a 50 ml flask and made up to mark with hexane. Absorbance was measured at 474nm using hexane as reference.

Total xanthophylls = (A_{474} D) / (W 236); Where

A_{474} = absorbance at 474nm

D = Dilution factor

W = sample weight

236 = specific absorbtivity of Trans- lutein (g/L)

2.3.2 Chromatographic separation-HPLC

Aliquots of the *Tagetes* extract obtained from the hexane extraction were isocratically separated using HPLC, over a C18 column in a normal mode. The colored fraction was separated using a mobile phase of a mixture of hexane and ethyl acetate (65:35). About 1 ml of the upper layer was diluted with hexane and 20 µL of it was injected to the column and was eluted at 1.0 ml/ min at room temperature. The pigments were monitored at 447 nm. The components profile was obtained using the relative percentage of HPLC chromatogram area.

3. Results and discussion

Both saponified and unsaponified extracts of marigold were analyzed and from the chromatogram it was observed that in both cases, the main component of oleoresin was Trans-Lutein. Saponification converts Xanthophyll esters to its free form and more peaks are found in the chromatogram of saponified product. Chromatogram peaks were identified by comparison to the retention time of standards and the peaks obtained were analogous to Cryptoxanthin, Cis-Lutein, Trans-Lutein, Trans-Zeaxanthin and some epoxides (Figure: 6, 7).

If the flowers are not properly stored and preserved or else are of poor quality, then assay show a low xanthophyll content. In the first run of the experiment, carried out using an unpreserved flower sample (A1), the yield and xanthophylls content was 4.62% and 54.87 g/Kg respectively. Higher value was observed when the experiment was repeated with well preserved (sample-A2) flowers (Table: 2). Xanthophyll isolation by solvent extraction method in contradictory to the new studies (enzyme based flower preservation, solid state fermentation etc) showed a less efficient

result due to the high temperature involved in isolation step and degradation of the component. The extracted oleoresin was enriched from 105.19 g/Kg Xanthophyll content to 226.88 g/Kg Xanthophyll content, by saponification and subsequent extraction with ethylene dichloride.

Although marigold flower is a cheaper source as a starting material for the isolation of lutein, its storage in seasonal times is very important. From the present work, it was found that, the sample without proper preservation had a diminution in the xanthophylls deduce the implication of preservation technique. A suitable technique for storage enhances the stability of the pigment in the flower and hence an exhaustive study in this area is required. This study involved an anaerobic and lactic acid treatment for the preservation of marigold flowers. Xanthophyll can retain some of the solvent from which they are isolated and purified. The solvents can be easily removed by drying the oleoresin at higher temperature, but in some instances the solvent hardly escapes from it. The traces of toxic organic solvents in the oleoresin makes it unfit for the human consumption as a food colorant. Still another disadvantage of this solvent extraction process is the hazardous organic wastes that face disposal problem.

4. Conclusion

The toxicological effects of the synthetic dyes in the food industry gave way to a renewed interest in the isolation of natural pigments. With the growing legislative restrictions on the use of synthetic colors, a reappraisal of natural plant pigments is taking place with a view to use them as possible colorants in foods. With the application of new innovations, natural pigments can become more cost effective, increase their competitiveness against certified dye, and dye products. According to the Code of Federal Regulations, marigold oleoresin should pursue the prescribed specification and only purified lutein can be used in food applications for human consumption. The antioxidant property of the lutein crafts it application even in making organic tea, which claims great medicinal value. Since large quantities of pesticides are used in the cultivation of marigold flower, the toxic components may be present in the marigold oleoresin. With the more studies of marigold extracts, showing its safety and non-toxicity, marigold flowers can be good source of natural orange colorant in food applications. Advanced biotechnology can improve the novel varieties of marigold having higher Lutein content to elevate Xanthophyll yield. As a food colorant, toxicity determination is valuable and hence the evaluation of solvents used and the study of toxicants in oleoresin are being in the experimental stage. The quantification of some of the toxicants-pesticides and trace metals, in the marigold flower and its oleoresin is also under study to scrutinize the use of marigold oleoresin as a natural food colorant without any impairment.

References

Attoe, E. L. & von Elbe, J. H. (1985). Oxygen involvement in betanine degradation: effect of antioxidants, *Journal of Food Science.*, 50: 106–110.

Ausich, RL, Sanders, DJ . (1997). Process for the formation, isolation and purification of comestible xanthophyll crystals from plants. US Patent 5, 648, 564.

Calnan, C.D. (1976). Quinazoline yellow SS in cosmetics. Contact Dermatitis, 2(3), 160- 166.

Delgado-Vargas, F. and Paredes-Lopez, O. (1997). Effects of enzymatic treatments of marigold flowers on lutein isomeric profiles, *Journal of Agricultural and Food Chemistry,* 45(4): 1097–1102.

Delgado-Vargas.F, Paredes-Lopez, O, Jimenez, A.R. (2000). Natural Pigments: Carotenoids, Anthocyanins, and Betalains — Characteristics, Biosynthesis, Processing, and Stability. *Critical Reviews in Food Science and Nutrition,* 40(3):173–289.

Dietmar, E, Breithaupt, Jorg Schlatterer. (2005). Lutein and Zeaxanthin in new dietary supplements — analysis and quantification. *European Food Research and Technology* 220: 648–652.

Gau, W., Plosche, H. J., Wunsche, C. (1983). Mass spectrometric identification of xanthophyll fatty acid esters from Marigold flowers (Tagetes Erecta) obtained by high performance liquid chromatography and counter current distribution. *Journal of Chromatography*, 262: 277–284.

Gladis Ester Scoles, Silvia Haydee Pattacini, Guillermo Federico Covas. (2000). Separation of the pigment of an Amaranth. Molecules, 5: 566,567.

Gregory, G.K.; Chen, T.S.; Philip, T. (1986). Quantitative analysis of lutein esters in marigold flowers by high performance liquid chromatography. *Journal of Food Science*, 51: 1093–1094.

Harborne, J. B., Marbry, T. J., And Marbry, H., (1988), The Flavanoids .London. Chapman & Hall.

Helrich, K. (1990). A.O.A.C. Official Methods of Analysis. 15th ed. Arlington, Virginia, 1048–1049.

Khachik, F . (1995). Process for isolation, purification, and recrystallization of lutein from saponified marigold oleoresin and uses thereof. US Patent 5, 382, 714.

Levi, LW . (2001). Trans-xanthophyll ester concentrates of enhanced purity and methods of making same. US Patent 6,191, 293.

Mabry, T.J; Drieiding. A.S. (1968). The Betalaines. In Recent Advances in Phytochemistry, (1968).

Madhavi, DL, Kagan DI . (2002). Process for the isolation of mixed carotenoids from plants. US Patent 6, 380, 442.

Navarrete-Bolanos,JL, Jimenez-Islas, H, Botello-Alvarez, E, Rico- Martinez, R. (2003). Mixed culture optimization for marigold flower ensilage via experimental design and response surface methodology. *Journal of Agricultural and Food Chemistry.* 51: 2206–2211.

Navarrete-Bolanos, Jose Luis, Jimenez-Islas Hugo, Botello-Alvarez Enrique, Rico- Martinez Ramiro, Paredes-Lopez, Octavio. (2004). An optimization study of solidstate fermentation: xanthophylls extraction from marigold flowers Applied Microbiology & Biotechnology, 65:383–390.

Paisan, K, Aroonsiri, S, Nontalee, C. (2002). Thermodynamics of adsorption of laccaic acid on silk, 53(2): 179–185.

Pasch, J. H. and von Elbe, J. H.. (1979). Betanine stability in buffered solutions containing organic acids, metal cations, antioxidants, or sequestrants, *Journal of Food Science,* 44: 72–74,81.

Philip, T . (1997). Purification of lutein-fatty acid esters from plant materials. US Patent 4, 048, 203.

Savarino, P, Viscardi, G, Quagliotto, P, Montoneri, B.E. (1999). Reactivity and effects of cyclodextrins in textile dyeing. Dyes and Pigments, 42(2):143–147.

Scotter, M.J, Castle, L. (2004). Food Additives and Contaminants, 21: 93–124.

Seddon. (1994). *Journal of American Medical Association*, 272 (18): 1413-1420.

Soboleva, M. I., Selezneva, G. D., Bondareva, E. S. (1978). Improvement of quality and shelf life of butter creams. Izvestiya-vyssikh- Uchebyki- zavenii, Pishchevanya Tekhnologiya. 2: 99–101.

Sowbhagya. H. B, S. R. Sampathu, Krishnamurthy. (2004). Natural Colorant from Marigold-Chemistry and Technology Food reviews International, 20(1): 33–50.

Strack, D., Steglich, W., Wray, V., (1993). Betalains. In: Methods in Plant Biochemistry Vol. 8, Academic Press, Orlando, 421–450.

Torgils, F, Luis, C, Oyvind, M.A. (1998). Colour and stability of pure anthocyanins influenced by pH including the alkaline region. Food Chemistry, 63(4): 435–440.

Verghese, J. (1998a). Focus on xanthophylls from Tagetes Erecta L the giant natural complex-I. Indian Spices 33(4): 8–13.

Verghese, J. (1998b). Focus on xanthophylls from Tagetes Erecta L the giant natural color complex-II. Indian Spices 34(1&2): 13–16.

Vernon-Carter, E. J., Gomez, S. A., Beristain, C. I., Mosqueira, G., Pedroza-Islas, R., and Moreno- Terrazas, R. C. (1996). Color degradation and coalescence kinetics of Aztec marigold oleoresin-in-water emulsions stabilized by mesquite or Arabic gums and their blends, *Journal of texture studies,* 27: 625–641.

Table 1. Composition of lutein fatty acid esters (%)

(Ref: Sowbhagya et al. 2004)

Xanthophyll Type	Gau et al	Helrich et al
Dipalmitate	35.5	37.57
Dimyristate	12.6	11.57
Myristate - palmitate	24.7	24.23
Palmitate-stearate	14.4	15.55
Di-stearate	2.4	3.63

Table 2. Comparative results of sample A1 and A2
(Xt – Xanthophyll content)

Sample	Moisture of flower (%)	Oleoresin Yield (%)	Xt (g/Kg)	T-Lutein in Xanthophyll (%)
A1	10.0	4.62	54.87	36.62
A2	12.1	9.12	105.19	70.28

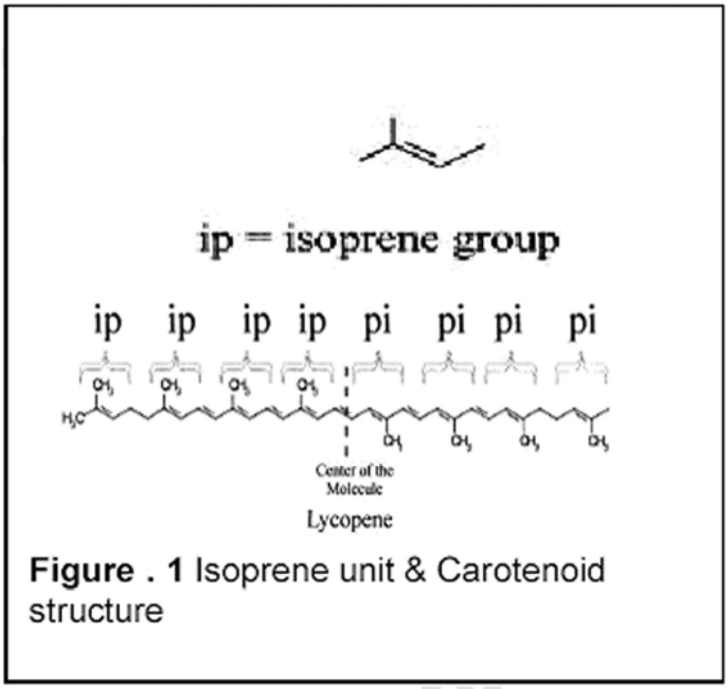

Figure . 1 Isoprene unit & Carotenoid structure

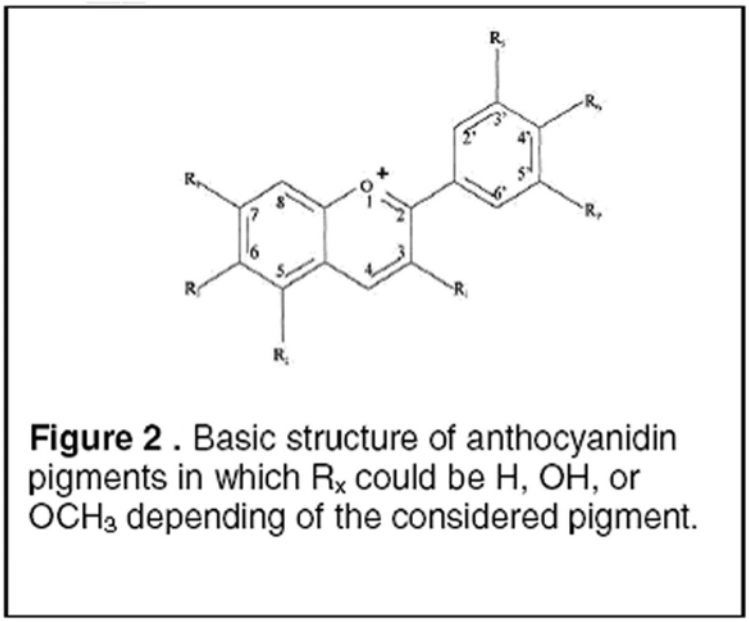

Figure 2 . Basic structure of anthocyanidin pigments in which R_x could be H, OH, or OCH_3 depending of the considered pigment.

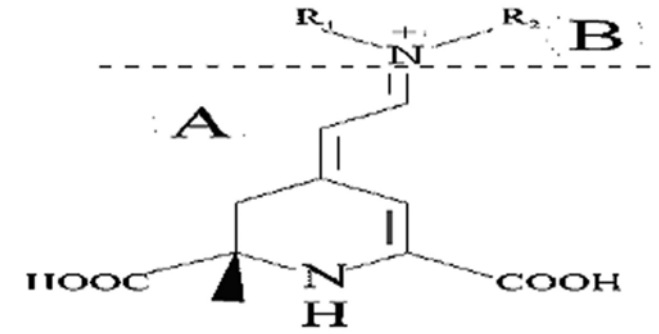

FIGURE 3. Betalain general formula.

(A) Betalamic acid moiety is present in all betalain molecules. (B) betacyanin or a betaxanthin, depending on the identity of the R_1 and R_2 residues.

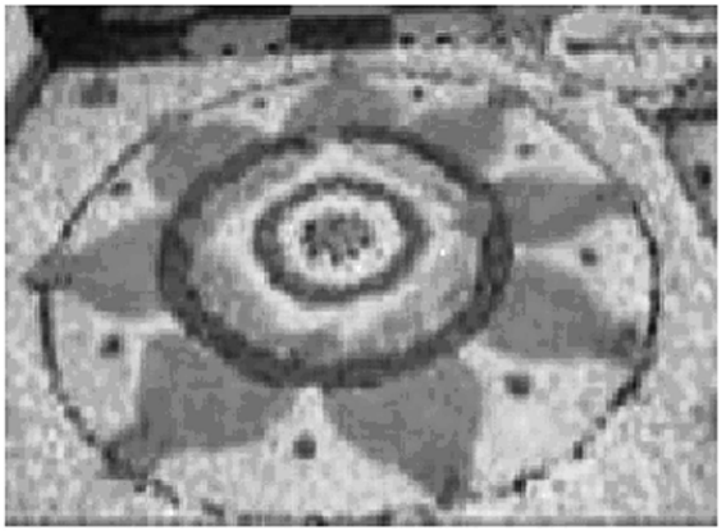

Figure . 4 Traditional decoration- "pookalam" using Marigold flower (Dark colored)

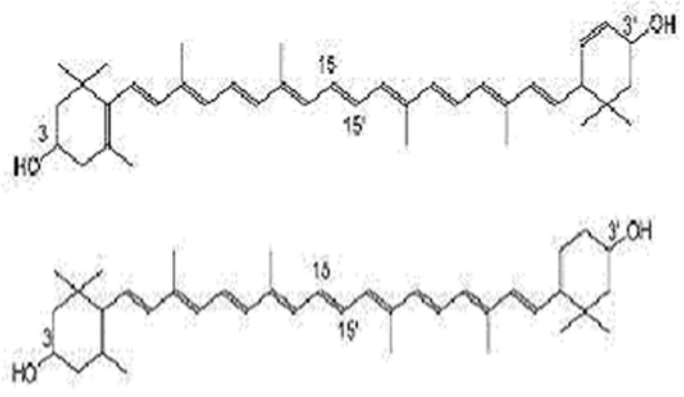

Figure 5 . Chemical structures of the xanthophylls lutein (β,Є-carotene,top) and zeaxanthin(β , β -carotene, bottom).

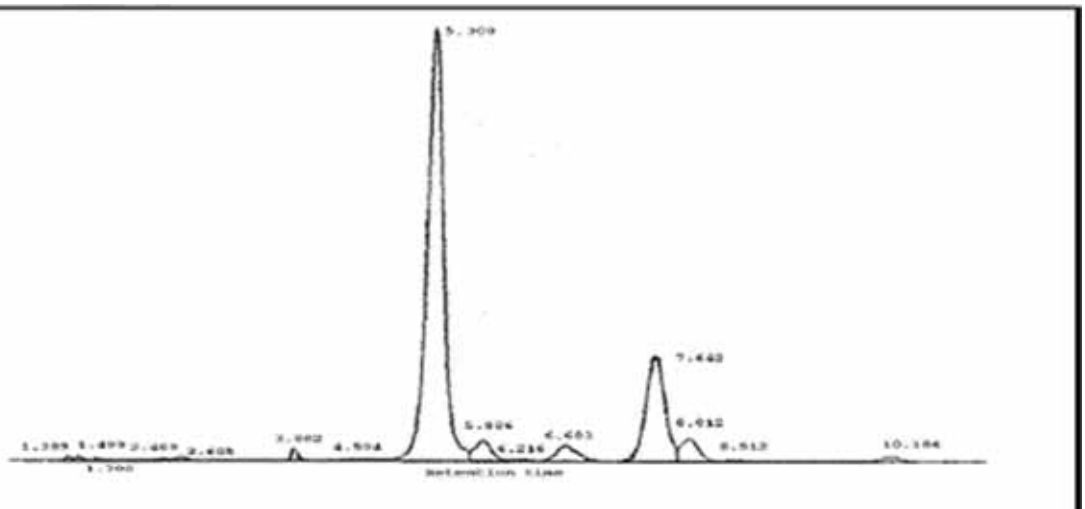

Figure 6 : HPLC Chromatogram of Marigold Oleoresin before saponification

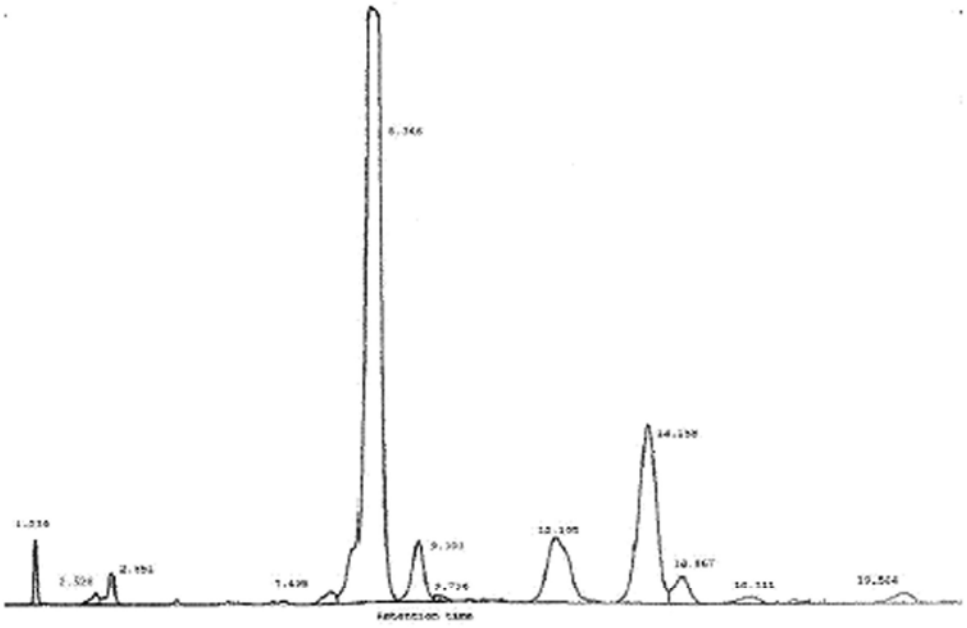

Figure .7 HPLC chromatogram of the saponified marigold oleoresin obtained as final product