Chemical Constituent and Lutein Extraction from Marigold

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Abstract

Marigold (*Tagetes erecta* L.), widely recognized for its ornamental and medicinal values, is a rich source of bioactive compounds, including lutein, a carotenoid with significant health benefits, particularly for eye health. Lutein's demand is increasing in the nutraceutical and food industries, where it is valued for its antioxidant properties and as a natural colorant. This study explores the chemical profile of marigold flowers and examines various methods for efficient lutein extraction. The research aims to enhance lutein yield and purity by optimizing extraction techniques. These findings advance the potential for large-scale lutein production and highlight marigold's role as a sustainable resource for natural bioactive compounds.

Keywords: Antioxidants in nutraceuticals, carotenoids, lutein extraction, marigold, supercritical fluid extraction, ultrasonic-assisted extraction

Introduction

Marigold (*Tagetes erecta* L.) is a highly adaptable plant native to Mexico and Central America, extensively grown for both ornamental and functional purposes across various sectors (Mahantesh *et al*., 2018).[1] Known for their vibrant yellow to orange blooms, marigolds are rich in bioactive compounds that lend significant medicinal as high carotenoid concentration, especially lutein, a powerful antioxidant, and agricultural importance (Hojnik *et al*., 2008).[2] It exhibits potent anti-inflammatory, antimicrobial, and insecticidal properties, making it valuable in traditional medicine and as a natural pesticide in farming (Gómez-Rodrıguez *et al*., 2003).[3] The plant's essential oils are known for their antifungal and antibacterial effects, whereas its flavonoids and phenolic compounds contribute antioxidant and anti-inflammatory benefits. It is also utilized in the food industry as a natural pigment and in cosmetics for its antioxidant benefits (Casella *et al*., 2021).[4] Marigold's ability to thrive in a range of climates and soil conditions enhances its role in sustainable agriculture, where it is often employed as a companion plant for pest control and soil improvement in mixed cropping systems.

Lutein is a naturally occurring carotenoid prominently found in marigold flowers, recognized for its vital role in promoting human health, particularly in safeguarding ocular function. As a potent antioxidant, lutein mitigates the effects of high-energy blue light, thereby reducing oxidative stress on the eyes and helping to prevent age-related conditions such as macular degeneration and cataracts (Chauhan *et al*., 2022).[5] Beyond its ocular benefits, lutein exhibits strong antioxidant properties, offering protection against cellular damage caused by free radicals. Its wide applications extend to the nutraceutical industry, where it is used in dietary supplements, the food industry as a natural pigment, and the cosmetic industry for its skin-protective benefits (Sreevidhya, 2010).[6] Since the human body cannot synthesize lutein, it must be obtained from external plant sources such as marigolds, spinach, kale, peas, and lettuce (Alves-Rodrigues and Shao, 2004; Roberts *et al*., 2009; Calvo, 2005).[7,8]

The increasing demand for lutein is largely driven by its proven benefits for eye health, including its role in preventing age-related macular degeneration (AMD) and cataracts, as well

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as its antioxidant effects that support overall cellular health. This heightened awareness of lutein's advantages has led to a growing market for dietary supplements and functional foods enriched with this carotenoid. Among various lutein sources, marigold is distinguished as a particularly effective crop for several reasons. Marigold not only contains a higher concentration of lutein compared to other sources but also ensures a stable and dependable supply due to its adaptability to a wide range of climatic conditions. In contrast to other lutein-rich plants such as spinach or kale, which may exhibit fluctuating lutein levels based on growing conditions, marigold offers a more consistent and concentrated lutein extract. Furthermore, its versatility in different soil types and environmental settings makes Marigold a prime candidate for sustainable agricultural practices, thereby positioning it as a leading crop to meet the escalating demand for lutein in the nutraceutical and food industries. Despite the widespread use of marigold as a lutein source, the efficiency of extracting this compound is often limited by the plant's complex chemical makeup. This paper delves into the chemical constituents of marigolds and their influence on the extraction process, focusing on improving lutein recovery through optimized methodologies. Understanding the interactions between lutein and other bioactive compounds in marigold is key to refining extraction processes for industrial applications in the food, pharmaceutical, and cosmetic industries.

Chemical Composition of Marigold

Marigold is distinguished by its diverse and rich chemical composition, which underpins its multifaceted applications in medicine, agriculture, and industry. The plant's chemical profile includes a variety of bioactive compounds such as carotenoids, flavonoids, phenolic acids, and essential oils, each contributing to its distinctive properties.

Carotenoids

Carotenoids are the primary pigments responsible for marigold's characteristic yellow to orange hues and are critical for their antioxidant and nutritional benefits.

Lutein

Lutein, a carotenoid predominantly present in leafy vegetables, marigolds, and select fruits, is highly regarded for its extensive health-promoting properties, particularly in ocular health (Calvo, 2005).[9] It selectively accumulates in the retina, where it exerts protective effects against AMD and cataracts by filtering blue light and mitigating oxidative damage (Giordano and Quadro, 2018; Sujak *et al*., 1999).[10,11] Beyond its ocular benefits, lutein functions as a potent antioxidant, contributing to skin protection by reducing ultraviolet (UV)-induced oxidative stress, thereby potentially delaying the onset of photoaging (J. E. Roberts and Dennison, 2015).[12] Moreover, lutein is associated with cardiovascular health, as it reduces inflammation and oxidative stress, which in turn may lower the risk of atherosclerosis and support cholesterol homeostasis (Leermakers *et al*., 2016).[13] Recent studies further indicate lutein's positive impact on cognitive function, particularly in the aging population, as well as its potential role in reducing cancer risk by neutralizing free radicals (Khachik *et al*., 1995).[14] Its anti-inflammatory properties also play a significant role in mitigating the risk of chronic diseases, such as diabetes and cardiovascular conditions. Consequently, dietary intake of lutein, either through natural sources or supplementation, presents a broad spectrum of health advantages, particularly in the prevention of degenerative diseases associated with aging. Lutein is the predominant carotenoid in marigolds, highly valued for its potent antioxidant properties and its role in ocular health. In general, it is crucial for protecting against AMD, cataracts, the neonatal period, and brain normal functioning (Giordano and Quadro, 2018; Erdman Jr *et al*., 2015).[15] Easy availability and numerous benefits make marigolds a key source for lutein extraction and supplementation.

Zeaxanthin

Zeaxanthin, a xanthophyll carotenoid abundant in various fruits, vegetables, and flowers, has been extensively studied for its health-promoting properties, particularly in ocular health (Lee and Demirci, 2022).[16] It plays a critical role in safeguarding the retina against oxidative stress and phototoxic blue light, thereby mitigating the risk of AMD and enhancing visual functions such as acuity and contrast sensitivity.(Ma and Lin, 2010; Mares, 2016).[17,18] Moreover, zeaxanthin contributes to cataract prevention by protecting the crystalline lens from oxidative damage. Beyond its ophthalmological benefits, zeaxanthin exhibits broader physiological effects, including photoprotection for the skin, neuroprotection with potential to reduce neurodegenerative risks, and cardiovascular support through its anti-inflammatory and antioxidant actions and liver boost (Murillo *et al*., 2019).[19] Thus, dietary intake or supplementation of zeaxanthin is associated with substantial benefits for both ocular and systemic health.

Beta-carotene

Beta-carotene (β-carotene), a potent antioxidant and pro-Vitamin A compound, provides several critical health benefits when included as part of a nutrient-rich diet. It plays a vital role in maintaining optimal ocular function, aiding in visual acuity, preventing night blindness, and lowering the risk of AMD (Johra *et al*., 2020).[20] Furthermore, β-carotene contributes to skin protection by mitigating damage from UV radiation and supporting skin rejuvenation. Its antioxidant capacity is linked to immune system enhancement and cardiovascular protection, particularly by inhibiting the oxidative modification of low-density lipoprotein (LDL) cholesterol (Bendich, 1991; Hughes, 2001; Wood *et al*., 2000).[21-23] Emerging research also suggests a potential protective effect of β-carotene against certain cancers, such as those affecting the lungs and skin. In addition, it promotes pulmonary health, especially in individuals with high exposure to environmental pollutants or tobacco smoke (Jun and Root, 2021).[24] Marigold also contains β-carotene, a precursor to Vitamin A. Although present in lower amounts than lutein, β-carotene contributes to essential functions such as vision, immune defense, and skin health.

Flavonoids

Flavonoids are polyphenolic compounds known for their antioxidant, anti-inflammatory, and antimicrobial properties. Although flavonoids enhance marigolds' medicinal value, they can complicate the extraction and purification of lutein by co-extracting with carotenoids.

Quercetin

Quercetin, a bioactive flavonoid prevalent in various fruits, vegetables, and plant sources, provides numerous health benefits due to its potent antioxidant, anti-inflammatory, and immunomodulatory properties (Babaei *et al*., 2018; Materska, 2008).[25,26] Quercetin is a major flavonoid in marigold, recognized for its substantial antioxidant and anti-inflammatory effects. As a robust antioxidant, quercetin mitigates oxidative stress by neutralizing free radicals, thereby safeguarding cellular integrity and potentially reducing the incidence of chronic conditions such as cancer and cardiovascular diseases. Its substantial anti-inflammatory activity supports the management of inflammatory disorders, including arthritis and chronic inflammation (Javadi *et al*., 2017; Sato and Mukai, 2020; Yuan *et al*., 2020).[27-29] Moreover, quercetin enhances immune function, potentially aiding in the body's defense against infections and exhibiting potential anticancer effects by inhibiting tumor proliferation and inducing apoptosis (Duo *et al*., 2012; Ren *et al*., 2017).[30,31] It also contributes to cardiovascular health by reducing blood pressure and lowering LDL cholesterol levels. In addition, quercetin's role as a natural antihistamine helps in mitigating allergy symptoms (Jafarinia *et al*., 2020),[32] whereas its neuroprotective effects may protect against neurodegenerative diseases. Its benefits extend to improving athletic performance, regulating blood glucose levels, and supporting weight management, making it a valuable component of a health-promoting diet (Huang *et al*., 2020; Huang *et al*., 2019).[33,34]

Rutin

Rutin, a bioactive flavonoid present in various fruits and vegetables, provides multiple health benefits. Its robust antioxidant capacity facilitates the neutralization of free radicals, mitigating oxidative stress and potentially reducing the incidence of chronic diseases, including cancer and cardiovascular disorders. Rutin also exhibits significant anti-inflammatory properties, which can help manage inflammatory conditions such as arthritis (Sun *et al*., 2017).[35] By fortifying vascular structures and enhancing circulatory function, rutin may lower the risk of vascular issues such as varicose veins and hemorrhoids. In addition, rutin contributes to blood pressure regulation (Ganga *et al*., 2019)[36] by improving endothelial function and reducing cholesterol levels. Its role in bolstering the immune system supports pathogen defense, whereas its anti-inflammatory effects may alleviate allergic reactions. Furthermore, preliminary studies indicate that rutin may be instrumental in promoting bone health and preserving cognitive function, potentially offering protection against neurodegenerative diseases. Rutin is another key flavonoid in marigold, exhibiting anti-inflammatory, antioxidant, and vascular protective properties (Patel and Patel, 2019).[37] It is utilized in dietary supplements to promote cardiovascular health and mitigate oxidative damage.

Phenolic acids

Phenolic acids in marigolds contribute significantly to their antioxidant capacity and overall therapeutic potential. These compounds, while beneficial for health, may need to be separated during the lutein extraction process to avoid interference with carotenoid purity.

Gallic acid

Gallic acid is a prominent phenolic acid with strong antioxidant properties. It helps neutralize free radicals and contributes to the plant's protective effects against oxidative stress (Badhani *et al*., 2015).[38]

p-Coumaric acid

This phenolic acid is noted for its antioxidant and potential anti-inflammatory and antimicrobial properties, enhancing the plant's medicinal and protective attributes (Boz, 2015).[39]

Essential oils

Marigold contains essential oils that are integral to its antimicrobial and insecticidal properties (Salehi *et al*., 2018).[40] These volatile oils are generally removed during the lutein extraction process, but their presence underscores the potential for marigolds to serve multiple industries beyond nutraceuticals.

Eucalyptol

Eucalyptol, or 1,8-cineole, is an essential oil with significant antimicrobial and anti-inflammatory properties (Ferdosi *et al*., 2022).[41] It is used in various applications, including natural pest control and medicinal formulations.

Linalool

Linalool is another essential oil found in marigolds, known for its antimicrobial and calming effects. It is commonly employed in aromatherapy and natural medicinal products (Mahmoud, 2013).[42]

Terpinen-4-ol

This essential oil possesses antifungal and antibacterial properties, enhancing marigold's efficacy in natural pest management and infection control (Moghaddam *et al*., 2021).[43]

Other bioactive compounds

Marigold also contains additional bioactive substances that contribute to its wide range of applications.

Saponins

Saponins are glycosides with potential anti-inflammatory and immune-enhancing effects. They also influence the plant's bitterness and foaming properties, impacting its use in traditional medicine and as a natural surfactant.

Tannins

Tannins are polyphenolic compounds with astringent properties, which play a role in wound healing and possess antimicrobial and anti-inflammatory activities.

Lutein Extraction Techniques

The extraction of lutein, a carotenoid with notable antioxidant properties, is a subject of significant interest in both research and industry. Effective extraction techniques are essential for isolating and purifying lutein from natural sources such as marigold, which is esteemed for its high lutein concentration. A range of extraction methods has been developed to optimize yield and purity while minimizing lutein degradation. These methods include traditional solvent extraction as well as more advanced techniques such as supercritical fluid extraction (SFE), ultrasonic-assisted extraction (UAE), and enzyme-associated extraction (Pal and Bhattacharjee, 2018; Surendranath *et al*., 2016).[44,45] Each technique presents unique benefits regarding efficiency, selectivity, and environmental impact. A thorough understanding and refinement of these extraction techniques is crucial for producing high-quality lutein suitable for dietary supplements, functional foods, and cosmetic products, thereby addressing the increasing demand for this important carotenoid. Lutein extraction from marigold must be conducted in a way that preserves the carotenoid's structural integrity while minimizing the coextraction of unwanted compounds. Several techniques have been developed to optimize this process, each offering different advantages based on the desired purity and yield.

Solvent extraction

Solvent extraction of lutein from marigolds is a widely employed method to isolate this valuable carotenoid due to its significant health benefits and industrial applications. The process involves using organic solvents to dissolve lutein from the marigold's flower petals, which are first dried and finely ground to enhance extraction efficiency. For better results, dried petals are grinded to powder form (Nath, 2016).[46] Common solvents used include hexane, acetone, ethanol, and ethyl acetate, each selected based on its ability to selectively dissolve lutein while minimizing the extraction of unwanted compounds (Nath, 2016).[46] The powder is mixed with the solvent, and the mixture is agitated to facilitate the dissolution of lutein (Hajare *et al*., 2013)[47] Following agitation, the solvent containing the lutein is separated from the residual plant material (Hajare *et al*., 2013)[47] and then evaporated to yield a concentrated lutein extract. This method, while effective in achieving high yields of lutein, requires careful consideration of solvent toxicity and environmental impact. Optimization of factors such as solvent-to-solid ratio, extraction time, and temperature is crucial to maximize the purity and efficiency of the extraction process.

Supercritical fluid extraction

SFE is a sophisticated method employed for extracting lutein from marigolds by utilizing the distinct properties of supercritical fluids, most commonly carbon dioxide (CO_2) (Anklam *et al*., 1998).[48] In its supercritical state, CO_2 demonstrates both liquid-like and gas-like characteristics (Sapkale *et al*., 2010),[49] enabling it to penetrate solid materials and selectively dissolve nonpolar compounds such as lutein. This technique provides several benefits over traditional solvent extraction, including enhanced extraction efficiency, reduced solvent usage, and a lower environmental footprint. SFE operates under high pressure and moderate temperatures, which helps prevent the thermal degradation of delicate compounds like lutein. Moreover, it allows for precise control over extraction parameters, resulting in high-purity lutein with minimal contaminants (Lang and Wai, 2001).[50] The use of CO_2 as a supercritical fluid is particularly beneficial due to its nontoxic and nonflammable properties, rendering it an environmentally friendly alternative also provides solvent-free analytes (Herrero *et al*., 2010).[51] In summary, SFE is an effective and sustainable method for producing high-quality lutein from marigolds, making it suitable for applications in nutraceuticals, pharmaceuticals, and cosmetics. The SFE process for isolating lutein from marigold flowers involves a series of methodical steps to achieve high efficiency and purity. Initially, marigold petals are dried and finely ground to enhance the surface area for extraction. This powdered material is then introduced into the extraction chamber of an SFE apparatus. CO_2 is utilized as a supercritical fluid due to its effective extraction capabilities and benign environmental impact (Da Silva *et al*., 2016).[52] The CO_2 is elevated to a supercritical state by increasing both temperature (beyond 31°C) and pressure (above 73.8 bar). In this supercritical condition, CO_2 acts as a solvent, penetrating the marigold matrix and dissolving lutein. The CO_2-lutein mixture is subsequently directed into a separator where the pressure is reduced, causing the CO_2 to revert to a gaseous phase, thereby concentrating the lutein (Sapkale *et al*., 2010).[49] The lutein extract is then collected and may be subjected to additional purification processes if required. The SFE system is cleaned postextraction to remove any residual material, ensuring it is prepared for future extractions. This technique is advantageous due to its high selectivity, minimal solvent usage, and capacity to maintain the bioactivity of lutein.

Ultrasonic-assisted extraction

UAE is an advanced method that enhances the efficiency of lutein extraction from marigolds by employing ultrasonic waves to improve solvent interaction and extraction kinetics (Shen *et al*., 2023).[53] This technique uses high-frequency sound waves to induce cavitation bubbles within the solvent, which generate localized shear forces and agitation. These forces effectively disrupt the cell walls of the marigold material, facilitating the release of lutein and other bioactive compounds into the solvent. UAE offers notable advantages over traditional extraction methods, including reduced extraction time, increased yield, and enhanced efficiency, and higher yield (Maheshwari *et al*., 2024).[54] It also allows for the use of milder solvents and lower temperatures, which helps maintain the stability and quality of lutein. Furthermore, UAE is considered an eco-friendly process due to its lower solvent consumption and reduced energy requirements (Oo *et al*., 2024).[55] By optimizing factors such as ultrasonic frequency, power, and extraction duration, UAE can substantially improve lutein recovery from marigolds, making it a valuable technique for producing high-quality lutein extracts for use in nutraceutical and industrial applications. To perform UAE, first prepare the plant material by cleaning, drying, and grinding it into a fine powder to increase the surface area for extraction. Mix the powdered material with an appropriate extraction solvent, such as ethanol, methanol, or water, to create a slurry. The slurry is then subjected to ultrasonic waves using an ultrasonic bath or probe. The ultrasonic waves create high-frequency pressure waves that generate cavitation bubbles within the solvent. The implosion of these bubbles produces shock waves and micro-turbulence that disrupt plant cell walls and enhance the release of bioactive compounds into the solvent. The extraction process is typically carried out at controlled temperatures and times to optimize yield and prevent thermal degradation of the compounds. After ultrasonic treatment, the mixture is filtered to remove solid residues (Zerajić *et al*., 2019).[56] The solvent containing the extracted compounds is then concentrated using methods like rotary evaporation. Finally, the extract may undergo further purification and analysis, such as high-performance liquid chromatography (HPLC), to identify and quantify the target compounds. UAE is favored for its ability to improve extraction efficiency, reduce extraction time, and minimize solvent usage compared to traditional methods.

Enzyme-assisted extraction

Enzyme-assisted extraction (EAE) represents an advanced technique for extracting lutein from marigolds by utilizing enzymatic activity to enhance the extraction process (Fu *et al*., 2018).[57] This method employs enzymes such as cellulases, pectinases, and proteases to degrade the cell wall components and polysaccharides within the plant material, thereby facilitating the liberation of lutein. By disrupting the cellular structure, EAE improves solvent penetration and lutein solubility, leading to higher extraction efficiency compared to traditional methods (Miękus *et al*., 2019).[58] The advantages of EAE include the use of milder extraction conditions, reduced reliance on organic solvents, and a lower environmental impact (Fu *et al*., 2019).[59] Moreover, EAE can enhance the purity of the lutein extract by reducing the co-extraction of undesired substances (Das *et al*., 2022).[60] The optimal performance of EAE depends on the careful selection and concentration of enzymes, as well as precise control of reaction conditions, making it a promising method for obtaining high-quality lutein for applications in nutrition, pharmaceuticals, and cosmetics. In the EAE of lutein from marigold flowers, the process begins with the preparation of the plant material by cleaning and grinding the dried flowers into a fine powder. The extraction employs specific enzymes, such as cellulases or pectinases, which are selected based on their efficacy in degrading plant cell walls (Miękus *et al*., 2019. The powdered material is then mixed with an enzyme-compatible buffer solution and incubated at temperatures between 30°C and 60°C to facilitate enzymatic hydrolysis (Nadar *et al*., 2018).[61] After enzymatic treatment, lutein is extracted by adding a suitable organic solvent, such as ethanol or methanol, and subjected to agitation or ultrasonic assistance to improve extraction efficiency. The mixture is subsequently filtered to remove insoluble residues (Das *et al*., 2022).[60] The resulting filtrate is concentrated using methods like rotary evaporation. The concentrated extract is purified through chromatographic techniques to isolate lutein, which is then quantified and confirmed using HPLC.

Optimization of Extraction Parameters

The efficiency of lutein extraction depends on various parameters, such as solvent type, temperature, time, and the ratio of solvent to plant material. Optimizing these factors is crucial to achieving high lutein yield while maintaining cost-effectiveness and environmental sustainability.

Temperature control

Temperature plays a critical role in enhancing the solubility of lutein in solvents. However, excessively high temperatures can lead to the degradation of sensitive compounds as the solubility and diffusion rates increase (Kang *et al*., 2016).[62] Therefore, finding the optimal temperature that maximizes yield without damaging lutein is essential.

Solvent-to-material ratio

A balanced solvent-to-material ratio is key to efficient extraction. While a higher solvent volume generally improves yield, excessive solvent can dilute the extract, increasing processing time and costs. This parameter should be carefully adjusted to optimize extraction while minimizing waste.

Extraction time

Longer extraction times may increase lutein yield, but excessive durations can lead to the extraction of unwanted compounds and the potential degradation of lutein. Techniques such as UAE or SFE can significantly reduce extraction time while maintaining high efficiency, providing an optimal balance between time and yield.

Purification and Quantification of Lutein

Purification and quantification are essential processes in refining lutein extracted from marigolds to ensure its purity and precise concentration for various applications. These processes involve advanced techniques to isolate lutein from other components and accurately measure its amount.

Purification of lutein

Purification techniques aim to separate lutein from contaminants and other co-extracted substances. Key methods include:

Column chromatography

This method utilizes a stationary phase, such as silica gel or alumina, and a mobile phase to separate lutein based on its interaction with the stationary phase. The extract is applied to the column, and lutein is eluted with an appropriate solvent or solvent mixture. Fractions containing lutein are then collected and assessed for purity (Coskun, 2016).[63]

High-performance liquid chromatography

HPLC is a precise method for purifying lutein. It involves a chromatographic column and a liquid mobile phase to separate lutein from other components (Belanger *et al*., 1997).[64] The lutein is then detected and quantified using a UV-Vis detector, typically calibrated to wavelengths around 445 nm, which is optimal for lutein.[64]

Flash chromatography

This technique accelerates the purification process using a pressurized system to speed up the flow of the mobile phase through the column, resulting in faster separation of lutein (Roge *et al*., 2011).[65]

Liquid-liquid partitioning

This method involves separating the extract between two immiscible liquids, such as water and an organic solvent (e.g. hexane or ethyl acetate) (Berthod and Carda-Broch, 2004).[66] Lutein, being lipophilic, preferentially moves into the organic phase, which facilitates its separation from more polar impurities.

Quantification of lutein

Accurate quantification is crucial for determining lutein concentration and ensuring consistency. Common quantification methods include:

High-performance liquid chromatography

HPLC is also used for precise quantification. The concentration of lutein is measured by comparing the peak area of the lutein sample with that of a known lutein standard, using calibration curves. This method offers high sensitivity and accuracy.

Spectrophotometry

This technique measures the absorbance of lutein at specific wavelengths using a UV-Vis spectrophotometer. Lutein has distinct absorbance peaks, typically around 445 nm, which allows for concentration determination by comparing sample absorbance to that of a lutein standard (Victor and Camarena-Bernard, 2023).[67]

Mass spectrometry

Mass spectrometry, often combined with HPLC, provides detailed molecular information about lutein, including its molecular weight and structure. This enhances the accuracy of quantification.

Colorimetric assays

These assays involve reacting lutein with specific reagents that produce a color proportional to its concentration. While simpler and faster, colorimetric assays may not offer the same level of precision as HPLC or spectrophotometry.

Thin-layer chromatography

Although less precise, thin-layer chromatography can be used for initial estimation and analysis of lutein concentration. This method involves separating lutein on a coated plate and visualizing it under UV light, with the intensity correlating to concentration (Rodić *et al*., 2012).[68]

After extraction, lutein must undergo purification to ensure its isolation from other carotenoids, flavonoids, and phenolic compounds. Several chromatographic techniques are used to achieve high purity and enable accurate quantification.

Conclusion

This study demonstrates the importance of understanding marigold's chemical complexity to improve lutein extraction processes. By optimizing extraction methods, this research not only enhances lutein yield and purity but also positions marigolds as a sustainable source of valuable bioactive compounds. The findings presented here contribute to expanding the use of marigold in nutraceutical and food industries, furthering its role as an important agricultural commodity.

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Conflicts of interest

There are no conflicts of interest.

References

1. Mahantesh KK, Prashanth P, Chandrashekhar R, Saidaih P., Siddappa P, Umesh BC. Evaluation of different African marigold (Tagetes species Linn.) genotypes for vegetative, floral and yield attributes under Southern Telangana condition. Int J Chem Stud 2018;6:3311-3315.
2. Hojnik M, Škerget M, Knez Ž. Extraction of lutein from Marigold flower petals–Experimental kinetics and modelling. LWT-Food Sci Technol 2008;41.
3 Gómez-Rodrıguez O, Zavaleta-Mejıa E, Gonzalez-Hernandez VA, Livera-Munoz M, Cárdenas-Soriano E. Allelopathy and microclimatic modification of intercropping with marigold on tomato early blight disease development. F Crop Res 2003;83:27-34.
4. Casella P *et al*. Optimization of lutein extraction from scenedesmus almeriensis using pressurized liquid extraction. Chem Eng Trans 2021;87:475-480.
5. Chauhan AS. *et al*. Valorizations of marigold waste for high-value products and their industrial importance: a comprehensive review. Resources 2022;11:91.
6. Sreevidhya MK. Extraction, preservation and utilization of natural colour from marigold (Tagetus erecta L.). Department of Processing Technology, College of Horticulture, Vellanikkara, 2010.
7. Alves-Rodrigues A, Shao A. The science behind lutein. Toxicol Lett 2004;150:57-83.
8. Roberts RL, Green J, Lewis B. Lutein and zeaxanthin in eye and skin health. Clin Dermatol 2009;27:195-201.
9. Calvo MM, Lutein: A Valuable Ingredient of Fruit and Vegetables. Crit Rev Food Sci Nutr 2005;45:671-696. doi: 10.1080/10408690590957034.
10. Giordano E. Quadro L. Lutein, zeaxanthin and mammalian development: Metabolism, functions and implications for health. Arch Biochem Biophys 2018;647:33-40.

11. Sujak A, Gabrielska J, Grudziński W, Borc R, Mazurek P, Gruszecki WI. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. Arch Biochem Biophys 1999;371:301-307.
12. Roberts JE, Dennison J, The Photobiology of Lutein and Zeaxanthin in the Eye. J Ophthalmol 2015; 2015:1-8. doi: 10.1155/2015/687173.
13. Leermakers ETM, *et al*. The effects of lutein on cardiometabolic health across the life course: a systematic review and meta-analysis. Am J Clin Nutr 2016;103:481-494.
14. Khachik F, Beecher GR, Smith Jr JC. Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. J Cell Biochem 1995;59:236-246.
15. Erdman Jr JW, *et al*. Lutein and brain function. Foods 2015;4:547-564.
16. Lee CC, Demirci M. Zeaxanthin BT - Handbook of Food Bioactive Ingredients: Properties and Applications. Jafari SM, Rashidinejad A, Simal-Gandara J, Eds. Cham: Springer International Publishing 2022. p. 1-34. doi: 10.1007/978-3-030-81404-5_53-1.
17. Mares J. Lutein and zeaxanthin isomers in eye health and disease. Annu Rev Nutr 2016;36:571-602.
18. Ma L, Lin X. Effects of lutein and zeaxanthin on aspects of eye health. J Sci Food Agric 2010;90:2-12.
19. Murillo AG, Hu S, Fernandez ML. Zeaxanthin: Metabolism, properties, and antioxidant protection of eyes, heart, liver, and skin. Antioxidants 2019;8:390.
20. Johra FT, Bepari AK, Bristy AT, Reza HM. A mechanistic review of β-carotene, lutein, and zeaxanthin in eye health and disease. Antioxidants 2020;9:1046.
21. Wood SM, Beckham C, Yosioka A, Darban H, Watson RR. β-Carotene and selenium supplementation enhances immune response in aged humans. Integr Med 2000;2:85-92. doi: https://doi.org/10.1016/S1096-2190(00)00009-3.
22. Hughes DA. Dietary carotenoids and human immune function. Nutrition 2001;17:823-827. doi: https://doi.org/10.1016/S0899-9007(01)00638-4.
23. BendichA. β-Carotene and the Immune Response. Proc Nutr Soc 1991;50:263-274. doi: 10.1079/PNS19910036.
24. Jun L, Root M. Association of carotenoid intake with pulmonary function. J Am Coll Nutr 2021;40:708-712.
25. Babaei F, Mirzababaei M, Nassiri-Asl M. Quercetin in food: possible mechanisms of its effect on memory. J Food Sci 2018;83:2280-2287.
26. Materska M. Quercetin and its derivatives: chemical structure and bioactivity-a review. Polish J food Nutr Sci 2008;58.
27. Javadi F, *et al*. The Effect of Quercetin on Inflammatory Factors and Clinical Symptoms in Women with Rheumatoid Arthritis: A Double-Blind, Randomized Controlled Trial. J Am Coll Nutr 2017;36:9-15. doi: 10.1080/07315724.2016.1140093.
28. Yuan K, *et al*. Quercetin alleviates rheumatoid arthritis by inhibiting neutrophil inflammatory activities. J Nutr Biochem 2020;84:108454. doi: https://doi.org/10.1016/j.jnutbio.2020.108454.
29. Sato S, Mukai Y. Modulation of Chronic Inflammation by Quercetin: The Beneficial Effects on Obesity. J Inflamm Res 2020;13:421-431. doi: 10.2147/JIR.S228361.
30. Ren K-W, *et al*. Quercetin nanoparticles display antitumor activity via proliferation inhibition and apoptosis induction in liver cancer cells," Int. J. Oncol., vol. 50, no. 4, pp. 1299-1311, 2017.
31 Duo J, Ying G-G, Wang G-W, Zhang L. Quercetin inhibits human breast cancer cell proliferation and induces apoptosis via Bcl-2 and Bax regulation. Mol Med Rep 2012;5:1453-1456.
32. Jafarinia M, *et al*. Quercetin with the potential effect on allergic diseases. Allergy, Asthma Clin Immunol 2020;16:1-11.
33. Huang H, Liao D, Dong Y, Pu R. Effect of quercetin supplementation on plasma lipid profiles, blood pressure, and glucose levels: a systematic review and meta-analysis. Nutr Rev 2020;78:615-626.
34. Huang H, Liao D, Dong Y, Pu R. Clinical effectiveness of quercetin supplementation in the management of weight loss: a pooled analysis of randomized controlled trials. Diabetes, Metab Syndr Obes Targets Ther 2019. p. 553-563.
35. Sun C, Wei J, Bi L. Rutin attenuates oxidative stress and proinflammatory cytokine level in adjuvant induced rheumatoid arthritis via inhibition of NF-κB. Pharmacology 2017;100:40-49.
36. Ganga RM, Goud PP, Nvl SR. Antihypertensive effect of rutin: Pharmacological and computational approach. Asian J Pharm Clin Res 2019;12:87-92.
37. Patel K, Patel DK. The Beneficial Role of Rutin, A Naturally Occurring Flavonoid in Health Promotion and Disease Prevention: A systematic review and update. Bioactive food as dietary interventions for arthritis and related inflammatory diseases. (Second Eds. Academic Press, 2019. p. 457-479. doi: https://doi.org/10.1016/B978-0-12-813820-5.00026-X.
38. Badhani B, Sharma N, Kakkar R. Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications. Rsc Adv 2015;5: 27540-27557.
39. Boz H. p-Coumaric acid in cereals: presence, antioxidant and antimicrobial effects. Int J Food Sci Technol 2015;50:2323-2328.
40. Salehi B, *et al*. Tagetes spp. essential oils and other extracts: Chemical characterization and biological activity. Molecules 2018;23:2847.
41. M. F. H. Ferdosi, I. H. Khan, and A. Javaid, "Composition of essential oil isolated from marigold (Tagetes erecta L.) flowers cultivated in Lahore, Pakistan. Bangladesh J Bot 2022;51:683-688.
42. Mahmoud GI. Biological effects, antioxidant and anticancer activities of marigold and basil essential oils. 2013;7:561-572. doi: 10.5897/JMPR12.350.
43. Moghaddam M, Pirbalouti AG, Babaei K, Farhadi N. Chemical compositions of essential oil from the aerial parts of Tagetes patula L. and Tagetes erecta L. cultivated in northeastern Iran. J Essent Oil Bear Plants 2021;24:990-997.
44. Pal S, Bhattacharjee P. Spray dried powder of lutein-rich supercritical carbon dioxide extract of gamma-irradiated marigold flowers: Process optimization, characterization and food application. Powder Technol 2018;327:512-523. doi: https://doi.org/10.1016/j.powtec.2017.12.085.
45. Surendranath R, Ganga M, Jawaharlal M, Anitha K. Extraction and quantification of marigold lutein using different solvent systems. culture 2016;19:24.
46. Nath S. Extraction and Quantification of Marigold Lutein Using Different Solvent Systems. 2016.
47. Hajare R, Ray A, Shreya TC, Avadhani MN, Selvaraj IC. Extraction and quantification of antioxidant lutein from various plant sources. International Journal of Pharmaceutical Sciences Review and Research, 2013;22:152-157.
48. Anklam E, Berg H, Mathiasson L, Sharman M, Ulberth F. Supercritical fluid extraction (SFE) in food analysis: A review. Food Addit Contam 1998;15:729-750. doi: 10.1080/02652039809374703.
49. Sapkale GN, Patil SM, Surwase US, Bhatbhage PK. A Review Supercritical Fluid Extraction 2010;8:729-743.
50. Lang Q, Wai CM. Supercritical fluid extraction in herbal and natural product studies—a practical review. Talanta 2001;53:771-782.
51. Herrero M, Mendiola JA, Cifuentes A, Ibáñez E. Supercritical fluid extraction: Recent advances and applications. J Chromatogr A 2010;1217:2495-2511. doi: https://doi.org/10.1016/j.chroma.2009.12.019.
52. Da Silva RPFF, Rocha-Santos TAP, Duarte AC. Supercritical fluid extraction of bioactive compounds. TrAC Trends Anal Chem 2016;76:40-51.
53. Shen L, *et al*. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. Ultrason Sonochem 2023;101:106646. doi: https://doi.org/10.1016/j.ultsonch.2023.106646.
54. Maheshwari N, Khanpit VV, Kannan A. Green ultrasound-assisted extraction and life cycle assessment of lutein from marigold flowers using biocompatible surfactants 2024;22:19-29. doi: doi:10.1515/ijcre-2023-0054.
55. Oo N, Shiekh KA, Jafari S, Kijpatanasilp I, Assatarakul K. Characterization of Marigold Flower (Tagetes erecta) Extracts and Microcapsules: Ultrasound-Assisted Extraction and Subsequent Microencapsulation by Spray Drying. Foods 2024;13:2436.
56. Zerajić SA, Savić-Gajić IM, Savić IM, Nikolić GS. The optimization of ultrasound-assisted extraction of total flavonoids from pot marigold (Calendulae officinalis L.) flowers. Adv Technol 2019;8:10-18.
57. Fu XQ, Ma N, Sun WP, Dang YY. Microwave and enzyme co-assisted aqueous two-phase extraction of polyphenol and lutein from marigold (Tagetes erecta L.) flower. Ind Crops Prod 2018;123:296-302.
58. Międkus NA, Iqbal K. Marszałek C, Puchalski, Świergiel A. Green Chemistry Extractions of Carotenoids from Daucus carota L.—Supercritical Carbon

Dioxide and Enzyme-Assisted Methods. Molecules 2019;24:4339. doi: 10.3390/molecules24234339.
59. Fu XQ, Zhang GL, Deng L, Dang YY. Simultaneous extraction and enrichment of polyphenol and lutein from marigold (Tagetes erecta L.) flower by an enzyme-assisted ethanol/ammonium sulfate system. Food Funct 2019;10:266–276.
60. Das S, Nadar SS, RathodVK. Enzyme-assisted Extraction of Bioactive Ingredients. in Natural Product Extraction: Principles and Applications, Prado J, Rostagno M, Eds. The Royal Society of Chemistry, 2022. doi: 10.1039/9781839165894-00269.
61. Nadar SS, Rao P, Rathod VK. Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review. Food Res Int 2018;108:309-330.
62. Kang JH, Kim S, Moon B. Optimization by response surface methodology of lutein recovery from paprika leaves using accelerated solvent extraction. Food Chem 2016;205:140-145. doi: 10.1016/j.foodchem.2016.03.013.
63. Coskun O. Separation techniques: chromatography. North Clin Istanbul 2016;3:156.
64. Belanger JMR, Paré JRJ, Sigouin M. High performance liquid chromatography (HPLC): principles and applications. In: Techniques and instrumentation in analytical chemistry. Canada: Elsevier; 1997;18:37-59.
65. Roge AB, Firke SN, Kawade RM, Sarje SK, Vadvalkar SM. Brief review on: flash chromatography. Int J Pharm Sci Res 2011;2:1930.
66. Berthod A, Carda-Broch S. Determination of liquid–liquid partition coefficients by separation methods J. Chromatogr. A. 2004;1037:3-14.
67. Victor P, Camarena-Bernard C. Lutein, violaxanthin, and zeaxanthin spectrophotometric quantification: A machine learning approach. J Appl Phycol 2023;35:73-84.
68. Rodić Z, Simonovska B, Albreht A, Vovk I. Determination of lutein by high-performance thin-layer chromatography using densitometry and screening of major dietary carotenoids in food supplements. J Chromatogr A 2012;1231:59-65.