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STUDY OF TEMPERATURE STABILITY OF PIGMENT OBTAINED FROM *clitoria ternatea* AND ITS APPLICATION AS NATURAL FOOD COLOUR

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ABSTRACT

Clitoria ternatea is often referred to as the butterfly pea flower. Butterfly peas are easy to grow and flower all year, especially in the summer. Shankapushpi, also known as butterfly pea, is a perennial twining herb found in tropical equatorial areas. The plants are extremely adaptable to changing temperatures and humidity. Plants and microorganisms that produce pigments such as anthocyanins, carotenoids, and chlorophyll are used as natural food colors. Natural coloring agents are gaining popularity, as synthetic colorings have been linked to food intolerance, allergies, irritability, hyperactivity, and sleep disorders in children. Blue pigments are less readily available than red, orange, and yellow pigments. As public awareness of food safety improves, researchers propose utilizing plant-based colorants as a safer alternative to hazardous synthetic dyes. In the present research study, for pigment extraction, *Clitoria ternatea* flowers and water were mixed in a 1:100 (w/v) ratio and used for each experiment. The extract is subjected to heat treatment & analyzed by UV-visible spectroscopy & calorimetrically for colour stability. The thermal stability of the pigment was studied in pressure-cooked rice (wada kolam). The results showed that the pigment has temperature stability and can serve as a natural food colour for the cooking process.

INTRODUCTION

Food colour can indicate flavour, safety, and nutritional value. Synthetic food colorants are widely utilised due to their excellent stability and affordability. However, consumer perception and demand have prompted the replacement of synthetic colourants with naturally produced alternatives. Natural pigment uses may be limited due to reduced stability, weaker tinctorial strength, interactions with food additives, and difficulties in matching desired colours. A single naturally derived colourant cannot replace a specific synthetic colourant in all applications. Fig. 1 depicts the overview of the primary environmental and biological sources of natural colourants. The chemical properties of common pigments, such as anthocyanins, carotenoids, betalains, and chlorophylls, are natural pigments, including anthocyanins for red and blue colourants, as well as potential future alternatives such as purple violacein and red pyranoanthocyanins (Sigurdson et al., 2017).

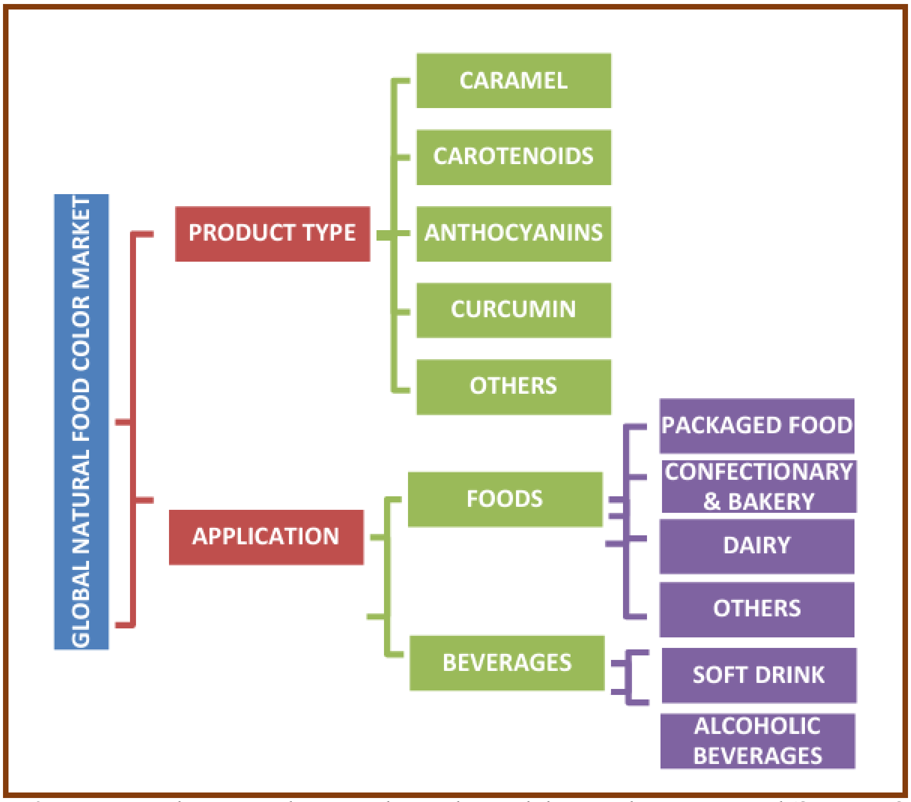

Fig. 1: Summary of major natural pigment classes in the market and their application in Food (Source : (Sen et al., 2019))

Clitoria ternatea is often known as butterfly pea blossom (Fig. 2). Butterfly peas are easy to grow and yield blossoms all year, especially during the summer. Butterfly pea blooms contain more anthocyanins than dragon fruit and Roselle (Suppadit et al., 2011). The Fabaceae family contains roughly 670 genera and nearly 20,000 species of trees, shrubs, vines, and herbs found throughout the world, including India's tropical regions. The flowers of the legume family are diverse, but bilaterally symmetrical. Fossil legume flowers from 50 million years ago were the first to show bilateral symmetry in blooming plants. *Clitoria ternatea L.*, often known as butterfly pea or Shankapushpi, is a perennial twining herb that grows in tropical equatorial environments (Jeyaraj et al., 2021). The plants are very tolerant to changing temperatures and humidity. They can tolerate frost and dry circumstances. Recently, there has been a renewed interest in its potential for medical applications as well as a natural source of food colourings and antioxidants to improve food product attractiveness. This flower contains a high concentration of polyacylated anthocyanins, which produce blue colour in neutral and low-acidic solutions (Marpaung et al., 2013). The flowers of *Clitoria ternatea* contain anthocyanins. Anthocyanins are plant pigments that produce the red, violet, and blue colours in plant blooms. However, the flower's limited shelf life restricts its commercial potential. *Clitoria ternatea* flower extract includes a high concentration of antioxidants, such as phenolic chemicals, flavonoids, and anthocyanins, which are good for human health (Jaafar et al., 2020; Pandey & Rizvi, 2009). Colourants improve food sensory and acceptability. Food colourants are classified as artificial or natural based on their origin. Artificial food colours are compounds generated from coal tar that usually contain an azo group (Dilrukshi et al., 2019; Lin et al., 2018). As public awareness of food safety improves, researchers advocate natural colourants derived from plants as a safer alternative to hazardous synthetic dyes. Customers regularly check the shelf-life date on packaging to determine the freshness and quality of perishable foods (Ghaani et al., 2016).

Fig. 2: *Clitoria ternatea* flower (Source: Plants and microbes produce natural food colors, such as anthocyanins, carotenoids, and chlorophyll (Sen et al., 2019).

Natural coloring agents are becoming more popular because synthetic colorings have been linked to food intolerance, allergies, irritability, hyperactivity, and sleep difficulties in children (Feketea & Tsabouri, 2017). Blue pigments are less readily available than red, orange, and yellow pigments. Fruits also contain anthocyanins, which contribute to their blue hue (Choo, 2018). Anthocyanin, the largest water-soluble pigment in plants, is found in various plant parts and has traditionally been used for food coloring and health purposes. However, its instability makes it unsuitable for commercial use. Anthocyanin has potential health benefits, including antioxidant, anticancer, antidiabetic, hypolipidemic, immunomodulatory, and antibiotic functions. Butterfly pea, a tropical flower, is a promising source of anthocyanins due to its high flower concentration and easy growth (Marpaung et al., 2013). *Clitoria ternatea* flower contains polyacylated anthocyanins, which are more stable than non-acylated anthocyanins and can be utilized as a natural food colorant (Buchweitz et al., 2012). *Clitoria ternatea* flower anthocyanin extract, like other anthocyanins, changes color with pH: red at pH 3. 3.2 or below, blue between pH 3. 3.2 and 5. 5.2, light blue from pH 5. 5.2 to 8. 8.2, and dark green from pH 8. 8.2 to 10. 2 (Escher et al., 2020). In India, *C. ternatea* flowers have long been used as an alternative therapy for snakebite and scorpion stings. An infusion of *C. ternatea* flowers increases menstruation and uterine contractions. In Thailand, the *C. ternatea* flower is used to promote hair growth and colour. *C. ternatea* blooms' blue colour is utilised as a natural food colouring in several cuisines. It is commonly used to manufacture blue glutinous or jasmine rice. In Southeast Asia, dried *C. ternatea* blossoms are used to make a popular herbal tea (Vidana Gamage et al., 2021), (JASMINE, 2014). The butterfly pea flower (*Clitoria ternatea*), a plant indigenous to Southeast Asia, is used to make blue tea, a form of herbal tea. It is a well-liked option for those seeking a soothing, antioxidant-rich beverage because of its striking blue hue and lack of caffeine (Vidana Gamage et al., 2021).

The current research study aims to extract a natural pigment from *Clitoria ternatea* (Butterfly pea) blossoms, to characterize the extracted pigment using spectroscopic methods (e. g., UV-Vis), and to assess the pigment's thermal stability under various temperature conditions, including those encountered during food processing (such as rice (wada kolam) cooking). The study will evaluate the pigment's durability and color intensity retention in actual food matrices, specifically rice cooked under pressure and by conventional methods in cooking utensils, to determine the efficacy of *Clitoria ternatea* pigment as a natural food colorant in high-temperature applications.

MATERIALS AND METHODS

Collection and Extraction of Pigment

The study was conducted between 2023 and 2024 at the Department of Microbiology, Yashvantrao Chavan Institute of Science, Satara, Maharashtra, India. *Clitoria ternatea* flowers were grown in the botanical garden of YCIS Satara. After harvesting, *Clitoria ternatea* flowers were rinsed and drained under running tap water. Petals were separated from sepals and dried in a hot air oven at 70°C. The dried sample was packed in a black bag and stored at room temperature. The raw ingredients (butterfly pea flower) and solvent (water) have a weight-to-volume ratio of 1:20. Each treatment used 10 g of dried material. The traditional method for harvesting *C. ternatea* flowers has been widely utilised since the 1970s. Extraction experiments on *C. ternatea* flowers with aqueous solvents showed the structure of phytochemicals, particularly anthocyanins (Terahara et al., 1989, 1998; Terahara & Oda, 1996). Simply 10 g of *C. ternatea* flowers was added to 200 mL of distilled water at varied temperatures, then centrifuged for 30 minutes. To prepare the aqueous extract, 10 g of the dried flower sample was combined with 200 mL of distilled water. Then the mixture was strained through the fine mesh & pigment extract was collected.

Study on the thermal stability of pigment at variable temperatures

To study the temperature stability of the pigment, 12 test tubes containing 5 ml of the extract were then placed in a water bath. The temperature of the water bath was kept at 60°C. Each tube was withdrawn after 5 minutes & tested for the following,

a) UV-Vis Spectroscopy

The UV-visible absorption spectra of the heat-treated extract were measured at room temperature with a UV-1600 spectrophotometer (Shimadzu, Kyoto, Japan) equipped with 10 mm quartz rectangular cells spanning in wavelength range from 400 to 580nm. Distilled water was utilised to change the baseline.

b) Colorimetry

The same heat-treated extract was then tested using a colorimeter. The solution was placed in a glass cuvette, and its absorbance was measured at 520 nm.

To assess any degradation of the pigment, absorbance measurements of pigment extracts (before and after heating) were taken using UV-Vis spectrophotometry, when appropriate.

Study on the thermal stability of pigment using rice (wada kolam variety)

Wada kolam rice variety is known for its sweet flavor and aroma. This thin, high-quality rice is well used in Maharashtra. During the experiment, the rice was chosen as a food matrix to represent a real-life food application. The extracted *Clitoria ternatea* pigment was uniformly blended into newly washed uncooked rice at a specific concentration (5 mL pigment extract per 100 g rice). In these experiments, the pigment-infused rice was then pressure-cooked to determine thermal stability during cooking at the pressure cooker temperature. The rice samples were cooked in a pressure cooker at around 100°C and 15 psi pressure. The heating time was set for 30 minutes to approximate typical domestic pressure cooking. After cooking, the rice was cooled and visually checked to ensure colour preservation in both sets. The surface colour intensity was measured visually.

RESULTS AND DISCUSSION

Extraction of pigment & study on the temperature stability of the pigment (UV-Vis Spectroscopy & colorimetry)

The pigment isolated from *Clitoria ternatea* flowers has a rich blue to purple tint, which is characteristic of anthocyanins. The extraction yield was found to be efficient under acidic circumstances, and UV-Vis spectroscopy verified the existence of anthocyanins with an absorption peak between 540 and 620 nm. The UV-Vis absorbance spectra of *Clitoria ternatea* pigment in boiled rice show minor degradation over time, with a gradual decrease in absorbance intensity. However, the pigment's shape remains unchanged, indicating structural stability. The pigment demonstrates good thermal stability in rice, even after 30 minutes of pressure cooking at 121°C & by conventional cooking too. The slight decrease in absorbance suggests minimal degradation but no significant color loss, making it a promising natural food colorant for thermally processed foods (Fig. 3).

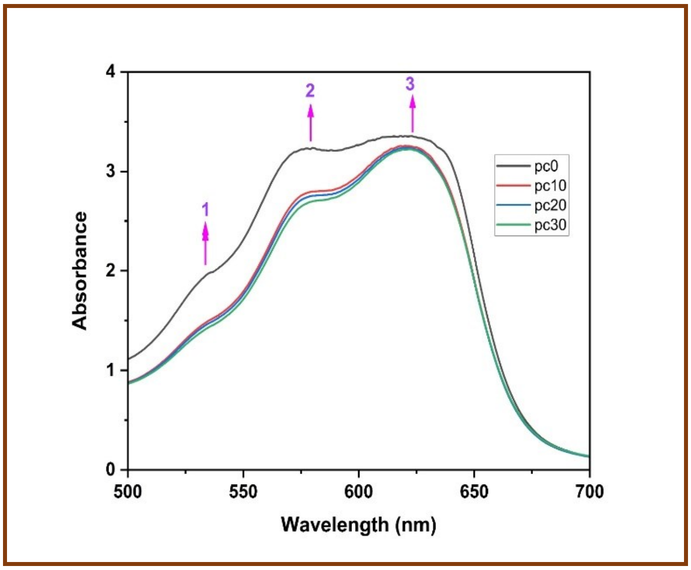

Fig. 3: UV-Visible spectroscopic analysis of the pigment

Table 1 shows optical density (OD) values of *Clitoria ternatea*, a natural food colorant, during a pressure cooking experiment. The initial OD value is highest at 0 minutes, but a gradual decrease suggests minor thermal breakdown of anthocyanins. After 30 minutes, the OD values stabilize and increase slightly, suggesting reversible structural changes or increased extraction due to rice starch barriers. This makes *Clitoria ternatea* a promising natural food colorant for thermally processed foods.

Table 1: Colorimetric analysis of the pigment

Sr. No.	Time (min)	Filter (nm)	Optical Density (OD)
1	0	520	0.77
2	10		0.59
3	20		0.58
4	30		0.56

Study on the temperature stability of pigment using rice (wada kolam variety)

The thermal stability of the pigment was evaluated at various temperatures. The results showed that the pigment maintained its colour intensity up to 100˚C for 30 min. However, under pressure cooking settings, the pigment demonstrated extraordinary durability, indicating its potential utility in high-temperature food preparation. One of the primary applications investigated was the addition of the pigment to boiling rice under pressure cooking conditions (121˚C, 15 psi for 30 minutes). The results showed, there was no substantial loss of colour intensity after 30 minutes of pressure cooking (Fig. 4). The pigment effectively coloured the rice, keeping a consistent shade without fading. The colour stability of boiled rice indicates that the pigment is robust to high-temperature cooking processes, making it a desirable natural food colourant for rice-based recipes. Steaming and boiling were effective in preserving the pigment colour, however, lengthy baking at high temperatures (>150˚C) resulted in deterioration.

Fig 4: Image of cooked rice with pigment

Clitoria ternatea pigment offers a natural, heat-resistant alternative to synthetic food colourants, suitable for rice, grain-based items, beverages, desserts, confectionery, and dairy products, but limited to lower baking temperatures. Anthocyanins are water-soluble pigments with significant roles in nature, including seed dispersal, pollination, and plant organ development. They are used in industries due to their color palette and antioxidant, anticancer, anti-inflammatory, and antimicrobial properties. However, their low stability is influenced by factors like pH, light, temperature, co-pigmentation, sulfites, ascorbic acid, oxygen, and enzymes. Understanding these factors is crucial to minimize their negative effects and enhance their health benefits (Enaru et al., 2021). Many researchers have examined how temperature & solvent affect natural extract yields. Water, alcohol, and acetone are among the most common solvents used for plant extracts in TLC. Solvent efficacy in extracting bioactive components from plants varies according to their intended use. UV-Vis spectroscopy was utilised to determine the absorption peaks and functional groups of dyes derived from *C. ternatea* flowers. (Jeyaraj et al., 2021) studied the antimicrobial, antioxidant, anti-diabetic & cytotoxic activities of *C. ternatea* flowers extract, results show these are beneficial to human beings& can be used as a promising candidate for functional food application.

APPLICATION

Clitoria ternatea flower extract is a natural blue colourant that is easy to use and has a longer shelf life than other plant-based colourants. *Clitoria ternatea* contains anthocyanins, which are antioxidants. Anthocyanin, which is found in edible fruits, vegetables, and flowers, has been demonstrated to help prevent diseases including diabetes and heart disease (Horbowicz et al., 2008; Siti Azima et al., 2017). In the current study, we used pigment extract from *Clitoria ternatea* as a natural colourant in rice, which produces a blue colour in cooked rice.

CONCLUSION

The study shows that *Clitoria ternatea's* anthocyanin pigment is stable under pressure cooking, & conventional cooking, making it a potential natural alternative to synthetic dyes in the food industry. However, further research is needed to evaluate its long-term storage stability, sensory attributes, consumer acceptance, and potential antioxidant and health benefits.

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